ISSN 0910－0865　Coden：KHKEEN

環境変異原研究

Environmental Mutagen Research

Vol.24 No.1 2002

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Environ. Mutagen Res., 24: 1-15 (2002) Original Article

Genotoxicity of extracts of Japanese traditional herbal medicines (Kampo)

Makoto Katami *, Haruo Kuboniwa, Shunichi Maemura and Toshihiko Yanagisawa

New Drug Discovery Laboratory, R & D Division, TSUMURA & CO.
3586, Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan

Summary

The possible genotoxicity potential of 128 Japanese traditional herbal medicines (Kampo) was investigated using a bacterial reverse mutation test (the Ames test), an in vivo micronucleus test (MN test) in mouse bone marrow cells and an unscheduled DNA synthesis test (UDS test) in rat hepatocytes. Of 128 Kampo extracts examined, 98 did not induce mutations in bacteria while 30 induced mutations weakly in *Salmonella typhimurium* TA1537. Extracts of Scutellariae Radix, a common herbal drug, and its major components, wogonin, baicalin, and baicalein showed positive responses in the Ames test. Among 30 Ames-positive Kampo extracts, 27 contained these major components of Scutellariae Radix extracts. It is possible that these components contribute to the mutagenicity of the 27 Kampo extracts. Thirty Ames-positive Kampo extracts were also evaluated for their ability to induce chromosomal aberrations and DNA damage in in vivo micronucleus and unscheduled DNA synthesis assays. None of these extracts induced micronuclei and UDS. Wogonin, baicalin and baicalein did not induce micronuclei in vivo. Based on the results in the present study, it is concluded that 98 Kampo extracts had no mutagenic potential in vitro, and that the other 30 Kampo extracts had no genotoxicity in vivo although they showed weak mutagenicity in bacteria.

Keywords: Japanese traditional herbal medicines (Kampo), genotoxicity, Ames test, micronucleus test, unscheduled DNA synthesis test

Introduction

Japanese traditional herbal medicines (Kampo) are mixtures of crude extracts of herbal drugs. Each Kampo is widely used to treat various types of disease in Japan. We market 129 Kampo extract preparations for prescription use. Based on their extensive history in clinical practice, Kampo are recognized as effective and safe biological agents. In order to verify the safety of Kampo extracts, we have conducted some toxicological studies in animals (Iijima et al., 1995 ; Minematsu et al., 1995a-b ; Katsumata et al., 1997 ; Shimazu et al., 1997). Although genotoxicity studies are useful for the prediction of carcinogenicity, relatively few Kampo extracts and herbal drugs have been previously investigated for genotoxicity (Fujita et al., 1992, 1994 ; Fujita and Sasaki, 1993 ; Kikuchi and Oshio, 1983 ; Morimoto et al., 1982 ; Nozaka et al., 1987, 1991, 1994a-b ; Tadaki et al., 1995 ; Watanabe et al., 1983 ; Xue-jun et al., 1991 ; Yamamoto et al., 1982). However, the data which do exist, show several inconsistencies.

In this study, the genotoxicity of 128 Kampo extracts was examined first in vitro followed by in vivo tests for samples that gave a positive in vitro response.

Materials and Methods

Test substances

Spray-dried extracts of 128 Kampo (Table 1), which were produced in Tsumura & Co., Tokyo, Japan, consist of hot-water extracts from some herbal drugs mixed at the ratios shown in Table 1. Freeze-dried water extracts of Scutellariae Radix and its components, wogonin, baicalin and baicalein, were obtained from Tsumura & Co., Tokyo, Japan.

Bacterial reverse mutation test (Ames test)

The Ames test was carried out by the usual method (Ames et al., 1975 ; Maron and Ames, 1983). The

* katami_makoto@mail. tsumura.co.jp
Received: March 9, 2001, accepted: September 13, 2001

Table 1 Summary of genotoxicity responses of 128 extracts of Japanese traditional herbal medicines (Kampo) in the bacterial reverse mutation test, the micronucleus test and the unscheduled DNA synthesis (UDS) test

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-1	Kakkon-to	Puerariae Radix 4.0, Zizyphi Fructus 3.0, Ephedrae Herba 3.0, Glycyrrhizae Radix 2.0, Cinnamomi Cortex 2.0, Paeoniae Radix 2.0, Zingiberis Rhizoma 2.0	N	−	−
TJ-2	Kakkon-to-ka-senkyu-shin'i	Puerariae Radix 4.0, Zizyphi Fructus 3.0, Ephedrae Herba 3.0, Glycyrrhizae Radix 2.0, Cinnamomi Cortex 2.0, Paeoniae Radix 2.0, Cnidii Rhizoma 2.0, Zingiberis Rhizoma 1.0, Magnoliae Flos 2.0	N	−	−
TJ-3	Otsuji-to	Angelicae Radix, 6.0, Bupleuri Radix 5.0, Scutellariae Radix 3.0, Glycyrrhizae Radix 2.0, Cimicifugae Rhizoma 1.0, Rhei Rhizoma 0.5	P	N	N
TJ-5	Anchu-san	Cinnamomi Cortex 4.0, Corydalis Tuber 3.0, Ostreae Testa 3.0, Foeniculi Fructus 1.5, Glycyrrhizae Radix 1.0, Amomi Semen 1.0, Alpiniae Officinarum Rhizoma 0.5	N	−	−
TJ-6	Jumi-haidoku-to	Platycodi Radix 3.0, Bupleuri Radix 3.0, Cnidii Rhizoma 3.0, Hoelen 3.0, Ledebouriellae Radix 1.5, Glycyrrhizae Radix 1.0, Schizonepetae Spica 1.0, Zingiberis Rhizoma 1.0, Quercus Cortex 3.0, Araliae Rhizoma et Radix 1.5	N	−	−
TJ-7[a)]	Hachimi-jio-gan	Rehmanniae Radix 6.0, Corni Fructus 3.0, Dioscoreae Rhizoma 3.0, Alismatis Rhizoma 3.0, Hoelen 3.0, Moutan Cortex 2.5, Cinnamomi Cortex 1.0, Aconiti Tuber 0.5	N	N	−
TJ-8[a)]	Dai-saiko-to	Bupleuri Radix 6.0, Pinelliae Tuber 4.0, Scutellariae Radix 3.0, Paeoniae Radix 3.0, Zizyphi Fructus 3.0, Aurantii Fructus Immaturus 2.0, Zingiberis Rhizoma 1.0, Rhei Rhizoma 1.0	P	N	N
TJ-9[a)]	Sho-saiko-to	Bupleuri Radix 7.0, Pinelliae Tuber 5.0, Scutellariae Radix 3.0, Zizyphi Fructus 3.0, Ginseng Radix 3.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	P	N	N
TJ-10[a)]	Saiko-keishi-to	Bupleuri Radix 5.0, Pinelliae Tuber 4.0, Scutellariae Radix 2.0, Glycyrrhizae Radix 2.0, Cinnamomi Cortex 2.0, Paeoniae Radix 2.0, Zizyphi Fructus 2.0, Ginseng Radix 2.0, Zingiberis Rhizoma 1.0	P	N	N
TJ-11	Saiko-keishi-kankyo-to	Bupleuri Radix 6.0, Scutellariae Radix 3.0, Trichosanthis Radix 3.0, Cinnamomi Cortex 3.0, Ostreae Testa 3.0, Glycyrrhizae Radix 2.0, Zingiberis Siccatum Rhizoma 2.0	P	N	N
TJ-12[a)]	Saiko-ka-ryukotsu-borei-to	Bupleuri Radix 5.0, Pinelliae Tuber 4.0, Cinnamomi Cortex 3.0, Hoelen 3.0, Scutellariae Radix 2.5, Zizyphi Fructus 2.5, Ginseng Radix 2.5, Ostreae Testa 2.5, Fossilia Ossis Mastodi 2.5, Zingiberis Rhizoma 1.0	P	N	N
TJ-14	Hange-shashin-to	Pinelliae Tuber 5.0, Scutellariae Radix 2.5, Glycyrrhizae Radix 2.5, Zizyphi Fructus 2.5, Ginseng Radix 2.5, Coptidis Rhizoma 1.0, Zingiberis Siccatum Rhizoma 2.5	P	N	N
TJ-15[a)]	Oren-gedoku-to	Scutellariae Radix 3.0, Coptidis Rhizoma 2.0, Gardeniae Fructus 2.0, Phellodendri Cortex 1.5	P	N	N
TJ-16	Hange-koboku-to	Pinelliae Tuber 6.0, Hoelen 5.0, Magnoliae Cortex 3.0, Perillae Herba 2.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-17	Gorei-san	Alismatis Rhizoma 4.0, Atractylodis Lanceae Rhizoma 3.0, Polyporus 3.0, Hoelen 3.0, Cinnamomi Cortex 1.5	N	−	−
TJ-18	Keishi-ka-jutsu-bu-to	Cinnamomi Cortex 4.0, Paeoniae Radix 4.0, Atractylodis Lanceae Rhizoma 4.0, Zizyphi Fructus 4.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0, Aconiti Tuber 0.5	N	−	−
TJ-19[a)]	Sho-seiryu-to	Pinelliae Tuber 6.0, Glycyrrhizae Radix 3.0, Cinnamomi Cortex 3.0, Schisandrae Fructus 3.0, Asiasari Radix 3.0, Paeoniae Radix 3.0, Ephedrae Herba 3.0, Zingiberis Siccatum Rhizoma 3.0	N	N	−
TJ-20	Boi-ogi-to	Astragali Radix 5.0, Sinomeni Caulis et Rhizoma 5.0, Atractylodis Lanceae Rhizoma 3.0, Zizyphi Fructus 3.0, Glycyrrhizae Radix 1.5, Zingiberis Rhizoma 1.0	N	−	−
TJ-21	Sho-hange-ka-bukuryo-to	Pinelliae Tuber 6.0, Hoelen 5.0, Zingiberis Rhizoma 1.5	N	−	−

(**Table 1** continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-22	Shofu-san	Gypsum Fibrosum 3.0, Rehmanniae Radix 3.0, Angelicae Radix 3.0, Atractylodis Lanceae Rhizoma 2.0, Ledebouriellae Radix 2.0, Akebiae Caulis 2.0, Anemarrhenae Rhizoma 1.5, Glycyrrhizae Radix 1.0, Sophorae Radix 1.0, Schizonepetae Spica 1.0, Arctii Fructus 2.0, Sesami Semen 1.5, Cicadae Periostracum 1.0	N	−	−
TJ-23[a)]	Toki-shakuyaku-san	Paeoniae Radix 4.0, Atractylodis Lanceae Rhizoma 4.0, Alismatis Rhizoma 4.0, Hoelen 4.0, Cnidii Rhizoma 3.0, Angelicae Radix 3.0	N	N	−
TJ-24[a)]	Kami-shoyo-san	Bupleuri Radix 3.0, Paeoniae Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Angelicae Radix 3.0, Hoelen 3.0, Gardeniae Fructus 2.0, Moutan Cortex 2.0, Glycyrrhizae Radix 1.5, Zingiberis Rhizoma 1.0, Menthae Herba 1.0	N	N	−
TJ-25[a)]	Keishi-bukuryo-gan	Cinnamomi Cortex 3.0, Paeoniae Radix 3.0, Persicae Semen 3.0, Hoelen 3.0, Moutan Cortex 3.0	N	N	−
TJ-26	Keishi-ka-ryukotsu-borei-to	Cinnamomi Cortex 4.0, Paeoniae Radix 4.0, Zizyphi Fructus 4.0, Ostreae Testa 3.0, Fossilia Ossis Mastodi 3.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.5	N	−	−
TJ-27	Mao-to	Armeniacae Semen 5.0, Ephedrae Herba 5.0, Cinnamomi Cortex 4.0, Glycyrrhizae Radix 1.5	N	−	−
TJ-28	Eppi-ka-jutsu-to	Gypsum Fibrosum 8.0, Ephedrae Herba 6.0, Atractylodis Lanceae Rhizoma 4.0, Zizyphi Fructus 3.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-29	Bakumondo-to	Ophiopogonis Tuber 10.0, Pinelliae Tuber 5.0, Zizyphi Fructus 3.0, Glycyrrhizae Radix 2.0, Ginseng Radix 2.0, Oryzae Fructus 5.0	N	−	−
TJ-30	Shimbu-to	Hoelen 4.0, Paeoniae Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Zingiberis Rhizoma 1.5, Aconiti Tuber 0.5	N	−	−
TJ-31	Goshuyu-to	Zizyphi Fructus 4.0, Evodiae Fructus 3.0, Ginseng Radix 2.0, Zingiberis Rhizoma 1.5	N	−	−
TJ-32	Ninjin-to	Glycyrrhizae Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Ginseng Radix 3.0, Zingiberis Siccatum Rhizoma 3.0	N	N	−
TJ-33	Daio-botampi-to	Persicae Semen 4.0, Moutan Cortex 4.0, Rhei Rhizoma 2.0, Benincasae Semen 6.0, Natrium Sulfuricum 1.8	N	−	−
TJ-34	Byakko-ka-ninjin-to	Gypsum Fibrosum 15.0, Anemarrhenae Rhizoma 5.0, Glycyrrhizae Radix 2.0, Ginseng Radix 1.5, Oryzae Fructus 8.0	N	N	−
TJ-35	Shigyaku-san	Bupleuri Radix 5.0, Paeoniae Radix 4.0, Aurantii Fructus Immaturus 2.0, Glycyrrhizae Radix 1.5	N	N	−
TJ-36	Moku-boi-to	Gypsum Fibrosum 10.0, Sinomeni Caulis et Rhizoma 4.0, Cinnamomi Cortex 3.0, Ginseng Radix 3.0	N	−	−
TJ-37	Hange-byakujutsu-temma-to	Aurantii Nobilis Pericarpium 3.0, Pinelliae Tuber 3.0, Atractylodis Rhizoma 3.0, Hoelen 3.0, Astragali Radix 1.5, Alismatis Rhizoma 1.5, Ginseng Radix 1.5, Phellodendri Cortex 1.0, Zingiberis Rhizoma 0.5, Gastrodiae Tuber 2.0, Hordei Fructus Germinatus 2.0, Zingiberis Siccatum Rhizoma 1.0	N	−	−
TJ-38	Toki-shigyaku-ka-go shuyu-shokyo-to	Zizyphi Fructus 5.0, Cinnamomi Cortex 3.0, Paeoniae Radix 3.0, Angelicae Radix 3.0, Akebiae Caulis 3.0, Glycyrrhizae Radix 2.0, Evodiae Fructus 2.0, Asiasari Radix 2.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-39	Ryo-kei-jutsu-kan-to	Hoelen 6.0, Cinnamomi Cortex 4.0, Atractylodis Lanceae Rhizoma 3.0, Glycyrrhizae Radix 2.0	N	−	−
TJ-40	Chorei-to	Alismatis Rhizoma 3.0, Polyporus 3.0, Hoelen 3.0, Asini Gelatinum 3.0, Kadinum 3.0	N	−	−
TJ-41[a)]	Hochu-ekki-to	Astragali Radix 4.0, Atractylodis Lanceae Rhizoma 4.0, Ginseng Radix 4.0, Angelicae Radix 3.0, Bupleuri Radix 2.0, Zizyphi Fructus 2.0, Aurantii Nobilis Pericarpium 2.0, Glycyrrhizae Radix 1.5, Cimicifugae Rhizoma 1.0, Zingiberis Rhizoma 0.5	N	N	−
TJ-43[a)]	Rikkunshi-to	Atractylodis Lanceae Rhizoma 4.0, Ginseng Radix 4.0, Pinelliae Tuber 4.0, Hoelen 4.0, Zizyphi Fructus 2.0, Aurantii Nobilis Pericarpium 2.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 0.5	N	N	−

(**Table 1** continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-45	Keishi-to	Cinnamomi Cortex 4.0, Paeoniae Radix 4.0, Zizyphi Fructus 4.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.5	N	−	−
TJ-46	Shichimotsu-koka-to	Paeoniae Radix 4.0, Angelicae Radix 4.0, Astragali Radix 3.0, Rehmanniae Radix 3.0, Cnidii Rhizoma 3.0, Phellodendri Cortex 2.0, Uncariae Ramulus et Uncus 3.0	N	−	−
TJ-47[a)]	Choto-san	Gypsum Fibrosum 5.0, Aurantii Nobilis Pericarpium 3.0, Ophiopogonis Tuber 3.0, Pinelliae Tuber 3.0, Hoelen 3.0, Ginseng Radix 2.0, Ledebouriellae Radix 2.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0, Uncariae Ramulus et Uncus 3.0, Chrysanthemi Flos 2.0	N	N	−
TJ-48	Juzen-taiho-to	Astragali Radix 3.0, Cinnamomi Cortex 3.0, Rehmanniae Radix 3.0, Paeoniae Radix 3.0, Cnidii Rhizoma 3.0, Atractylodis Lanceae Rhizoma 3.0, Angelicae Radix 3.0, Ginseng Radix 3.0, Hoelen 3.0, Glycyrrhizae Radix 1.5	N	−	−
TJ-50	Keigai-rengyo-to	Scutellariae Radix 1.5, Phellodendri Cortex 1.5, Coptidis Rhizoma 1.5, Platycodi Radix 1.5, Aurantii Fructus Immaturus 1.5, Schizonepetae Spica 1.5, Bupleuri Radix 1.5, Gardeniae Fructus 1.5, Rehmanniae Radix 1.5, Paeoniae Radix 1.5, Cnidii Rhizoma 1.5, Angelicae Radix 1.5, Menthae Herba 1.5, Angelicae Dahuricae Radix 1.5, Ledebouriellae Radix 1.5, Forsythiae Fructus 1.5, Glycyrrhizae Radix 1.0	P	N	N
TJ-51	Juncho-to	Rehmanniae Radix 6.0, Angelicae Radix 3.0, Scutellariae Radix 2.0, Aurantii Fructus Immaturus 2.0, Armeniacae Semen 2.0, Magnoliae Cortex 2.0, Rhei Rhizoma 2.0, Persicae Semen 2.0, Glycyrrhizae Radix 1.5, Cannabidis Semen2.0	P	N	N
TJ-52	Yokuinin-to	Coicis Semen 8.0, Atractylodis Lanceae Rhizoma 4.0, Angelicae Radix 4.0, Ephedrae Herba 4.0, Cinnamomi Cortex 3.0, Paeoniae Radix 3.0, Glycyrrhizae Radix 2.0	N	−	−
TJ-53	Sokei-kakketsu-to	Paeoniae Radix 2.5, Rehmanniae Radix 2.0, Cnidii Rhizoma 2.0, Atractylodis Lanceae Rhizoma 2.0, Angelicae Radix 2.0, Persicae Semen 2.0, Hoelen 2.0, Achyranthis Radix 1.5, Aurantii Nobilis Pericarpium 1.5, Sinomeni Caulis et Rhizoma 1.5, Ledebouriellae Radix 1.5, Gentianae Scabrae Radix 1.5, Glycyrrhizae Radix 1.0, Angelicae Dahuricae Radix 1.0, Zingiberis Rhizoma 0.5, Clematidis Radix 1.5, Notopterygii Rhizoma 1.5	N	N	−
TJ-54	Yoku-kan-san	Atractylodis Lanceae Rhizoma 4.0, Hoelen 4.0, Cnidii Rhizoma 3.0, Angelicae Radix 3.0, Bupleuri Radix 2.0, Glycyrrhizae Radix 1.5, Uncariae Ramulus et Uncus 3.0	N	−	−
TJ-55	Ma-kyo-kan-seki-to	Gypsum Fibrosum 10.0, Armeniacae Semen 4.0, Ephedrae Herba 4.0, Glycyrrhizae Radix 2.0	N	−	−
TJ-56	Gorin-san	Hoelen 6.0, Scutellariae Radix 3.0, Glycyrrhizae Radix 3.0, Rehmanniae Radix 3.0, Plantaginis Semen 3.0, Alismatis Rhizoma 3.0, Angelicae Radix 3.0, Akebiae Caulis 3.0, Gardeniae Fructus 2.0, Paeoniae Radix 2.0, Kadinum 3.0	P	N	N
TJ-57	Unsei-in	Rehmanniae Radix 3.0, Paeoniae Radix 3.0, Cnidii Rhizoma 3.0, Angelicae Radix 3.0, Scutellariae Radix 1.5, Phellodendri Cortex 1.5, Coptidis Rhizoma 1.5, Gardeniae Fructus 1.5	P	N	N
TJ-58	Seijo-bofu-to	Scutellariae Radix 2.5, Platycodi Radix 2.5, Gardeniae Fructus 2.5, Cnidii Rhizoma 2.5, Glehniae Radix cum Rhizoma 2.5, Angelicae Dahuricae Radix 2.5, Forsythiae Fructus 2.5, Coptidis Rhizoma 1.0, Glycyrrhizae Radix 1.0, Aurantii Fructus Immaturus 1.0, Schizonepetae Spica 1.0, Menthae Herba 1.0	P	N	N
TJ-59	Ji-zuso-ippo	Cnidii Rhizoma 3.0, Atractylodis Lanceae Rhizoma 3.0, Forsythiae Fructus 3.0, Ledebouriellae Radix 2.0, Glycyrrhizae Radix 1.0, Schizonepetae Spica 1.0, Carthami Flos 1.0, Rhei Rhizoma 0.5, Lonicerae Caulis et Folium 2.0	N	−	−

(Table 1 continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-60	Keishi-ka-shakuyaku-to	Paeoniae Radix 6.0, Cinnamomi Cortex 4.0, Zizyphi Fructus 4.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	N	N	−
TJ-61	Tokaku-joki-to	Persicae Semen 5.0, Cinnamomi Cortex 4.0, Rhei Rhizoma 3.0, Glycyrrhizae Radix 1.5, Natrium Sulfuricum 0.9	N	−	−
TJ-62	Bofu-tsusho-san	Scutellariae Radix 2.0, Glycyrrhizae Radix 2.0, Platycodi Radix 2.0, Gypsum Fibrosum 2.0, Atractylodis Rhizoma 2.0, Rhei Rhizoma 1.5, Schizonepetae Spica 1.2, Gardeniae Fructus 1.2, Paeoniae Radix 1.2, Cnidii Rhizoma 1.2, Angelicae Radix 1.2, Menthae Herba 1.2, Ledebouriellae Radix 1.2, Ephedrae Herba 1.2, Forsythiae Fructus 1.2, Zingiberis Rhizoma 0.3, Kadinum 3.0, Natrium Sulfuricum 0.7	P	N	N
TJ-63	Goshaku-san	Atractylodis Lanceae Rhizoma 3.0, Aurantii Nobilis Pericarpium 2.0, Angelicae Radix 2.0, Pinelliae Tuber 2.0, Hoelen 2.0, Glycyrrhizae Radix 1.0, Platycodi Radix 1.0, Aurantii Fructus Immaturus 1.0, Cinnamomi Cortex 1.0, Magnoliae Cortex 1.0, Paeoniae Radix 1.0, Zingiberis Rhizoma 1.0, Cnidii Rhizoma 1.0, Zizyphi Fructus 1.0, Angelicae Dahuricae Radix 1.0, Ephedrae Herba 1.0	N	−	−
TJ-64	Sha-kanzo-to	Rehmanniae Radix 6.0, Ophiopogonis Tuber 6.0, Cinnamomi Cortex 3.0, Zizyphi Fructus 3.0, Ginseng Radix 3.0, Zingiberis Rhizoma 1.0, Cannabidis Semen 3.0, Glycyrrhizae Radix 3.0, Asini Gelatinum 2.0	P	N	N
TJ-65	Kihi-to	Astragali Radix 3.0, Ginseng Radix 3.0, Atractylodis Rhizoma 3.0, Hoelen 3.0, Polygalae Radix 2.0, Zizyphi Fructus 2.0, Angelicae Radix 2.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0, Saussureae Radix 1.0, Zizyphi Spinosi Semen 3.0, Longanae Arillus 3.0	N	−	−
TJ-66	Jinso-in	Pinelliae Tuber 3.0, Hoelen 3.0, Puerariae Radix 2.0, Platycodi Radix 2.0, Aurantii Nobilis Pericarpium 2.0, Zizyphi Fructus 1.5, Ginseng Radix 1.5, Glycyrrhizae Radix 1.0, Aurantii Fructus Immaturus 1.0, Perillae Herba 1.0, Zingiberis Rhizoma 0.5, Peucedani Radix 2.0	N	−	−
TJ-67	Nyoshin-san	Cyperi Rhizoma 3.0, Cnidii Rhizoma 3.0, Atractylodis Lanceae Rhizoma 3.0, Angelicae Radix 3.0, Scutellariae Radix 2.0, Cinnamomi Cortex 2.0, Ginseng Radix 2.0, Arecae Semen 2.0, Coptidis Rhizoma 1.0, Glycyrrhizae Radix 1.0, Caryophylli Flos 1.0, Saussureae Radix 1.0	P	N	N
TJ-68[a)]	Shakuyaku-kanzo-to	Glycyrrhizae Radix 6.0, Paeoniae Radix 6.0	N	N	−
TJ-69	Bukuryo-in	Hoelen 5.0, Atractylodis Lanceae Rhizoma 4.0, Aurantii Nobilis Pericarpium 3.0, Ginseng Radix 3.0, Aurantii Fructus Immaturus 1.5, Zingiberis Rhizoma 1.0	N	−	−
TJ-70	Koso-san	Cyperi Rhizoma 4.0, Perillae Herba 2.0, Aurantii Nobilis Pericarpium 2.0, Glycyrrhizae Radix 1.5, Zingiberis Rhizoma 1.0	N	−	−
TJ-71	Shimotsu-to	Rehmanniae Radix 3.0, Paeoniae Radix 3.0, Cnidii Rhizoma 3.0, Angelicae Radix 3.0	P	N	N
TJ-72	Kam-baku-taiso-to	Zizyphi Fructus 6.0, Glycyrrhizae Radix 5.0, Tritici Fructus 20.0	N	−	−
TJ-73	Saikan-to	Bupleuri Radix 5.0, Pinelliae Tuber 5.0, Scutellariae Radix 3.0, Zizyphi Fructus 3.0, Ginseng Radix 2.0, Coptidis Rhizoma 1.5, Glycyrrhizae Radix 1.5, Zingiberis Rhizoma 1.0, Trichosanthis Semen 3.0	P	N	N
TJ-74	Choi-joki-to	Rhei Rhizoma 2.0, Glycyrrhizae Radix 1.0, Natrium Sulfuricum 0.5	P	N	N
TJ-75	Shikunshi-to	Atractylodis Lanceae Rhizoma 4.0, Ginseng Radix 4.0, Hoelen 4.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0, Zizyphi Fructus 1.0	N	−	−
TJ-76	Ryutan-shakan-to	Rehmanniae Radix 5.0, Angelicae Radix 5.0, Akebiae Caulis 5.0, Scutellariae Radix 3.0, Plantaginis Semen 3.0, Alismatis Rhizoma 3.0, Glycyrrhizae Radix 1.0, Gardeniae Fructus 1.0, Gentianae Scabrae Radix 1.0	P	N	N

(**Table 1** continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-77	Kyuki-kyogai-to	Rehmanniae Radix 5.0, Paeoniae Radix 4.0, Angelicae Radix 4.0, Glycyrrhizae Radix 3.0, Cnidii Rhizoma 3.0, Artemisiae Folium 3.0, Asini Gelatinum 3.0	N	−	−
TJ-78	Ma-kyo-yoku-kan-to	Coicis Semen 10.0, Ephedrae Herba 4.0, Armeniacae Semen 3.0, Glycyrrhizae Radix 2.0	N	−	−
TJ-79	Heii-san	Atractylodis Lanceae Rhizoma 4.0, Magnoliae Cortex 3.0, Aurantii Nobilis Pericarpium 3.0, Zizyphi Fructus 2.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 0.5	N	−	−
TJ-80	Saiko-seikan-to	Bupleuri Radix 2.0, Scutellariae Radix 1.5, Phellodendri Cortex 1.5, Coptidis Rhizoma 1.5, Trichosanthis Radix 1.5, Glycyrrhizae Radix 1.5, Platycodi Radix 1.5, Gardeniae Fructus 1.5, Rehmanniae Radix 1.5, Paeoniae Radix 1.5, Cnidii Rhizoma 1.5, Angelicae Radix 1.5, Menthae Herba 1.5, Forsythiae Fructus 1.5, Arctii Fructus 1.5	P	N	N
TJ-81	Nichin-to	Pinelliae Tuber 5.0, Hoelen 5.0, Aurantii Nobilis Pericarpium 4.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-82	Keishi-ninjin-to	Cinnamomi Cortex 4.0, Glycyrrhizae Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Ginseng Radix 3.0, Zingiberis Siccatum Rhizoma 2.0	N	−	−
TJ-83	Yoku-kan-san-ka-chimpi-hange	Pinelliae Tuber 5.0, Atractylodis Lanceae Rhizoma 4.0, Hoelen 4.0, Cnidii Rhizoma 3.0, Aurantii Nobilis Pericarpium 3.0, Angelicae Radix 3.0, Bupleuri Radix 2.0, Glycyrrhizae Radix 1.5, Uncariae Ramulus et Uncus 3.0	N	N	−
TJ-84	Daio-kanzo-to	Rhei Rhizoma 4.0, Glycyrrhizae Radix 2.0	N	−	−
TJ-85	Shimpi-to	Ephedrae Herba 5.0, Armeniacae Semen 4.0, Magnoliae Cortex 3.0, Aurantii Nobilis Pericarpium 2.5, Glycyrrhizae Radix 2.0, Bupleuri Radix 2.0, Perillae Herba 1.5	N	−	−
TJ-86	Toki-inshi	Angelicae Radix 5.0, Rehmanniae Radix 4.0, Paeoniae Radix 3.0, Cnidii Rhizoma 3.0, Ledebouriellae Radix 3.0, Astragali Radix 1.5, Schizonepetae Spica 1.5, Glycyrrhizae Radix 1.0, Tribuli Fructus 3.0, Polygoni Multiflori Radix 2.0	N	−	−
TJ-87	Rokumi-gan	Rehmanniae Radix 5.0, Corni Fructus 3.0, Dioscoreae Rhizoma 3.0, Alismatis Rhizoma 3.0, Hoelen 3.0, Moutan Cortex 3.0	N	−	−
TJ-88	Nijutsu-to	Pinelliae Tuber 4.0, Atractylodis Lanceae Rhizoma 3.0, Scutellariae Radix 2.5, Cyperi Rhizoma 2.5, Aurantii Nobilis Pericarpium 2.5, Atractylodis Rhizoma 2.5, Hoelen 2.5, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0, Clematidis Radix 2.5, Arisaematis Tuber 2.5, Araliae Radix 2.5	P	N	N
TJ-89	Ji-daboku-ippo	Cinnamomi Cortex 3.0, Cnidii Rhizoma 3.0, Nupharis Rhizoma 3.0, Glycyrrhizae Radix 1.5, Rhei Rhizoma 1.0, Caryophylli Flos 1.0, Quercus Cortex 3.0	N	−	−
TJ-90	Seihai-to	Angelicae Radix 3.0, Ophiopogonis Tuber 3.0, Hoelen 3.0, Scutellariae Radix 2.0, Platycodi Radix 2.0, Armeniacae Semen 2.0, Gardeniae Fructus 2.0, Mori Cortex 2.0, Zizyphi Fructus 2.0, Aurantii Nobilis Pericarpium 2.0, Glycyrrhizae Radix 1.0, Schisandrae Fructus 1.0, Zingiberis Rhizoma 1.0, Phyllostachysis Caulis in Taeniam 2.0, Asparagi Radix 2.0, Fritillariae Bulbus 2.0	P	N	N
TJ-91	Chikujo-untan-to	Pinelliae Tuber 5.0, Bupleuri Radix 3.0, Ophiopogonis Tuber 3.0, Hoelen 3.0, Platycodi Radix 2.0, Aurantii Fructus Immaturus 2.0, Cyperi Rhizoma 2.0, Aurantii Nobilis Pericarpium 2.0, Coptidis Rhizoma 1.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0, Ginseng Radix 1.0, Phyllostachysis Caulis in Taeniam 3.0	N	−	−
TJ-92	Jiin-shiho-to	Cyperi Rhizoma 3.0, Bupleuri Radix 3.0, Paeoniae Radix 3.0 Anemarrhenae Rhizoma 3.0, Aurantii Nobilis Pericarpium 3.0, Angelicae Radix 3.0, Ophiopogonis Tuber 3.0, Atractylodis Rhizoma 3.0, Hoelen 3.0, Glycyrrhizae Radix 1.0, Menthae Herba 1.0, Lycii Radicis Cortex 3.0, Fritillariae Bulbus 2.0	N	−	−

(**Table 1** continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-93	Jiin-koka-to	Atractylodis Lanceae Rhizoma 3.0, Rehmanniae Radix 2.5, Paeoniae Radix 2.5, Aurantii Nobilis Pericarpium 2.5, Angelicae Radix 2.5, Ophiopogonis Tuber 2.5, Phellodendri Cortex 1.5, Glycyrrhizae Radix 1.5, Anemarrhenae Rhizoma 1.5, Asparagi Radix 2.5	N	−	−
TJ-95	Goko-to	Gypsum Fibrosum 10.0, Armeniacae Semen 4.0, Ephedrae Herba 4.0, Mori Cortex 3.0, Glycyrrhizae Radix 2.0	N	−	−
TJ-96[a)]	Saiboku-to	Bupleuri Radix 7.0, Pinelliae Tuber 5.0, Hoelen 5.0, Scutellariae Radix 3.0, Magnoliae Cortex 3.0, Zizyphi Fructus 3.0, Ginseng Radix 3.0, Glycyrrhizae Radix 2.0, Perillae Herba 2.0, Zingiberis Rhizoma 1.0	P	N	N
TJ-97	Dai-bofu-to	Astragali Radix 3.0, Rehmanniae Radix 3.0, Paeoniae Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Angelicae Radix 3.0, Ledebouriellae Radix 3.0, Cnidii Rhizoma 2.0, Glycyrrhizae Radix 1.5, Achyranthis Radix 1.5, Zizyphi Fructus 1.5, Ginseng Radix 1.5, Notopterygii Rhizoma 1.5, Eucommiae Cortex 3.0, Zingiberis Siccatum Rhizoma 1.0, Aconiti Tuber 1.0	N	−	−
TJ-98	Ogi-kenchu-to	Paeoniae Radix 6.0, Astragali Radix 4.0, Cinnamomi Cortex 4.0, Zizyphi Fructus 4.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-99	Sho-kenchu-to	Paeoniae Radix 6.0, Cinnamomi Cortex 4.0, Zizyphi Fructus 4.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-100	Dai-kenchu-to	Ginseng Radix 3.0, Zanthoxyli Fructus 2.0, Zingiberis Siccatum Rhizoma 5.0	N	−	−
TJ-101	Shoma-kakkon-to	Puerariae Radix 5.0, Paeoniae Radix 3.0, Cimicifugae Rhizoma 2.0, Glycyrrhizae Radix 1.5, Zingiberis Rhizoma 0.5	N	−	−
TJ-102	Toki-to	Angelicae Radix 5.0, Pinelliae Tuber 5.0, Cinnamomi Cortex 3.0, Magnoliae Cortex 3.0, Paeoniae Radix 3.0, Ginseng Radix 3.0, Astragali Radix 1.5, Zanthoxyli Fructus 1.5, Glycyrrhizae Radix 1.0, Zingiberis Siccatum Rhizoma 1.5	N	−	−
TJ-103	Sansonin-to	Hoelen 5.0, Cnidii Rhizoma 3.0, Anemarrhenae Rhizoma 3.0, Glycyrrhizae Radix 1.0, Zizyphi Spinosi Semen 10.0	N	−	−
TJ-104	Shin'i-seihai-to	Gypsum Fibrosum 5.0, Ophiopogonis Tuber 5.0, Scutellariae Radix 3.0, Gardeniae Fructus 3.0, Anemarrhenae Rhizoma 3.0, Cimicifugae Rhizoma 1.0, Lilii Bulbus 3.0, Magnoliae Flos 2.0, Eriobotryae Folium 2.0	P	N	N
TJ-105	Tsu-do-san	Aurantii Fructus Immaturus 3.0, Rhei Rhizoma 3.0, Angelicae Radix 3.0, Glycyrrhizae Radix 2.0, Carthami Flos 2.0, Magnoliae Cortex 2.0, Aurantii Nobilis Pericarpium 2.0, Akebiae Caulis 2.0, Sappan Lignum 2.0, Natrium Sulfuricum 1.8	N	−	−
TJ-106	Unkei-to	Ophiopogonis Tuber 4.0, Pinelliae Tuber 4.0, Angelicae Radix 3.0, Glycyrrhizae Radix 2.0, Cinnamomi Cortex 2.0, Paeoniae Radix 2.0, Cnidii Rhizoma 2.0, Ginseng Radix 2.0, Moutan Cortex 2.0, Evodiae Fructus 1.0, Zingiberis Rhizoma 1.0, Asini Gelatinum 2.0	N	N	−
TJ-107[a)]	Gosha-jinki-gan	Rehmanniae Radix 5.0, Achyranthis Radix 3.0, Corni Fructus 3.0, Dioscoreae Rhizoma 3.0, Plantaginis Semen 3.0, Alismatis Rhizoma 3.0, Hoelen 3.0, Moutan Cortex 3.0, Cinnamomi Cortex 1.0, Aconiti Tuber 1.0	N	N	−
TJ-108	Ninjin-yoei-to	Rehmanniae Radix 4.0, Angelicae Radix 4.0, Atractylodis Rhizoma 4.0, Hoelen 4.0, Ginseng Radix 3.0, Cinnamomi Cortex 2.5, Polygalae Radix 2.0, Paeoniae Radix 2.0, Aurantii Nobilis Pericarpium 2.0, Astragali Radix 1.5, Glycyrrhizae Radix 1.0, Schisandrae Fructus 1.0	N	−	−
TJ-109	Sho-saiko-to-ka-kikyo-sekko	Gypsum Fibrosum 10.0, Bupleuri Radix 7.0, Pinelliae Tuber 5.0, Scutellariae Radix 3.0, Platycodi Radix 3.0, Zizyphi Fructus 3.0, Ginseng Radix 3.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	P	N	N
TJ-110	Rikko-san	Gardeniae Fructus 2.0, Cimicifugae Rhizoma 2.0, Ledebouriellae Radix 2.0, Glycyrrhizae Radix 1.5, Gentianae Scabrae Radix 1.0	N	−	−

(**Table 1** continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-111	Seishin-renshi-in	Ophiopogonis Tuber 4.0, Hoelen 4.0, Scutellariae Radix 3.0, Plantaginis Semen 3.0, Ginseng Radix 3.0, Astragali Radix 2.0, Glycyrrhizae Radix 1.5, Nelumbinis Semen 4.0, Lycii Radicis Cortex 2.0	P	N	N
TJ-112	Chorei-to-go-shimotsu-to	Rehmanniae Radix 3.0, Paeoniae Radix 3.0, Cnidii Rhizoma 3.0, Alismatis Rhizoma 3.0, Polyporus 3.0, Angelicae Radix 3.0, Hoelen 3.0, Asini Gelatinum 3.0, Kadinum 3.0	N	−	−
TJ-113	San'o-shashin-to	Scutellariae Radix 3.0, Coptidis Rhizoma 3.0, Rhei Rhizoma 3.0	P	N	N
TJ-114[a)]	Sairei-to	Bupleuri Radix 7.0, Alismatis Rhizoma 5.0, Pinelliae Tuber 5.0, Scutellariae Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Zizyphi Fructus 3.0, Polyporus 3.0, Ginseng Radix 3.0, Hoelen 3.0, Glycyrrhizae Radix 2.0, Cinnamomi Cortex 2.0, Zingiberis Rhizoma 1.0	P	N	N
TJ-115	Irei-to	Magnoliae Cortex 2.5, Atractylodis Lanceae Rhizoma 2.5, Alismatis Rhizoma 2.5, Polyporus 2.5, Aurantii Nobilis Pericarpium 2.5, Atractylodis Rhizoma 2.5, Hoelen 2.5, Cinnamomi Cortex 2.0, Zingiberis Rhizoma 1.5, Zizyphi Fructus 1.5, Glycyrrhizae Radix 1.0	N	−	−
TJ-116	Bukuryo-in-go-hange-koboku-to	Pinelliae Tuber 6.0, Hoelen 5.0, Atractylodis Lanceae Rhizoma 4.0, Magnoliae Cortex 3.0, Aurantii Nobilis Pericarpium 3.0, Ginseng Radix 3.0, Perillae Herba 2.0, Aurantii Fructus Immaturus 1.5, Zingiberis Rhizoma 1.0	N	−	−
TJ-117	Inchin-gorei-san	Alismatis Rhizoma 6.0, Atractylodis Lanceae Rhizoma 4.5, Polyporus 4.5, Hoelen 4.5, Cinnamomi Cortex 2.5, Artemisiae Capillaris Spica 4.0	N	−	−
TJ-118	Ryo-kyo-jutsu-kan-to	Hoelen 6.0, Atractylodis Rhizoma 3.0, Glycyrrhizae Radix 2.0, Zingiberis Siccatum Rhizoma 3.0	N	−	−
TJ-119	Ryo-kan-kyo-mi-shin-ge-nin-to	Armeniacae Semen 4.0, Pinelliae Tuber 4.0, Hoelen 4.0, Schisandrae Fructus 3.0, Glycyrrhizae Radix 2.0, Asiasari Radix 2.0, Zingiberis Siccatum Rhizoma 2.0	N	−	−
TJ-120	Oren-to	Pinelliae Tuber 6.0, Coptidis Rhizoma 3.0, Glycyrrhizae Radix 3.0, Cinnamomi Cortex 3.0, Zizyphi Fructus 3.0, Ginseng Radix 3.0, Zingiberis Siccatum Rhizoma 3.0	N	−	−
TJ-121	Sammotsu-ogon-to	Rehmanniae Radix 6.0, Scutellariae Radix 3.0, Sophorae Radix 3.0	P	N	N
TJ-122	Haino-san-kyu-to	Platycodi Radix 4.0, Glycyrrhizae Radix 3.0, Aurantii Fructus Immaturus 3.0, Paeoniae Radix 3.0, Zizyphi Fructus 3.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-123	Toki-kenchu-to	Paeoniae Radix 5.0, Cinnamomi Cortex 4.0, Zizyphi Fructus 4.0, Angelicae Radix 4.0, Glycyrrhizae Radix 2.0, Zingiberis Rhizoma 1.0	N	−	−
TJ-124	Senkyu-chacho-san	Cyperi Rhizoma 4.0, Cnidii Rhizoma 3.0, Schizonepetae Spica 2.0, Menthae Herba 2.0, Angelicae Dahuricae Radix 2.0, Ledebouriellae Radix 2.0, Glycyrrhizae Radix 1.5, Notopterygii Rhizoma 2.0, Theae Folium 1.5	N	−	−
TJ-125	Keishi-bukuryo-gan-ka-yokuinin	Coicis Semen 10.0, Cinnamomi Cortex 4.0, Paeoniae Radix 4.0, Persicae Semen 4.0, Hoelen 4.0, Moutan Cortex 4.0	N	−	−
TJ-126	Mashinin-gan	Rhei Rhizoma 4.0, Aurantii Fructus Immaturus 2.0, Armeniacae Semen 2.0, Magnoliae Cortex 2.0, Paeoniae Radix 2.0, Cannabidis Semen 5.0	N	−	−
TJ-127	Mao-bushi-saishin-to	Ephedrae Herba 4.0, Asiasari Radix 3.0, Aconiti Tuber 1.0	N	−	−
TJ-128	Keihi-to	Atractylodis Lanceae Rhizoma 4.0, Hoelen 4.0, Dioscoreae Rhizoma 3.0, Ginseng Radix 3.0, Alismatis Rhizoma 2.0, Aurantii Nobilis Pericarpium 2.0, Glycyrrhizae Radix 1.0, Nelumbinis Semen 3.0, Crataegi Fructus 2.0	N	−	−
TJ-133	Dai-joki-to	Magnoliae Cortex 5.0, Aurantii Fructus Immaturus 3.0, Rhei Rhizoma 2.0, Natrium Sulfuricum 1.3	N	−	−
TJ-134	Keishi-ka-shakuyaku-daio-to	Paeoniae Radix 6.0, Cinnamomi Cortex 4.0, Zizyphi Fructus 4.0, Glycyrrhizae Radix 2.0, Rhei Rhizoma 2.0, Zingiberis Rhizoma 1.0	N	−	−

(**Table 1** continued)

Code No.	Names of Kampo	Constituents and combination ratio of herbal drugs	Genotoxicity response		
			Ames	MN	UDS
TJ-135	Inchinko-to	Gardeniae Fructus 3.0, Rhei Rhizoma 1.0, Artemisiae Capillaris Spica 4.0	N	−	−
TJ-136	Seisho-ekki-to	Atractylodis Lanceae Rhizoma 3.5, Ginseng Radix 3.5, Ophiopogonis Tuber 3.5, Astragali Radix 3.0, Aurantii Nobilis Pericarpium 3.0, Angelicae Radix 3.0, Phellodendri Cortex 1.0, Glycyrrhizae Radix 1.0, Schisandrae Fructus 1.0	N	−	−
TJ-137	Kami-kihi-to	Astragali Radix 3.0, Bupleuri Radix 3.0, Atractylodis Lanceae Rhizoma 3.0, Ginseng Radix 3.0, Hoelen 3.0, Polygalae Radix 2.0, Gardeniae Fructus 2.0, Zizyphi Fructus 2.0, Angelicae Radix 2.0, Glycyrrhizae Radix 1.0, Zingiberis Rhizoma 1.0, Saussureae Radix 1.0, Zizyphi Spinosi Semen 3.0, Longanae Arillus 3.0	N	−	−
TJ-138	Kikyo-to	Glycyrrhizae Radix 3.0, Platycodi Radix 2.0	N	−	−

Ames: Bacterial reverse mutation test (Ames test), MN: Micronucleus test using mouse bone marrow cells,
UDS: Unscheduled DNA synthesis (UDS) tests using rat hepatocytes, P: Positive responses,
N: Negative responses, −: Not tested, [a)] Already published (Kuboniwa et al., 1999a-q).

Scutellariae Radix extracts and their major components were also submitted to the Ames test. *Salmonella typhimurium* strains (TA100, TA1535, TA98 and TA1537) and an *Escherichia coli* strain (WP2*uvr*A) were obtained from the Institute for Fermentation, Osaka, Japan. Each assay mix of 1.0 mL included 0.1 mL of S9 as an exogenous metabolic activation system, 8 μmol of $MgCl_2$, 33 μmol of KCl, 5 μmol of glucose-6-phosphate, 4 μmol of NADPH, 4 μmol of NADH and 100 μmol of sodium phosphate buffer. The maximum dose levels of test substances were determined by dose-finding studies. The dose at which growth was inhibited was used as the maximum dose level. In the case of no growth inhibition, 5000 μg/plate was selected as the maximum dose level as recommended in regulatory guidelines. All test substances were suspended in distilled water. As positive controls, 2-(2-furyl) -3- (5-nitro-2-furyl) acrylamide (CASRN 3688-53-7), 2-aminoanthracene (CASRN 613-13-8), 9-aminoacridine hydrochloride hydrate (CASRN 52417-22-8), sodium azide (CASRN 26628-22-8) and *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine (CASRN 63885-23-4) were used.

The tests were carried out by the plate incorporation method in the presence or absence of the S9mix. After incubation for 48 h, the number of revertant colonies was counted. If the number of revertant colonies in a test substance treatment group increased dose-dependently more than 2-fold over the number of revertant colonies in the negative controls, it was declared positive. Mutagenic potencies were calculated as the number of induced revertant colonies per μg substance.

Micronucleus test

The micronucleus test was conducted to assess chromosomal damage in vivo according to the Japanese guideline for nonclinical studies (Japanese Ministry of Health and Welfare, 1994). Thirty Ames-positive and 17 Ames-negative Kampo extracts (Table 1) and wogonin, baicalin and baicalein were tested.

Male Crj：CD-1 (ICR) mice, purchased from Charles River Japan, Inc., Yokohama, Japan, were used at 8 weeks old. Dose levels of the test substances were determined experimentally, or from technical limits for administration. All test substances were suspended in distilled water. Mice were treated with the test substances by a single, or repeated oral administration for 2 or 4 days. Bone marrow cells were flushed out from femora with fetal bovine serum at 24-72 h after the final administration, smeared on glass slides, and stained with Giemsa or Acridine orange for microscopy. The incidence of micronucleated polychromatic erythrocytes (MNPCE) was evaluated based on observation of 1000 polychromatic erythrocytes (PCE) per mouse. Statistical analysis was done by the method of Kastenbaum and Bowman (Kastenbaum and Bowman, 1970). Mitomycin C (CASRN 50-07-7, 2 mg/kg, i.p. injection) was used as a positive control. If the incidence of MNPCE in a treated group increased significantly and dose-dependently in comparison with negative control groups, it was declared positive.

UDS test

UDS test was carried out by the method of Furihata et al. (Furihata and Mori, 1994；Sawada et al., 1993) to evaluate the induction of DNA damage in vivo. Thirty Ames-positive Kampo extracts (Table 1) were tested. Male F344/du Crj rats, purchased from Charles River Japan, Inc., Yokohama Japan, were used at 8 weeks old. Dose levels of the test substances were determined by the technical limits of administration. Test substances were suspended in distilled water and administered to rats by gavage. Hepatocytes were isolated by perfusion with collage-

Table 2 Positive responses on 30 extracts of Japanese traditional herbal medicines (Kampo) in the *S. typhimurium* TA1537 reverse mutation test

Code No.	Names of Kampo	Metabolic activation	Mutagenicity[a]	Negative control value[b]	Mutagenic potency[c]
TJ-3	Otsuji-to	+	20/150	8	0.080
TJ-8[d]	Dai-saiko-to	+	29/1500	11	0.012
TJ-9[d]	Sho-saiko-to	+	16/150	8	0.053
TJ-10[d]	Saiko-keishi-to	+	16/150	8	0.053
TJ-11	Saiko-keishi-kankyo-to	+	38/400	13	0.063
TJ-12[d]	Saiko-ka-ryukotsu-borei-to	+	30/1500	12	0.012
TJ-14	Hange-shashin-to	+	26/400	13	0.033
TJ-15[d]	Oren-gedoku-to	+	17/50	8	0.180
TJ-50	Keigai-rengyo-to	+	54/3000	17	0.012
TJ-51	Juncho-to	+	26/500	12	0.028
TJ-56	Gorin-san	+	47/600	17	0.050
TJ-57	Unsei-in	−	12/313	5	0.022
		+	22/625	10	0.019
TJ-58	Seijo-bofu-to	+	35/600	17	0.030
TJ-62	Bofu-tsusho-san	+	24/313	10	0.045
TJ-64	Sha-kanzo-to	−	31/3000	7	0.008
TJ-67	Nyoshin-san	+	37/600	18	0.032
TJ-71	Shimotsu-to	−	26/4000	9	0.004
TJ-73	Saikan-to	+	44/1000	18	0.026
TJ-74	Choi-joki-to	−	31/2500	10	0.008
TJ-76	Ryutan-shakan-to	+	27/500	12	0.030
TJ-80	Saiko-seikan-to	+	22/31	11	0.355
TJ-88	Nijutsu-to	+	21/50	9	0.240
TJ-90	Seihai-to	+	18/625	8	0.016
TJ-96[d]	Saiboku-to	+	35/1250	10	0.020
TJ-104	Shin'i-seihai-to	+	48/800	19	0.036
TJ-109	Sho-saiko-to-ka-kikyo-sekko	+	16/150	7	0.060
TJ-111	Seishin-renshi-in	+	35/600	12	0.038
TJ-113	San'o-shashin-to	+	25/132	9	0.121
TJ-114[d]	Sairei-to	+	20/313	10	0.032
TJ-121	Sammotsu-ogon-to	−	14/150	7	0.047
		+	38/400	12	0.065

[a] No. of revertant colonies/dose (in μg) of Kampo incorporated in the plate, [b] No. of revertant colonies in negative control group, [c] Net number of revertant colonies per μg, [d] Already published (Kuboniwa et al., 1999a-d; Kuboniwa et al., 1999o-q), TA1537: *S. typhimurium* TA1537, +: Presence of metabolic activation (S9mix), −: Absence of metabolic activation (S9mix).

nase at 2 and 12 h after administration, and were cultured (1.0×10^5 cells/mL) in William's E (WE) medium for 2 h. The medium was then replaced with fresh WE medium containing 370 KBq/mL (10 μCi/mL) of thymidine [methyl-^{3}H] for labeling DNA of hepatocytes. For autoradiography processing, cultured hepatocytes were harvested and dipped in emulsion for X-ray. After storage at −20 ℃ for 10 to 11 days, the exposed DNA of hepatocytes was developed for observation as grains. The number of grains over the nucleus (Nuclear grains) and in three adjacent nuclear-size areas over the cytoplasm (Cytoplasmic grains) were counted. Net nuclear grains were calculated as Nuclear grains minus the mean of Cytoplasmic grains over the three respective areas. Dimethylnitrosamine (CASRN 62-75-9) was used at 5 mg/kg as a positive control. If the mean of Net nuclear grains in the treated groups increased more than 5, the test substance was declared positive.

Results

Ames test

Of 128 Kampo extracts, 98 were negative in the Ames test either in the presence or absence of the exogenous metabolic activation system (data not shown). In the positive control groups, the number of revertant colonies was markedly increased (data not shown). Thirty Kampo extracts (TJ-3, TJ-8, TJ-9, TJ-10, TJ-11, TJ-12, TJ-14, TJ-15, TJ-50, TJ-51, TJ-56, TJ-57, TJ-58, TJ-62, TJ-64, TJ-67, TJ-71, TJ-73, TJ-74, TJ-76, TJ-80, TJ-88, TJ-90, TJ-96, TJ-104, TJ-109, TJ-111, TJ-113, TJ-114 and TJ-121), exhibited a weak but reproducible positive effect in *S. typhimurium* TA1537 (Tables 1 and 2).

Table 3 Positive responses on extracts of Scutellariae Radix and its main components in the bacterial reverse mutation tests

Names of herbal drugs or components	Tester strain for detection of positive responses	Metabolic activation	Mutagenicity[a)]	Negative control value[b)]	Mutagenic potency[c)]
Scutellariae Radix	TA1537	−	11/50	5	0.120
		+	28/50	10	0.360
Wogonin[d)]	TA100	+	468/50	92	7.520
	TA1537	+	127/50	13	2.800
Baicalin[d)]	TA1537	+	31/50	13	0.360
Baicalein[d)]	TA1537	−	15/50	5	0.200
		+	41/50	13	0.560

[a)] No. of revertant colonies/dose (in μg) of herbal drugs or components incorporated in the plate,
[b)] No. of revertant colonies in negative control group, [c)] Net number of revertant colonies per μg,
[d)] Component in Scutellariae Radix,
TA1537: *S. typhimurium* TA1537, TA100: *S. typhimurium* TA100,
+: Presence of metabolic activation (S9mix), −: Absence of metabolic activation (S9mix).

Extracts of Scutellariae Radix, which is a common herbal drug used in 27 of the 30 Ames-positive Kampo extracts, and its major components, wogonin, baicalin and baicalein, induced positive responses in TA1537 and wogonin was also positive in TA100 (Table 3).

Mouse bone marrow MN test

All 30 Ames-positive and 17 Ames-negative Kampo extracts did not increase the MNPCE frequencies. The incidence of MNPCE ranged from 0.0 to 0.4% per mouse in all the treatment groups as shown in Table 4 for the Ames-positive extracts ; this range was comparable to that of the negative controls. The major components of Scutellariae Radix, wogonin, baicalin, and baicalein were also negative in the MN test ; their MNPCE frequencies ranged from 0.0 to 0.5% per mouse (Table 5). In the positive control groups, the incidence of MNPCE was significantly increased as expected (data not shown).

The ratio of PCE to total erythrocytes in the treatment groups did not decrease indicating an absence of cytotoxicity (data not shown).

Rat liver UDS test

None of the Ames-positive Kampo extracts elicited UDS induction at 2 and 12 h after administration (Table 6). The range of Net nuclear grains was −0.5 to −3.4 in all the treatment groups (Table 6), and was comparable to the negative controls. The positive control groups showed the expected responses (data not shown).

Discussion

In this present study, the genotoxicity of Kampo extracts in vitro and in vivo has been examined. Out of 128 Kampo extracts evaluated by the Ames bacterial mutation test, 98 were negative and the remaining 30 extracts showed a positive response. Previously, Rokumigan and Juzen-taiho-to were reported with contradictory Ames test results (Nozaka et al., 1994a ; Fujita et al., 1992, 1994 ; Fujita and Sasaki, 1993). In the present study, they were judged negative in the Ames test. In contrast, Otsuji-to, Dai-saiko-to, Sho-saiko-to, Saiko-ka-ryukotsu-borei-to, Hange-shashin-to, Oren-gedoku-to, Keigai-rengyo-to, Unsei-in and Saiboku-to were positive in the present study although they were reported negative elsewhere (Nozaka et al., 1994a). Such contradictory findings might be due to variations in the origin of the herbal drugs, with some herbs having many subspecies the components of which are likely to be altered by climatic and geographical conditions. Their variations can easily affect the results of in vitro assays such as the Ames test.

The extracts of Scutellariae Radix are used quite widely as a component of Kampo. In the present study, it was compounded in 27 of the 30 Ames-positive Kampo extracts. These Scutellariae Radix extracts and components wogonin, baicalin and baicalein were positive in the Ames test, therefore these compounds may have contributed to the positive responses of these 27 Kampo extracts in the Ames test. However, more extensive analyses are needed to further probe the components of these Ames-positive Kampo extracts, as well as the components of the 3 Ames-positive extracts that did not contain Scutellariae Radix.

Several extracts of herbal drugs or their components, such as flavonoids, anthraquinones, alkaloids and their derivatives, are known to be mutagenic (Brown and Dietrich, 1979 ; Brown, 1980 ; Czeczot et al., 1990 ; Fujita et al., 1993, 1994 ; Kikuchi and Oshio, 1983 ; Morimoto et al., 1982, 1983 ; Nagao et al., 1981 ; Nozaka et al., 1987, 1990, 1991, 1994b ; Sugimura et al., 1977 ; Tadaki et al., 1995 ; Watanabe et al., 1983 ; Xue-jun et al., 1991 ; Yamamoto et al., 1982). These components may have contributed to the positive responses of the Kampo extracts studied here.

All 30 Ames-positive and 17 Ames-negative Kampo

Table 4 Micronucleus test for 30 extracts of Japanese traditional herbal medicines (Kampo) showing positive responses in the bacterial reverse mutation test

Code No.	Names of Kampo	Dose (mg/kg)	Sampling time (h)	Range of MNPCE/PCE(%)[a]
TJ-3	Otsuji-to	2500, 5000, 10000	24[b]	0.0～0.3
TJ-8[c]	Dai-saiko-to	500, 1000, 2000	24, 48, 72	0.0～0.3
TJ-9[c]	Sho-saiko-to	2500, 5000, 10000	24	0.0～0.3
TJ-10[c]	Saiko-keishi-to	500, 1000, 2000	24, 48, 72	0.0～0.4
TJ-11	Saiko-keishi-kankyo-to	2500, 5000, 10000	24[b]	0.1～0.3
TJ-12[c]	Saiko-ka-ryukotsu-borei-to	250, 500, 1000, 2000	24, 48	0.0～0.2
TJ-14	Hange-shashin-to	2000, 4000, 8000	24[b]	0.0～0.4
TJ-15[c]	Oren-gedoku-to	500, 1000, 2000	24, 48, 72	0.0～0.4
TJ-50	Keigai-rengyo-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-51	Juncho-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-56	Gorin-san	2500, 5000, 10000	24[b]	0.0～0.3
TJ-57	Unsei-in	2500, 5000, 10000	24[b]	0.0～0.3
TJ-58	Seijo-bofu-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-62	Bofu-tsusho-san	2500, 5000, 10000	24[b]	0.0～0.4
TJ-64	Sha-kanzo-to	2500, 5000, 10000	24[b]	0.0～0.3
TJ-67	Nyoshin-san	2500, 5000, 10000	24[b]	0.0～0.4
TJ-71	Shimotsu-to	2500, 5000, 10000	24[b]	0.0～0.3
TJ-73	Saikan-to	2500, 5000, 10000	24[b]	0.0～0.3
TJ-74	Choi-joki-to	1250, 2500, 5000	24[b]	0.0～0.3
TJ-76	Ryutan-shakan-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-80	Saiko-seikan-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-88	Nijutsu-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-90	Seihai-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-96[c]	Saiboku-to	500, 1000, 2000	24, 48, 72	0.0～0.4
TJ-104	Shin'i-seihai-to	2500, 5000, 10000	24[b]	0.0～0.3
TJ-109	Sho-saiko-to-ka-kikyo-sekko	2500, 5000, 10000	24[b]	0.0～0.2
TJ-111	Seishin-renshi-in	2000, 4000, 8000	24[b]	0.0～0.2
TJ-113	San'o-shashin-to	2500, 5000, 10000	24[b]	0.0～0.4
TJ-114[c]	Sairei-to	500, 1000, 2000	24, 48, 72	0.0～0.4
TJ-121	Sammotsu-ogon-to	2500, 5000, 10000	24[b]	0.0～0.3

[a] Minimum and maximum incidences (%) of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in 5 mice,
[b] Time after final administration,
[c] Already published (Kuboniwa et al., 1999a-d; Kuboniwa et al., 1999o-q).

Table 5 Micronucleus test for main components of Scutellariae Radix showing positive responses in the bacterial reverse mutation test

Names of the components	Dose (mg/kg)	Sampling time (h)[a]	Range of MNPCE/PCE (%)[b]
Wogonin	500, 1000, 2000	24, 48	0.0～0.4
Baicalin	500, 1000, 2000	24, 48	0.0～0.5
Baicalein	500, 1000, 2000	24, 48	0.0～0.4

[a] Time after final administration,
[b] Minimum and maximum incidences (%) of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in 5 mice.

extracts and major components of Scutellariae Radix were subjected to the mouse bone marrow MN test. None induced micronuclei even at doses as high as 2000 mg/kg or more. These 30 Ames-positive Kampo extracts are usually recommended for human consumption at doses of 1250-7000 mg/body/day (ca. 21-117 mg/kg/day). Thus the human exposure levels of Kampo extracts are at least one order of magnitude below those shown to be negative in the MN test. The 30 Ames-positive Kampo extracts were also evaluated by the rat liver UDS test. None showed any DNA damaging potential under the test conditions.

In conclusion, the majority (98 of 128) of Kampo extracts tested were not mutagenic in the Ames test. On further testing of the 30 Ames-positive extracts in a mouse bone marrow MN test and a rat liver UDS test, none were found to elicit an effect. Overall, these results suggest that all Kampo extracts evaluated here pose little or no risk for human health from the viewpoint of genotoxicity.

Table 6 Unscheduled DNA synthesis (UDS) test for 30 extracts of Japanese traditional herbal medicines (Kampo) showing positive responses in the bacterial reverse mutation test

Code No.	Names of Kampo	Dose (mg/kg)	Time after treatment (h)	No. of Net nuclear grains[a)]
TJ-3	Otsuji-to	4000	2	− 1.2 ± 1.5
			12	− 1.0 ± 1.7
TJ-8[b)]	Dai-saiko-to	2000	2	− 2.3 ± 0.6
			12	− 2.6 ± 1.0
TJ-9[b)]	Sho-saiko-to	2000	2	− 2.5 ± 0.9
			12	− 3.3 ± 0.3
TJ-10[b)]	Saiko-keishi-to	2000	2	− 2.8 ± 0.3
			12	− 2.4 ± 1.1
TJ-11	Saiko-keishi-kankyo-to	2500	2	− 2.2 ± 0.2
			12	− 3.1 ± 0.9
TJ-12[b)]	Saiko-ka-ryukotsu-borei-to	2000	2	− 2.4 ± 0.5
			12	− 2.7 ± 0.3
TJ-14	Hange-shashin-to	2000	2	− 2.3 ± 0.3
			12	− 2.8 ± 1.3
TJ-15[b)]	Oren-gedoku-to	2000	2	− 2.2 ± 0.2
			12	− 2.3 ± 0.5
TJ-50	Keigai-rengyo-to	4000	2	− 1.7 ± 1.1
			12	− 2.2 ± 0.7
TJ-51	Juncho-to	4000	2	− 1.3 ± 1.8
			12	− 0.8 ± 1.8
TJ-56	Gorin-san	2500	2	− 1.3 ± 0.4
			12	− 2.6 ± 0.7
TJ-57	Unsei-in	4000	2	− 1.8 ± 0.8
			12	− 3.4 ± 0.2
TJ-58	Seijo-bofu-to	4000	2	− 0.5 ± 1.6
			12	− 1.0 ± 1.9
TJ-62	Bofu-tsusho-san	4000	2	− 2.7 ± 0.1
			12	− 2.2 ± 1.0
TJ-64	Sha-kanzo-to	4000	2	− 2.8 ± 0.9
			12	− 3.3 ± 0.2
TJ-67	Nyoshin-san	4000	2	− 1.2 ± 0.4
			12	− 2.2 ± 0.4
TJ-71	Shimotsu-to	4000	2	− 0.7 ± 1.6
			12	− 1.2 ± 1.8
TJ-73	Saikan-to	2500	2	− 0.6 ± 1.7
			12	− 0.9 ± 1.8
TJ-74	Choi-joki-to	3000	2	− 0.8 ± 1.7
			12	− 1.2 ± 1.8
TJ-76	Ryutan-shakan-to	3000	2	− 0.8 ± 1.6
			12	− 1.6 ± 2.3
TJ-80	Saiko-seikan-to	4000	2	− 1.7 ± 0.1
			12	− 1.5 ± 0.6
TJ-88	Nijutsu-to	2500	2	− 2.0 ± 0.4
			12	− 2.4 ± 0.7
TJ-90	Seihai-to	4000	2	− 0.7 ± 1.5
			12	− 1.2 ± 2.0
TJ-96[b)]	Saiboku-to	2000	2	− 3.1 ± 0.6
			12	− 2.3 ± 0.7
TJ-104	Shin'i-seihai-to	4000	2	− 0.5 ± 1.2
			12	− 1.8 ± 2.1
TJ-109	Sho-saiko-to-ka-kikyo-sekko	2500	2	− 2.3 ± 0.6
			12	− 3.0 ± 0.9
TJ-111	Seishin-renshi-in	2000	2	− 3.0 ± 0.4
			12	− 2.2 ± 0.9
TJ-113	San'o-shashin-to	3000	2	− 2.8 ± 0.3
			12	− 2.3 ± 0.9
TJ-114[b)]	Sairei-to	2000	2	− 1.0 ± 0.4
			12	− 2.3 ± 0.2
TJ-121	Sammotsu-ogon-to	4000	2	− 0.6 ± 1.7
			12	− 0.9 ± 1.6

[a)] Mean ± S.D. of 3 rats, [b)] Already published (Kuboniwa et al., 1999a-d; Kuboniwa et al., 1999o-q).

Acknowledgment

We thank Dr. Makoto Hayashi, National Institute of Health Sciences, for giving counsel to the contents of our manuscript.

References

Ames, B.N., J. McCann and E. Yamasaki (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res., 31, 347-364.

Brown, J.P. and P.S. Dietrich (1979) Mutagenicity of anthraquinone and benzanthrone derivatives in the Salmonella/microsome test: Activation of anthraquinone glycosides by enzymic extracts of rat cecal bacteria, Mutat. Res., 66, 9-24.

Brown, J. P. (1980) A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds, Mutat. Res., 75, 243-277.

Czeczot, H., B. Tudek, J. Kusztelak, T. Szymczyk, B. Dobrowolska, G. Glinkowska, J. Malinowski and H. Strzelecka (1990) Isolation and studies of the mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs, Mutat. Res., 240, 209-216.

Fujita, H., C. Sumi and M. Sasaki (1992) Mutagenicity of water extract of Rokumigan with *Salmonella typhimurium*, Ann. Rep. Tokyo Metr. Res. Lab. P.H., 43, 228-230 (in Japanese).

Fujita, H. and M. Sasaki (1993) Mutagenicity of water extracts of Rokumigan and component crude drugs with *Salmonella typhimurium*, Ann. Rep. Tokyo Metr. Res. Lab. P. H., 44, 274-277 (in Japanese).

Fujita, H., N. Aoki and M. Sasaki (1994) Mutagenicity of Juzen-taiho-to with *Salmonella typhimurium*, Ann. Rep. Tokyo Metr. Res. Lab. P. H., 45, 185-187 (in Japanese).

Furihata, C. and H. Mori (1994) Recommendations for the performance of UDS tests *in vitro* and *in vivo*, Environ. Mutat. Res. Commun. 16, 161-164 (in Japanese).

Hayashi, M. (1991) The micronucleus test, Scientist Inc., Tokyo, pp. 31-55 (in Japanese).

Iijima, O.T., S. Minematsu, S. Maemura, L.A. Waterson and C. Gopinath (1995) A single oral dose toxicity study and a 13-week repeated dose study with a 4-week recovery period of TSUMURA Hochu-ekki-to (TJ-41) in rats, Jpn. Pharmacol. Ther., 23 (suppl.7), 209-224 (in Japanese).

Japanese Ministry of Health and Welfare, Pharmaceuticals and Cosmetics Division, Pharmaceutical Affairs Bureau (Ed) (1994) Japanese guidelines for nonclinical studies of drugs manual, YAKUJI NIPPO, Ltd., Tokyo, pp.46, 105-107 (in Japanese).

Kastenbaum, M.A. and K.O. Bowman (1970) Tables for determining the statistical significance of mutation frequencies, Mutat. Res., 9, 527-549.

Katsumata, Y., K. Yasuda, M. Uematsu, H. Takei and S. Maemura (1997) Reproductive and developmental toxicity study of TSUMURA Toki-shakuyaku-san (TJ-23) in rats by oral administration—Single study design—, Jpn. Pharmacol. Ther., 25, No. 6, 59-72 (in Japanese).

Kikuchi, Y. and H. Oshio (1983) Mutagenicity tests on Rhubarb extract and its related substances, Proc. Symp. WAKAN-YAKU, 16, 6-10 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu, M. Onishi, K. Saigou and F. Aruga (1999a) Mutagenicity studies of Dai-saiko-to (TJ-8), Jpn. Pharmacol. Ther., 27 (suppl.6), 5-11 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu and M. Onishi (1999b) Mutagenicity studies of Sho-saiko-to (TJ-9), Jpn. Pharmacol. Ther., 27 (suppl.6), 13-19 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu, M. Onishi and F. Aruga (1999c) Mutagenicity studies of Saiboku-to (TJ-96), Jpn. Pharmacol. Ther., 27 (suppl.6), 21-27 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu, M. Onishi and F. Aruga (1999d) Mutagenicity studies of Sairei-to (TJ-114), Jpn. Pharmacol. Ther., 27 (suppl.6), 29-35 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou, F. Aruga and Y. Miwa (1999e) Mutagenicity studies of Hochu-ekki-to (TJ-41), Jpn. Pharmacol. Ther., 27 (suppl.6), 37-42 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou and F. Aruga (1999f) Mutagenicity studies of Rikkunshi-to (TJ-43), Jpn. Pharmacol. Ther., 27 (suppl.6), 43-48 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi and F. Aruga (1999g) Mutagenicity studies of Choto-san (TJ-47), Jpn. Pharmacol. Ther., 27 (suppl.6), 49-55 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, K. Saigou, Y. Nagasawa and Y. Miwa (1999h) Mutagenicity studies of Shakuyaku-kanzo-to (TJ-68), Jpn. Pharmacol. Ther., 27 (suppl. 6), 57-63 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou and F. Aruga (1999i) Mutagenicity studies of Gosha-jinki-gan (TJ-107), Jpn. Pharmacol. Ther., 27 (suppl.6), 65-70 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou, F. Aruga and Y. Miwa (1999j) Mutagenicity studies of Kami-shoyo-san (TJ-24), Jpn. Pharmacol. Ther., 27 (suppl.6), 71-76 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou, F. Aruga and Y. Miwa (1999k) Mutagenicity studies of Hachimi-jio-gan (TJ-7), Jpn. Pharmacol. Ther., 27 (suppl.6), 77-82 (in Japanese).

Kuboniwa, H., S. Maemura, S, Minematsu, M. Onishi and F. Aruga (1999l) Mutagenicity studies of Toki-shakuyaku-san (TJ-23), Jpn. Pharmacol. Ther., 27 (suppl.6), 83-88 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou, F. Aruga and Y. Miwa (1999m) Mutagenicity studies of Sho-seiryu-to (TJ-19), Jpn. Pharmacol. Ther., 27 (suppl.6), 89-94 (in Japanese).

Kuboniwa, H., S. Maemura, S. Minematsu, M. Onishi, K. Saigou, F. Aruga and Y. Miwa (1999n) Mutagenicity studies of Keishi-bukuryo-gan (TJ-25), Jpn. Pharmacol. Ther., 27 (suppl.6), 95-100 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu, M. Onishi, K. Saigou and F. Aruga (1999o) Mutagenicity studies of Saiko-keishi-to (TJ-10), Jpn. Pharmacol. Ther., 27 (suppl.6), 101-107 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu, M. Onishi, K. Saigou, F. Aruga and Y. Miwa (1999p) Mutagenicity studies of Saiko-ka-ryukotsu-borei-to (TJ-12), Jpn. Pharmacol. Ther., 27 (suppl.6), 109-115 (in Japanese).

Kuboniwa, H., M. Katami, S. Maemura, S. Minematsu, M. Onishi and F. Aruga (1999q) Mutagenicity studies of Oren-gedoku-to (TJ-15), Jpn. Pharmacol. Ther., 27 (suppl.6), 117-123 (in Japanese).

Maron, D. M. and B. N. Ames (1983) Revised methods for the Salmonella mutagenicity test, Mutat. Res., 113, 173-215.

Minematsu, S., H. Takei, K. Sudo, K. Honda, Y. Fujii and T. Oyama (1995a) A single oral dose toxicity study of TSUMURA Sho-saiko-to (TJ-9) in rats, Jpn. Pharmacol. Ther., 23 (suppl.7), 29-32 (in Japanese).

Minematsu, S., S. Maemura, C.J. Perry and M. Mulhern (1995b) A single oral dose toxicity study and a 13-week repeated dose study with a 4-week recovery period of TSUMURA Choto-san (TJ-47) in rats, Jpn. Pharmacol. Ther., 23 (suppl.7), 241-257 (in Japanese).

Morimoto, I., F. Watanabe, T. Osawa and T. Okitsu (1982) Mutagenicity screening of crude drugs with *Bacillus subtilis* rec-assay and Salmonella/microsome reversion assay, Mutat. Res., 97, 81-102.

Morimoto, I., T. Nazaka, F. Watanabe, M. Ishino, Y. Hirose and T. Okitsu (1983) Mutagenic activities of gentisin and isogentisin from *Gentianae radix* (Gentianaceae), Mutat. Res., 116, 103-117.

Nagao, M., N. Morita, T. Yahagi, M. Shimizu, M. Kuroyanagi, M. Fukuoka, K. Yoshihira, S. Natori, T. Fujino and T. Sugimura (1981) Mutagenicities of 61 flavonoids and 11 related compounds, Environ. Mutagen., 3, 401-419.

Nozaka, T., I. Morimoto, M. Ishino, T. Okitsu, H. Kondoh, K. Kyogoku, Y. Sugawara and H. Iwasaki (1987) Mutagenic principles in Sinomeni Caulis *et* Rhisoma. Ⅰ. The structure of a mutagenic alkaloid, *N*-demethyl-*N*-formyldehydronuciferine, in the neutral fraction of the methanol extract, Chem. Pharm. Bull., 35 (7), 2844-2848.

Nozaka, T., F. Watanabe, S. Tadaki, M. Ishino, I. Morimoto, J. Kunitomo, H. Ishii and S. Natori (1990) Mutagenicity of isoquinoline alkaloids, especially of the aporphine type, Mutat. Res., 240, 267-279.

Nozaka, T., S. Yamada, Y. Hirose, M. Ishino and A. Tanaka (1991) Mutagenicity screening of water extracts from crude drugs, Annual Report of Saitama Institute of Public Health, 25, 51-65 (in Japanese).

Nozaka, T., S. Yamada, Y. Hirose, M. Ishino and A. Tanaka (1994a) Mutagenicity screening of the dry extract preparations of Chinese medicines, Annual Report of Saitama Institute of Public Health, 27, 42-43 (in Japanese).

Nozaka, T., S. Yamada, Y. Hirose, M. Ishino and A. Tanaka (1994b) Mutagenicity screening of water extracts from crude drugs and quercetin as mutagenic component, Jpn. J. Toxicol. Environ. Health, 40 (1), 66-70 (in Japanese).

Sawada, S., S. Asakura, H. Daimon, K. Yamatsu and C. Furihata (1993) A practical method for the in vivo UDS assay in rat liver, Japanese J. of Mutagenicity Tests on Chemicals, 2 (3), 147-153 (in Japanese).

Shimazu H., Y. Katsumata, K. Yasuda, H. Matsumoto, S. Minematsu and S. Maemura (1997) Reproductive and developmental toxicity study of TSUMURA Saiboku-to (TJ-96) in rats by oral administration—Single study design—, Jpn. Pharmacol. Ther., 25 (6), 89-103 (in Japanese).

Sugimura, T., M. Nagao, T. Matsushima, T. Yahagi, Y. Seino, A. Shirai, M. Sawamura, S. Natori, K. Yoshihira, M. Fukuoka and M. Kuroyanagi (1977) Mutagenicity of flavone derivatives, Proc. Japan Acad., 53 (B), No.4, 194-197.

Tadaki, S., S. Yamada, N. Miyazawa, T. Nozaka, and A. Tanaka (1995) Clastogenicity of Eucommiae Cortex and Astragali Radix, Jpn. J. Toxicol. Environ. Health, 41 (6), 463-469 (in Japanese).

Watanabe, F., I. Morimoto, T. Nozaka, M. Koyama and T. Okitsu (1983) Mutagenic screening of hot water extracts from crude drugs, Shoyakugaku Zasshi, 37 (3), 237-240 (in Japanese).

Xue-jun, Y., L. De-xiang, W. Hechuan and Z. Yu (1991) A study on the mutagenicity of 102 raw pharmaceuticals used in Chinese traditional medicine, Mutat. Res., 260, 73-82.

Yamamoto, H., T. Mizutani and H. Nomura (1982) Studies on the mutagenicity of crude drug extracts. Ⅰ, YAKUGAKU ZASSHI, 102 (6), 596-601 (in Japanese).

Environ. Mutagen Res., 24 : 17-21 (2002)

Original Article

Effect of a p53 codon 237 mutation on X-ray induced *HPRT* mutations

Shigeko Morimoto[1], Masamitsu Honma[3] and Fumio Yatagai[1,2]*

[1]Division of Radioisotope Technology and [2]Cellular Physiology Laboratory, The Institute of Physical and Chemical Research, Saitama 351-0198, Japan; [3]Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, 158-8501, Japan

Summary

To determine the influence of mutant p53 on *HPRT* mutations induced by ionizing radiation, we irradiated human lymphoblastoid cell lines TK6 (wild-type p53) and WI-L2-NS (mutant p53 at codon 237) with X-rays. We applied multiplex PCR analysis for independent mutants arising spontaneously or induced by X-ray exposure. X-rays enhanced the *HPRT* mutation frequency, yielding values that were 6 times the spontaneous level in TK6 and 6.8 times that level in WI-L2-NS. Point mutations (all exon regions amplified by PCR), which comprised the major portion of spontaneous mutations (60 ～ 80%) in both cell lines, were decreased by X-ray irradiation. In contrast, the proportion of complete deletions (no exon region amplified) was increased. These tendencies of decrease and increase after irradiation were the same as those reported by Phillips et al. (1995) but not statistically proved by chi-2 test. Although such test also can not show the statistic significance in the following differences, the proportion of partial deletions (some exon regions amplified) increased in TK6 but decreased in WI-L2-NS, reflecting the relatively high spontaneous level (29%) of such mutations in WI-L2-NS relative to the level (8%) in TK6. The high level of spontaneous partial deletions in p53 mutant cells was primarily attributable to a frequent (6/8) specific event-deletion of exons 2 to 5. X-ray reduced that frequency to a more ordinary level (1/8). As reported by Phillips et al. (1995), we also did not detect any effect of the codon 237 of p53 mutation on the distribution of point mutations, partial deletions, or complete deletions in X-ray-induced *HPRT* mutants.

Keywords: human lymphoblastoid cell, *HPRT* (hypoxanthine phosphoribosyltransferase) mutation, multiplex PCR (polymerase chain reaction) analysis, X-ray exposure

Introduction

Since p53 protein is critical in the maintenance of genomic stability, we set out to study the influence of p53 gene status on the specificity of spontaneous and radiation-induced mutations in cultured human cells. Such information might provide insight into the mechanisms of mutation induction in relation to DNA replication, transcription, and repair. The closely related human lymphoblastoid cell lines TK6 and WI-L2-NS are derived from WI-L2 cells originally isolated from human spleen (Levy et al., 1968). WI-L2-NS contains an AT*G* (Methionine) to AT*A* (Isoleucine) homozygous change in codon 237 of the p53 gene, while TK6 contains the wild-type (Xia et al., 1995). WI-L2-NS is more resistant than TK6 to X-rays, and this resistance is accompanied by higher mutability at the hypoxanthine phosphoribosyltransferase (*HPRT*) locus (Amundson et al., 1993; Zhen et al., 1995). These differences in radiation resistance may reflect differences in an error-prone (Amundson et al., 1993), or recombinational repair (Xia et al., 1994) system.

Inactivation of wild-type p53 leads to loss of G1/S checkpoint control and to genomic instability, including an increased spontaneous level of homologous recombination (Mekeel et al., 1997; Willers et al., 2000). Enhanced spontaneous and radiation-induced intrachromosomal homologous recombination was observed in mouse L cells with p53 mutations at codons 175, 248, or 273 (Bertrand et al., 1997; Saintigny et al., 1999). WTK1 cells, derived from WI-L2-N2, which carry the same p53 muta-

* yatagai@postman.riken.go.jp

received: November 5, 2001, accepted: January 29, 2002

Table 1 Primers used in multiplex PCR analysis of *HPRT* mutations

Band size	Primer	Position	Sequence
393bp	ex2pF	S 14674 to 14700	CACCTAAATTTCTCTGATAGACTAAGG
	ex2pR	A 15066 to 15044	GATACTAAGTAATTAGTAAGGCC
459bp	ex3pF	S 16519 to 16540	GTGGAAGTTTAATGACTAAGAG
	ex3pR	A 16977 to 16954	GTATATATCCTCCAAGGTGACTAG
332bp	ex4pF	S 27754 to 27776	GCTATGGATATTAGCTAGCTAAC
	ex4pR	A 28085 to 28063	GCTTCCAAGGGTTAAATAACCCA
236bp	ex5pF	S 31500 to 31522	AGCATCTAAAAACAAGAGTTTGG
	ex5pR	A 31735 to 31712	AACTGATGCAGAGGAATTTCTCTC
258bp	ex6pF	S 34817 to 34841	GATTTTGGTGAGAATTACTGTGCTG
	ex6pR	A 35074 to 35052	CACTTAATCCCCCTTCAAATGAG
900bp	ex78pF	S 39596 to 39619	ACCAAGTGCCTTGTCTGTAGTGTC
	ex78pR	A 40495 to 40471	TCCTAAATCTTCCTCAACCATGTTC
794bp	ex9pF	S 41261 to 41281	ACTAATGTGATAGACTACTGC
	ex9pR	A 42054 to 42032	GAACTGCTGACAAAGATTCACTG

tions at codon 237 as WI-L2-NS, show higher spontaneous mutation frequencies at the *TK* locus as well as at the *HPRT* locus (Honma et al., 1997a). WTK1 cells also demonstrate a high frequency of *TK* allelic loss and unbalanced translocation (Honma et al., 1997b). This mutant p53 gene is constitutively expressed (Xia et al., 1995) and results in loss of p21 transactivation following genotoxic stress (Wenz et al., 1998). In human gliomas overexpression of p21 have been implicated in radio-resistance (Kokunai and Tamaki 1999). That observation, however, is difficult to reconcile with other reports showing radioresistance in p21-uninduced cells. A lower frequency and/or delayed appearance of apoptosis in WI-L2-NS cells relative to wild-type cells after X-ray exposure may be related to such resistance (Xia et al., 1995, Zhen et al., 1995).

After X-ray irradiation, TK6 and WTK1 cells show no significant difference in *HPRT* mutation spectrum (distribution of point mutations, partial deletions, and complete deletions) (Phillips et al., 1995). If WTK1 loci other than p53 also mutated during establishment of the cell line, those mutations might mask the influence of mutant p53 on mutational specificity. Thus, in this study, we aimed to confirm the reported characteristic in WI-L2-NS, a related cell line carrying the same p53 mutation.

Materials and Methods

Cell culture and exposure to X-rays

Human TK6 and WI-L2-NS lymphoblastoid cells were cultured at 37℃ in RPMI 1640 medium (Gibco-BRL) supplemented with 1 mM α-ketoglutaric acid and 10% fetal bovine serum (REHATUIN) in a humidified atmosphere containing 5% CO_2. They were cultured for two days in RPMI medium containing CHAT (10 μM cytidine, 200 μM hypoxanthine, 0.2 μM aminopterin, and 17.5 μM thymidine) and one more day in CHT medium (CHAT excluding aminopterin). Cells were then resuspended in fresh RPMI at 8×10^5 cells/ml and exposed to X-rays at 1 Gy/min (Softex-Rigaku, Japan, 250 kVp).

Determination of cell survival and selection of mutant clones

Determination of cell survival and selection of *HPRT* mutants were based upon a limiting dilution methodology (Kagawa et al., 1999). Briefly, following X-ray irradiation, the cells were incubated for 4 days in normal medium to allow phenotypic expression and then treated with 6-thioguanine (6-TG; 5 μg/ml final concentration). The treated cells were incubated in eighty 96-well dishes of (4×10^4 cells/ml per well) for 2 weeks for selection of 6-TG resistant mutants. Each 96-well dish was prepared from a single phenotypic expression dish. One *HPRT* mutant clone was picked from each 96-well dish and characterized by PCR analysis to make a mutational spectrum consisting of independent mutant clones. Spontaneous mutants were collected by the same procedure, but with no phenotypic expression period. Putative *HPRT* mutant clones were diluted 20-fold in the selection medium and incubated for another 5 days to confirm their *HPRT* status. Cell survival was also measured after 2 weeks incubation in 96-well dishes (inoculation of approximately 1.6 cells per well).

Multiplex PCR analysis of genomic DNA

ISOGEN™ (Nippon Gene, Japan) was used for the extraction of genomic DNA from *HPRT* mutant clones. All exon regions of the *HPRT* locus, except the exon 1 region, were amplified by multiplex PCR (Gibbs et al., 1990). The PCR reaction conditions, including primers (Table 1), were essentially the same as in our previous study (Kagawa et al., 1999). All the PCR products were analyzed in a 2% agarose gel.

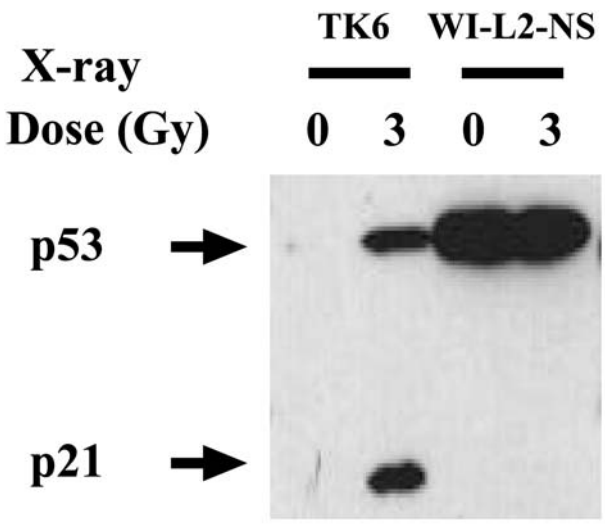


Fig. 1 Western blot analysis of p53 and p21 protein expression by TK6 and WI-L2-NS cells 2h after 3 Gy of X-ray, C-ion, and Fe-ion irradiation.

Western blot analysis

Total protein was extracted from the cells 2h after they were irradiated, and 60 μg of the extract was loaded into each lane in a 15% SDS-PAGE mini-gel. The protein was electrophoresed, blotted onto a PVDF membrane (Millipore), and probed with monoclonal antibodies against p53 (DO-1, Santa Cruz Biotech) and p21 (EA10, Oncogene Sciences).

Results

***HPRT* mutation induction**

Western blot analysis showed that p53 protein was induced in TK6 2 hours after exposure to 3 Gy X-ray radiation, while mutant p53 was constitutively produced in WI-L2-NS (Fig. 1). Induction of p21 protein was accompanied by an increase in p53 protein only in TK6 but not in WI-L2-NS. We know from survival curves that WI-L2-NS is more resistant than TK6 to X-ray irradiation ($P = 0.025$ at D_{10} by Student T-test) (data not shown). That is in accordance with a previous study (Amundson et al., 1993), as is our previous observation of higher mutability in WI-L2-NS than in TK6 after X-ray exposure (Amundson et al., 1993, Zhen et al., 1995).

In this study, we compared the *HPRT* mutation spectrum between both cell lines after X-ray irradiation reduced cell survival to approximately 10%. Exposure of TK6 cells to 1.7 Gy of X-rays reduced cell survival to 12% and increased the *HPRT* mutation frequency from the spontaneous level of $2.0 \pm 0.7 \times 10^{-6}$ to 12.0×10^{-6}. Exposure of WI-L2-NS cells to 2.5 Gy of X-rays reduced survival to 14% and increased *HPRT* mutation frequency from $3.4 \pm 0.5 \times 10^{-6}$ to 23.0×10^{-6}.

Comparison of *HPRT* mutation spectra

Table 2 shows the results of multiplex PCR analysis for independently arising *HPRT* mutants. In both cell lines, the majority of spontaneous *HPRT* mutations were point mutations (PCR amplification was normal). The proportion of spontaneous partial deletions (only some exon regions were amplified) was higher in WI-L2-NS (29%) than in TK6 (8%). Interestingly, 6 of the 8 partial deletions observed in WI-L2-NS were of type b (exon regions 2-5 were not amplified (Table 2[b)]). This "hot-spot" existed only in WI-L2-NS and after X-ray exposure, when the frequency of that deletion was reduced to 1/7. The frequency of spontaneous complete deletions (no exon region was amplified) was similar for the two cell lines, ~10% of the total mutations.

X-ray irradiation reduced the proportion of point mutations from the spontaneous level in both strains. It increased the proportion of partial deletions in TK6, but decreased it in WI-L2-NS. This result suggests that the induction of partial deletions is more efficient in TK6 than in WI-L2-NS. X-ray irradiation increased the proportion of complete deletions in both strains, which suggests that the DNA double-strand breaks caused by X-ray exposure were involved in induction of deletion mutations. Statistical analysis of the data revealed no significant difference between the two cell lines in the distribution of point mutations, partial deletions, and complete deletions. Among the 6 and 7 partial deletions observed in TK6 and WI-L2-NS, respectively, we found only one that reflected the same deletion pattern (f) (Table 2[b)]). A single complex deletion mutation (k* in Table 2[b)]) involving non-contiguous exons was recovered in WI-L2-NS cells exposed to X-rays. The same complex deletion has also been identified in human lymphoblastoid cells exposed to accelerated carbon- and neon-ion beams (Kagawa et al., 1999).

Discussion

Increase of mutation frequency in TK6 and WI-L2-NS by X-ray irradiation is consistent with those reported by Nelson et al. (1994). The relatively high proportion of partial deletions among the *HPRT* mutations occurring spontaneously in p53 mutant cells compared to 53 wild-type cells suggests the possible involvement of mutant p53 function in the generation of spontaneous mutations. The distribution of *HPRT* mutational class (point mutation, partial deletion, and complete deletion) obtained after X-ray irradiation was not influenced by this p53 mutation. This tendency is consistent with a similar finding for TK6 and WTK1 cells reported by Phillips et al. (1995). They, however, noted that a particular type of partial deletion - a deletion inside the *HPRT* locus - was about twice as frequent in WTK1 as in TK6 cells both before and after X-ray exposure. Similarly we found a distinct difference in the

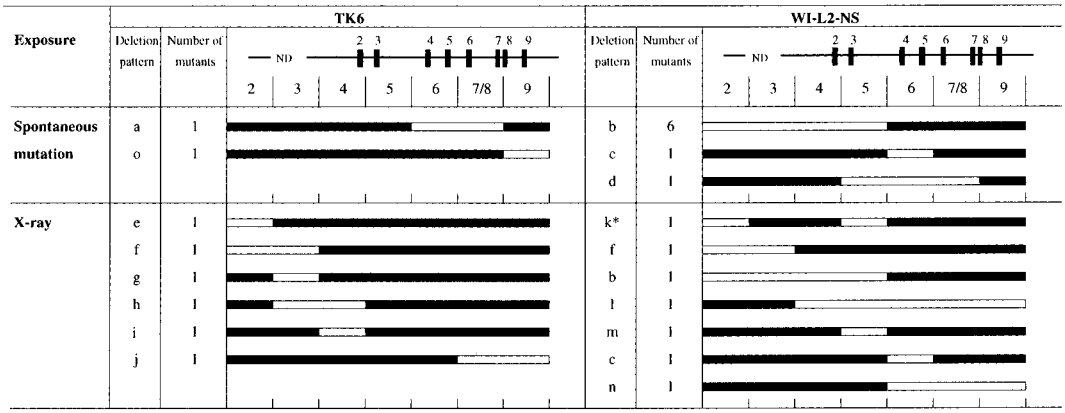
Table 2 Analysis of independent *HPRT* mutants by multiplex PCR

a) Distribution of *HPRT* mutation classes

	TK6		WI-L2-NS	
	Spont.	X-rays (1.7 Gy)	Spont.	X-rays (2.5 Gy)
Point mutation	20(80)	32(67)	17(60)	25(52)
Partial deletion	2(8)	6(13)	8(29)	7(15)
Complete deletion	3(12)	10(20)	3(11)	16(33)
Total number of mutants	25	48	28	48

The numbers in parenthesis represent relative proportion of each mutation class as %.

b) Detailed characteristics of partial deletion mutants



a ～ o: PCR amplification pattern (deletion profile) of exon regions

■: indicates exon regions of *HPRT* that were amplified by multiplex PCR.

*: Complex deletion mutation (see text)

pattern of partial deletions recovered after X-ray exposure.

The p53 protein is believed to contribute to genetic stability through DNA homologous recombination in TK6 (Honma et al., 2000). The function of mutant p53 has been suggested to be involved in this homologous recombination resulting in the enhancement of recombinogenic activity associated with higher mutability (Xia et al., 1994, Bertrand et al., 1997, Mekeel et al., 1997, Saintigny et al., 1999). Such high recombinogenic activity in WTK1 (which has the same p53 mutation as WI-L2-NS) is also suggested by spontaneously and radiation-induced enhancement of LOH at the *TK* locus (Honma et al., 1997a & b, Wiese et al., 2001). A novel integrated plasmid-based recombination assay performed to elucidate the effect of p53 status on homologous and nonhomologous recombination resulting in deletions could not account for how those deletions arose (Gebow et al., 2000).

Acknowledgments

This research was supported in part by a Grant-in-Aid from the Science and Technology Agency of Japan and in part by the "Ground Research for Space Utilization" program promoted by NASDA and the Japan Space Forum.

References

Amundson, S. A., F. Xia, K. Wolfson and H. L. Liber (1993) Different cytotoxic and mutagenic responses induced by X-rays in two human lymphoblastoid cell lines derived from a single donor, Mutat. Res., 286(2): 233-241.

Bertrand, P., D. Rouillard, A. Boulet, C. Levalois, T. Soussi and B. S. Lopez (1997) Increase of spontaneous intrachromosomal homologous recombination in mammalian cells expressing a mutant p53 protein, Oncogene, 14(9): 1117-1122.

Gebow, D., N. Miselis and H. L. Liber (2000) Homologous and nonhomologous recombination resulting in deletion: effects of p53 status, microhomology, and repetitive DNA length and orientation, Mol. Cell. Biol., 20(11): 4028-4035.

Gibbs, R. A., P. N. Nguyen, A. Edwards, A. B. Civitello and C. T. Caskey (1990) Multiplex DNA deletion detection and exon sequencing of the hypoxanthine phosphoribosyltransferase gene in Lesch-Nyhan families, Genomics, 7(2): 235-244.

Honma, M., M. Hayashi and T. Sofuni (1997a) Cytotoxic and mutagenic responses to X-rays and chemical mutagens in normal and p53-mutated human lymphoblastoid cells, Mutat. Res., 374(1): 89-98.

Honma, M., L. S. Zhang, M. Hayashi, K. Takeshita, Y. Nakagawa, N. Tanaka and T. Sofuni (1997b) Illegitimate recombination leading to allelic loss and unbalanced translocation in p53-mutated human lymphoblastoid cells, Mol. Cell. Biol., 17(8): 4774-4781.

Honma, M., M. Momose, H. Tanabe, H. Sakamoto, Y. Yu, J. B. Little, T. Sofuni and M. Hayashi (2000) Requirement of wild-type p53

protein for maintenance of chromosomal integrity, Mol. Carcinog, 28(4): 203-214.

Kagawa, Y., T. Shimazu, A. J. Gordon, N. Fukunishi, N. Inabe, M. Suzuki, M. Hirano, T. Kato, M. Watanabe, F. Hanaoka and F. Yatagai (1999) Complex hprt deletion events are recovered after exposure of human lymphoblastoid cells to high-LET carbon and neon ion beams, Mutagenesis, 14(2): 199-205.

Kokunai, T. and N. Tamaki (1999) Relationship between expression of p21WAF1/CIP1 and radioresistance in human gliomas, Jpn. J. Cancer. Res., 90(6): 638-646.

Levy, J. A., M. Virolainen and V. Defendi (1968) Human lymphoblastoid lines from lymph node and spleen, Cancer, 22(3): 517-524.

Mekeel, K. L., W. Tang, L. A. Kachnic, C. M. Luo, J. S. DeFrank and S. N. Powell (1997) Inactivation of p53 results in high rates of homologous recombination, Oncogene, 14(15): 1847-1857.

Nelson, S. L., C. R. Giver and A. J. Grosovsky (1994) Spectrum of X-ray-induced mutations in the human hprt gene, Carcinogenesis, 15(3): 495-502.

Phillips, E. N., F. Xia, K. T. Kelsey and H. L. Liber (1995) Spectra of spontaneous and X-ray-induced mutations at the *hprt* locus in related human lymphoblast cell lines that express wild-type or mutant p53, Radiat. Res., 143(3): 255-262.

Saintigny, Y., D. Rouillard, B. Chaput, T. Soussi and B. S. Lopez (1999) Mutant p53 proteins stimulate spontaneous and radiation-induced intrachromosomal homologous recombination independently of the alteration of the transactivation activity and of the G1 checkpoint, Oncogene, 18(24): 3553-3563.

Wenz, F., E. I. Azzam and J. B. Little (1998) The response of proliferating cell nuclear antigen to ionizing radiation in human lymphoblastoid cell lines is dependent on p53, Radiat. Res., 149(1): 32-40.

Wiese, C., S. S. Gauny, W. C. Liu, C. L. Cherbonnel-Lasserre and A. Kronenberg (2001) Different mechanisms of radiation-induced loss of heterozygosity in two human lymphoid cell lines from a single donor, Cancer Res., 61(3): 1129-1137.

Xia, F., S. A. Amundson, J. A. Nickoloff and H. L. Liber (1994) Different capacities for recombination in closely related human lymphoblastoid cell lines with different mutational responses to X-irradiation, Mol. Cell. Biol., 14(9): 5850-5857.

Xia, F., X. Wang, Y. H. Wang, N. M. Tsang, D. W. Yandell, K. T. Kelsey and H. L. Liber (1995) Altered p53 status correlates with differences in sensitivity to radiation-induced mutation and apoptosis in two closely related human lymphoblast lines, Cancer Res., 55(1): 12-15.

Zhen, W., C. M. Denault, K. Loviscek, S. Walter, L. Geng and A. T. Vaughan (1995) The relative radiosensitivity of TK6 and WI-L2-NS lymphoblastoid cells derived from a common source is primarily determined by their p53 mutational status, Mutat. Res., 346(2): 85-92.

Environ. Mutagen Res., 24 : 23-27 (2002)

Original Article

A chromosomal aberration study of fibrillated PVA fiber in cultured mammalian cells

Takashi Hayashi * and Fumiaki Arai

Ina Research Inc. 8047 Nishiminowa, Ina-shi, Nagano-ken 399-4501, Japan

Summary

We studied the effect of a modified form of polyvinyl alcohol fiber (43.4, 86.8, and 173.5 μg/mL), an asbestos substitute, with and without a metabolic activation system on chromosomal aberrations in a Chinese hamster cell line (CHL/IU cells). The fiber did not increase the frequency of polyploidy or of cells with structural aberrations under various exposure conditions.

Keywords: chromosomal aberrations, PVA fiber, Kuralon® RM182, asbestos, asbestos substitute

Introduction

Asbestos was extensively used in the manufacture of insulating materials, abrasives, flame retardants, and cement until the 1970s, when inorganic polymer asbestos fibers were found to cause mesotheliomas following a long latent period and were classified as a direct-acting carcinogen (Borow et al., 1973; Stanton, 1977; Chamberlain and Brown, 1978; McDonald and McDonald, 1978, 1980). As a result, worldwide regulatory authorities prohibited its manufacture and use (Asbestos International Association, 1983, 1984), and in the 1980s, several types of polyvinyl alcohol (PVA) fibers were developed as substitute reinforcement materials (Mizobe, 1985; Hikasa, 1986, 1994; Rosenbruch et al., 1992; Koshi, 1997). The most popular of those, Kuralon® RM182 and RMH182, cannot be subjected to routine in vitro tests because the size of the fibers—approximately 14 μm in width (diameter) and 4 to 6 mm in length—makes them unsuitable for such tests. Following several unsuccessful attempts, however, we were able to modify a special type of PVA fiber, Kuralon® RMU182, which is not of cement reinforcing grade, into a testable form.

Here we describe a chromosomal aberration study of fibrillated PVA fiber in cultured mammalian cells.

* haya-ta@ina-research.co.jp

Received: August 21, 2000, accepted: February 20, 2002

Materials and Methods

Test article

A sample of Kuralon® was cut and disintegrated in a disk refiner to accomplish fibrillation and was placed in a tube, sealed and irradiated with γ-rays (25 kGy) by Kuraray Co., Ltd. The actual test article was provided to us as a white suspension in distilled water (1.735 mg/mL). The distribution of the dimensions of the fibrillated Kuralon®, referred to as "A-3"; Fig. 1, is shown in Table 1. The sealed tubes were placed in an airtight container and stored at approximately 4℃. On the day of treatment, the stock suspension was sonicated and applied to each treatment level as it is while changing its volume.

Positive control articles

Mitomycin C (MMC: CAS 50-07-7), 1000 μg/mg potency, or 99.5% pure N-nitrosodimethylamine (DMN: CAS 62-75-9), both from Wako Pure Chemical Industries, Ltd., was used as the positive control in the absence (continuous treatment) or presence (short treatment), respectively, of the metabolic activation system. The compounds were dissolved in physiological saline J.P. (Otsuka Pharmaceutical Factory, Inc.) and filtered through a 0.20 μm membrane (Corning®, Iwaki Glass Company Ltd.).

Negative control article

Japanese Pharmacopeia water for injection (Otsuka Pharmaceutical Factory, Inc.) was used as the negative control and was stored at room temperature.

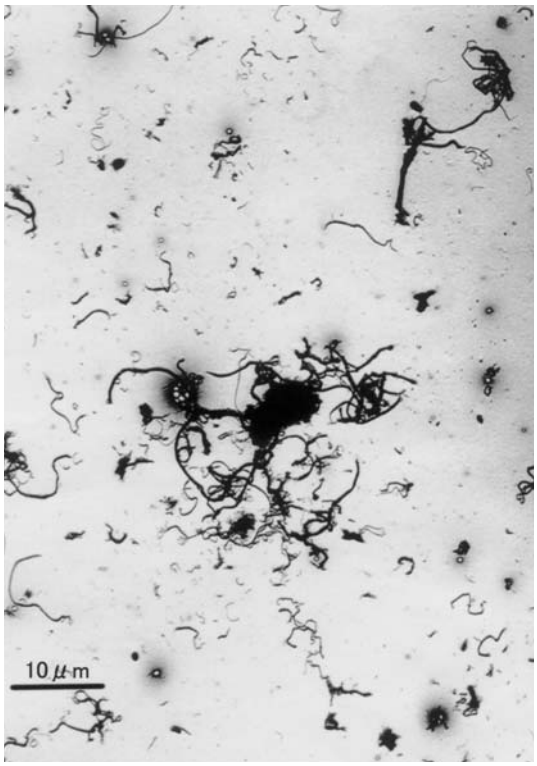


Fig. 1 An electron micrograph of fibrillated PVA fiber

Table 1 Distribution of dimensions of fibrillated PVA fiber Kuralon® (A-3)

Distribution of dimensions		No. of fibers	Fiber content (%)
Length (μm)	L < 1	3	2.2
	1 ≦ L < 3	87	64.0
	3 ≦ L < 5	25	18.4
	5 ≦ L < 10	15	11.0
	10 ≦ L < 30	6	4.4
	30 ≦ L < 500	0	0.0
	500 ≦ L	0	0.0
	Total	136	100
Width (diameter) (μm)	W < 0.1	1	0.7
	0.1 ≦ W < 0.2	66	48.5
	0.2 ≦ W < 0.3	51	37.5
	0.3 ≦ W < 0.4	13	9.6
	0.4 ≦ W < 0.5	4	2.9
	0.5 ≦ W < 1	1	0.7
	1 ≦ W < 30	0	0.0
	30 ≦ W	0	0.0
	Total	136	100
Aspect ratio (L/W)	L/W < 3	0	0.0
	3 ≦ L/W < 5	9	6.6
	5 ≦ L/W < 10	44	32.4
	10 ≦ L/W < 20	50	36.8
	20 ≦ L/W < 30	21	15.4
	30 ≦ L/W < 40	6	4.4
	40 ≦ L/W < 50	2	1.5
	50 ≦ L/W	4	2.9
	Total	136	100

Standard control article

The standard control article, zimbabwe chrysotile, was purchased from Wako Pure Chemical Industries, Ltd. To make a 50 μg/mL suspension, we added an appropriate amount to water for injection and pulverized/stirred it in a mixer (Bio-mixer®, Nihonseiki Kaisha Ltd.) for 3 minutes. The suspension was autoclaved and stored at approximately 4℃.

Cell line and culture conditions

We purchased CHL/IU cells from Dainippon Pharmaceutical Co., Ltd. Frozen aliquots (approximately -196℃) of the preserved cell suspension were thawed and serially subcultured in Eagle's minimum essential medium (Kohjin Bio Co., Ltd., 87-603) supplemented with 10% (v/v) heat-inactivated calf bovine serum (Cansera International Inc., Lot No. 2608312) every 2-5 days. The cells were checked during this acclimation period and judged to be normal if they had a satisfactory proliferation rate, were free of mycoplasma, and had a modal chromosomal number of 25. The passage numbers of the cells used for continuous treatment (24 hrs or 48 hrs) and short treatment (6 hrs) were 17 and 19, respectively.

S9 mix

We prepared the S9 mix from a purchased S9 fraction (Oriental Yeast Co., Ltd., Lot No. 97112809) and Cofactor-C (Oriental Yeast Co., Ltd). The S9 fraction was derived from the livers of 7-week-old male Sprague-Dawley rats pre-treated with phenobarbital and 5,6-benzoflavone. The S9 mix was prepared just prior to use and preserved in an ice water bath until completion of the tests. The components of 1 mL S9 mix were as follows: 0.3 mL S9 fraction, 0.1 mL 50 mmol/L $MgCl_2$, 0.1 mL 330 mmol/L KCl, 0.1 mL 50 mmol/L G-6-P, 0.1 mL 40 mmol/L NADP, 0.1 mL distilled water, and 0.2 mL 20 mmol/L HEPES buffer solution (pH 7.2).

Treatment

We based treatment levels on the results of a preliminary study in which the A-3 did not inhibit cell growth at up to 173.5 μg/mL, the concentration of the stock suspension. We applied that concentration and 86.8 and 43.4 μg/mL for both continuous and short treatments. We used zimbabwe chrysotile at 50 μg/mL, which produced chromosomal aberrations in a preliminary study.

CHL/IU cell suspension was seeded at 3×10^4/5 mL onto a plastic plate (diam. 60 mm) and incubated for 3 days. For continuous treatment, 0.125 to 0.5 mL of the medium was removed from each culture and then replaced with an equal volume of test or control article,

Table 2 Chromosomal aberration study of fibrillated PVA fiber in cultured CHL/IU cells —Continuous treatment—

Compound	Dose (μg/mL)	Trt-Rec[a] (hr)	Number of cells with structural aberrations[b]								Polyploid cells[b]	
									Total		(Pcc[c] in polyploid cells)	
			gap	ctb[d]	cte[e]	csb[f]	cse[g]	oth[h]	+ gap(%)	- gap(%)	No. of cells	%
Water for injection	24-0	0	0	0	0	0	0	0.0	0.0	3	1.5	
Fibrilated PVA Fiber	43.4	24-0	0	0	0	0	0	0	0.0	0.0	0	0.0
Fibrilated PVA Fiber	86.8	24-0	0	0	0	0	0	0	0.0	0.0	1	0.5
Fibrilated PVA Fiber	173.5	24-0	0	0	0	0	0	0	0.0	0.0	2	1.0
Mitomycin C	0.2	24-0	6	67	100	0	2	0	58.5	58.5	3	1.5
Untreated	-	24-0	1	0	0	0	0	0	0.5	0.0	0	0.0
Zimbabwe Chrysotile	50	24-0	1	4	3	1	0	0	4.0	3.5	34 (8)	17.0 (23.5)
Water for injection	-	48-0	0	0	0	0	0	0	0.0	0.0	1	0.5
Fibrilated PVA Fiber	43.4	48-0	1	0	0	0	0	0	0.5	0.0	1	0.5
Fibrilated PVA Fiber	86.8	48-0	0	0	0	0	0	0	0.0	0.0	1	0.5
Fibrilated PVA Fiber	173.5	48-0	0	0	0	0	0	0	0.0	0.0	2	1.0
Mitomycin C	0.2	48-0	14	126	176	1	3	0	92.5	91.0	4	2.0
Untreated	-	48-0	3	0	0	0	0	0	1.5	0.0	1	0.5
Zimbabwe Chrysotile	50	48-0	3	7	6	4	1	0	8.0	7.0	95 (11)	47.5 (11.6)

[a] Treatment time - Recovery time

[b] Results are based on counts of 200 cells

[c] Premature chromosome condensation (=PVZ: pulverization)

[d] Chromatid breaks [e] Chromatid exchanges [f] Chromosome breaks [g] Chromosome exchanges [h] Others

and the plates were incubated an additional 24 or 48 hours. For short treatment, 2.075 to 2.8 mL of the medium was removed and replaced with 0.075 to 0.3 mL test or control article plus 0.5 mL S9 mix (final S9 concentration, 5% v/v), and the plates were then incubated 6 hours more. When treatment was complete in short treatment, the treatment medium was removed and replaced with fresh medium and the cultures were incubated for an additional 18 hours. For both treatments, we added a colcemid (final colcemid concentration, 0.2 μg/mL) to each culture at approximately 2 hours before cell harvesting. After that, the cells were trypsinized and incubated in 0.075 mol/L KCl hypotonic solution for 15 min at 37℃ and fixed several times with freshly prepared Carnoy fixative (methanol : acetic acid = 3 : 1). Two drops of the cell suspension were spread onto degreased glass slides and stained with Giemsa solution. We examined 100 metaphase cells per plate by a blind method and scored structural chromosome aberrations and polyploidy (Ishidate, 1987; Japan Environmental Mutagen Society, 1988). We further classified structural aberrations into the following categories: gaps, chromatid breaks (ctb), chromatid exchanges (cte), chromosome breaks (csb), chromosome exchanges (cse), and others. A gap was defined as a short achromatic region (length equal to or slightly longer than the width of a chromatid) existing along the major axis of a chromatid or chromosome. If the achromatic region was considerably larger, it was classified as a break.

The results were judged according to the following criteria: negative (−) if less than 5% of cells were aberrant, equivocal (±) if 5.0-9.0% cells were aberrant, and positive (+) if 10% or more cells were aberrant and the incidence of cells with chromosomal aberrations, excluding cells with gap only, increased in a dose-dependent manner.

Results

For 24- and 48-hour continuous treatment, Table 2, results were negative in the PVA fiber-treated groups. In the standard control group, results were negative for structural aberrations but polyploidy was frequently and positively observed and the incidence increased over time. Among the polyploid cells, premature chromosome condensation (pulverization) was evident, but the incidence did not change over time.

The same results were obtained for short treatments in both the presence and absence of S-9 (Table 3).

Discussion

The harmful action of asbestos is attributed to its size and shape, which enables it to invade cells (Pott, 1980; Stanton, 1977; Stanton et al., 1981). More than 90% of asbestos used until recently was chrysotile, which is less than 1 μm in width (diameter) and more than 5 mm in length, and most chrysotile was used as a reinforcing ingredient in cement material. Following the prohibition of the manufacture and use of asbestos (Asbestos International Association, 1983, 1984), substitutes were developed (Mizobe, 1985; Hikasa, 1986, 1994). The most popular substitutes for cement reinforcing material are the PVA fibers Kuralon® RM182 and RMH182, whose per-

Table 3 Chromosomal aberration study of fibrillated PVA fiber in cultured CHL/IU cells — Short treatment —

Compound	Dose (μg/mL)	Trt-Rec[a] (hr)	S9 mix	Number of cells with structural aberration[b]								Polyploid cells[b]	
										Total		(Pcc[c] in polyploid cells)	
				gap	ctb[d]	cte[e]	csb[f]	cse[g]	oth[h]	+ gap(%)	- gap(%)	No. of cells	%
Water for injection	-	6-18	+	0	0	0	0	0	0	0.0	0.0	2	1.0
Fibrilated PVA Fiber	43.4	6-18	+	1	0	0	0	0	0	0.5	0.0	0	0.0
Fibrilated PVA Fiber	86.8	6-18	+	0	0	0	0	0	0	0.0	0.0	1	0.5
Fibrilated PVA Fiber	173.5	6-18	+	2	0	0	0	0	0	1.0	0.0	1	0.5
N-Nitrosodimethylamine	1000	6-18	+	5	28	31	0	0	0	22.0	20.5	3	1.5
Untreated	-	6-18	+	1	1	0	0	0	0	1.0	0.5	2	1.0
Zimbabwe Chrysotile	50	6-18	+	4	6	4	1	0	0	4.5	4.0	45 (9)	22.5 (20.0)
Water for injection	-	6-18	-	0	0	0	0	0	0	0.0	0.0	0	0.0
Fibrilated PVA Fiber	43.4	6-18	-	0	0	1	0	0	0	0.5	0.5	3	1.5
Fibrilated PVA Fiber	86.8	6-18	-	0	0	0	1	0	0	0.5	0.5	2	1.0
Fibrilated PVA Fiber	173.5	6-18	-	2	1	0	0	0	0	1.0	0.5	2	1.0
N-Nitrosodimethylamine	1000	6-18	-	0	0	1	0	0	0	0.5	0.5	1	0.5
Untreated	-	6-18	-	0	0	0	0	0	0	0.0	0.0	4	2.0
Zimbabwe Chrysotile	50	6-18	-	2	2	1	0	0	0	2.0	1.0	60 (7)	30.0 (11.7)

[a] Treatment time - Recovery time
[b] Results are based on counts of 200 cells
[c] Premature chromosome condensation (=PVZ: pulverization)
[d] Chromatid breaks [e] Chromatid exchanges [f] Chromosome breaks [g] Chromosome exchanges [h] Others

formance is comparable to that of chrysotile (Mizobe, 1985; Hikasa, 1986, 1994). Although those materials are not fibrillated under ordinary conditions (Saito et al., 1999), a special type of PVA fiber, Kuralon® RMU182, which is not of cement reinforcing grade, can be fibrillated because this fiber was produced with PVA resin of higher degree of polymerization and was highly drawn on the fiber production stage more than the case of conventional fibers. We therefore fibrillated a sample of Kuralon® RMU182 and investigated its clastogenicity.

Although asbestos and the majority of its substitutes, such as a glass fiber, mineral wool, and ceramic fiber, generally induce cytotoxicity and/or clastogenicity in in vitro assay systems, the fibrillated PVA fiber (A-3) tested in this study induced neither. Zimbabwe chrysotile that is known its in vitro clastogenicities and in vivo toxicities (Daniel, 1983; Koshi et al., 1986, 1991; Smith and Wright, 1996) did induce polyploidy and structural chromosome aberrations under the same test conditions. We believe that the geometric characteristics of the PVA fiber Kuralon® RM series may be responsible this result and that the materials may be safer than asbestos and other asbestos substitutes even if it is fibrillated.

References

Asbestos International Association (1983) Asbestos International Association Reports: Asbestos banning situation in the world - as of August 15, 1983, Japan Asbestos Association, Sekimen, No. 455, 6-10. (in Japanese)

Asbestos International Association (1984) Asbestos International Association Reports: Asbestos banning situation in the world - as of July 1, 1984, Japan Asbestos Association, Asbestos, No. 465, 6-8. (in Japanese)

Asbestos International Association (1984) Asbestos International Association Reports; Asbestos banning situation in the world (continued) - as of July 1, 1984, Japan Asbestos Association, Sekimen, No. 466, 6-8. (in Japanese)

Borow, M., A. Conston, L. Livornese and N. Schalet (1973) Mesothelioma following exposure to Sekimen, A review of 72 cases, Chest, 64, 641-646.

Chamberlain, M. and R.C. Brown (1978) The cytotoxic effects of asbestos and other mineral dust in tissue culture cell lines, Brit. J. Exptl. Pathol., 59, 183-189.

Daniel, F. B. (1983) In vitro assessment of asbestos genotoxicity, Environ. Health Perspect, 53, 163-167.

Hikasa, J. (1986) Substitute fibre for asbestos, Sen-i Kikai Gakkaishi (Sen-i Kogaku), 39 (4), 22-28. (in Japanese)

Hikasa, J. (1994) Synthetic fibers - Vinylon, Sen-i Gakkaishi (Sen-i to Kogyo), 50 (6), 136-138. (in Japanese)

Ishidate, M. Jr. (Ed) (1987) Data book of chromosomal aberration test in vitro (revised edition), L.I.C. Inc., Tokyo. (in Japanese)

Japan Environmental Mutagen Society, Section on MMS. (Ed) (1988) Atlas of chemicals-induced chromosomal aberrations, Asakura Shoten, Tokyo. (in Japanese)

Koshi, K., N. Kohyama, T. Myojo and K. Fukuda (1986) Biological effects of asbestos and its substitutes: Cell toxicity, hemolytic action and clastogenic activity in vitro (Fiscal Year 1985-1987), Kankyo Hozen Kenkyu, 94-1 - 94-19. (in Japanese)

Koshi, K. (1991) Biological effects of man-made mineral fibers by in vitro tests, Aerosol Res., 6 (1), 25-33. (in Japanese)

Koshi, K. (1997) Biological Effects of asbestos substitute fibers, Roudou no Kagaku, 52 (2), 26-31. (in Japanese)

McDonald, A.D. and J.C. McDonald (1978) Mesothelioma after crocidolite exposure during gas mask manufacture, Environ. Res., 17, 340-346.

McDonald, A.D. and J.C. McDonald (1980) Malignant mesothelioma in North America, Cancer, 46, 1650-1656.

Mizobe, A. (1985) Fibers for reinforcing cement and concrete, Sen-i

Gakkaishi (Sen-i to Kogyo), 41 (6), 14-19. (in Japanese)
Pott, F. (Ed) (1980) Animal experiments on biological effects of mineral fibers. In: Biological Effects of Mineral Fibers, Wagner JC. IARC Sci. Publ. No. 30, Lyon, IARC. pp. 261-272.
Rosenbruch, M., K.H. Friedrichs and H.-W. Schlipköter (1992) Zur gesundheitlichen Bedeutung der zur Herstellung von Faserzement verwendeten Asbestersatzfasern, Zbl Arbeitsmed, 42, 355-362.
Saito, T., I. Sone, J. Hikasa, J. Morisawa, Y. Hayashi and K. Miyazaki (1999) Fibrillation of synthetic fibers as a reinforcement, The Report on Works of Kochi Prefectural Paper Technology Center, 4, 76-88. (in Japanese)
Smith, A.H. and C.C. Wright (1996) Chrysotile asbestos is the main cause of pleural mesothelioma, Amer. J. of Industrial Medicine, 30, 252-266.
Stanton, M.F. (1977) Carcinogenicity of fibrous glass: Pleural response in the rat in relation to fiber dimension,. N. Cancer Inst., 58, 587-603.
Stanton, M.F., M. Layard, A. Tegerls (1981) Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals, J. Natl. cancer Inst., 67, 965-975.

Environ. Mutagen Res., 24 : 29-35 (2002)

Original Article

Antioxidative effects of fluvastatin and its metabolites in cultured human endothelial cells using single cell gel electrophoresis

Tomonori Aoki[1], Atsumune Imaeda[1*] and Hisamitsu Nagase[2]

[1]Safety Research Laboratory, Tanabe Seiyaku Co., Ltd.,
3-16-89 Kashima, Yodogawa-ku, Osaka 532-8505, Japan
[2]Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan

Summary

In the present study, we investigated the antioxidative effects of fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, and its metabolites (M2, M3, and M4) on oxidative DNA damage in cultured human umbilical vein endothelial cells (HUVEC), as well as the effects of other inhibitors of this enzyme, pravastatin and simvastatin. Single cell gel electrophoresis was used to evaluate the protective effects of fluvastatin on reactive oxygen species (ROS)-induced DNA damage in HUVEC exposed to either *t*-butylhydroperoxide or hydrogen peroxide. Fluvastatin and its metabolites showed protective effects on DNA damage as potent as the reference antioxidants, ascorbic acid, trolox, and probucol. It was suggested that fluvastatin has the potential to protect cells against ROS-induced DNA damage, although pravastatin and simvastatin did not exhibit clear protective effects. Fluvastatin may contribute to reduce ROS in humans and also reduce ROS related diseases.

Keywords: fluvastatin, HUVEC, oxidative DNA damage, antioxidant, single cell gel electrophoresis assay

Introduction

Oxidative stress may be an important factor in the development of various diseases. In atherosclerosis, for example, an increased plasma concentration of low-density lipoprotein (LDL) has been considered to be a major risk factor. The impaired removal of plasma LDL in such patients results in abnormal persistence of LDL in the plasma, allowing more time for oxidative modification to occur (Hussein et al., 1997). Oxidative modification of LDL has been reported to be one of the important steps in the progression of atherogenesis (Goldstein et al., 1979; Leonhardt et al., 1997). In addition, it has been indicated that oxygen-derived free radicals may play a major role as initiators and promoters of DNA damage and in mutations that may be related to cancer, diabetes, heart disease, and other age-related diseases (Ames, 1983; Wiseman and Halliwell, 1996; Anderson et al., 1997; Araki, 1997; Leinonen et al., 1998).

Fluvastatin (FV) is a highly potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is used as a hypolipidemic drug (Plosker and Wagstaff, 1996). FV and its metabolites (Fig. 1) (Dain et al., 1993) have been proved to have antioxidative activities (Suzumura et al., 1999a; Suzumura et al., 1999b). FV was confirmed to scavenge hydroxyl radical (HO•) generated by the Fenton's reaction using an electron spin resonance (ESR) spectrometer (Yamamoto et al., 1998). The antioxidative activities are related to their unique chemical structures (Nakashima et al., 1999; Nakamura et al., 2000).

In a previous study, FV showed protective effects against the oxidation of LDL in vitro (Hussein et al., 1997), and a reduction in the level of serum thiobarbituric acid-reactive substances in cholesterol-fed rabbits (Mitani et al., 1996; Hussein et al., 1997). Thus FV may help to reduce the risk of atherosclerosis by protecting LDL from oxidative modification as well as reducing the plasma LDL levels (Suzumura et al., 1999c).

In the present study, we investigated the protective

* a-ima@tanabe.co.jp
Received: February 20, 2002, accepted: March 30, 2002

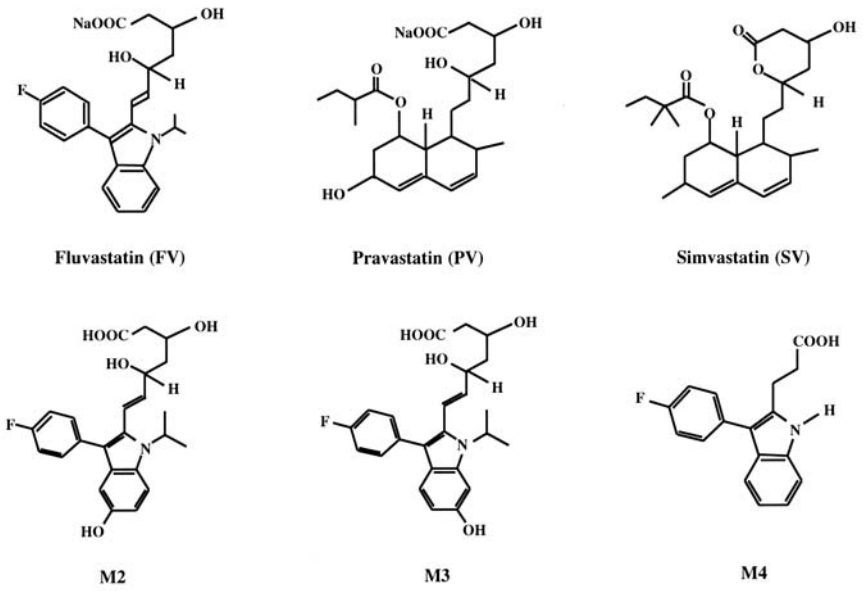


Fig. 1 Structures of fluvastatin and its major metabolites (M2, M3, and M4), pravastatin, and simvastatin

Table 1 Test materials used in this study

Test material	Abbreviation or formula
Fluvastatin	FV
Fluvastatin metabolites	M2, M3, M4
Pravastatin	PV
Simvastatin	SV
Ascorbic acid	AsA
Trolox	TX
Probucol	PB
Hydroxyl radical	HO•
Hydrogen peroxide	H_2O_2
tert-Butylhydroperoxide	*t*-BuOOH

effects of FV and its human metabolites (M2, M3, and M4) against oxidative DNA damage using normal human umbilical vein endothelial cells (HUVEC) in vitro. We also compared the protective effects of FV on oxidative DNA damage with those of two other HMG-CoA reductase inhibitors, pravastatin (PV) and simvastatin (SV) (Fig. 1).

Materials and Methods

Reagents

Fluvastatin (FV) was donated from the Department of Pharmacology, Novartis Pharma (Ibaraki, Japan). FV metabolites (M2, M3, and M4) and Probucol (PB) were synthesized by the Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd. (Saitama, Japan) (Fig. 1). *t*-Butylhydroperoxide (*t*-BuOOH), dimethyl sulfoxide (DMSO), and hydrogen peroxide (H_2O_2) were purchased from Katayama Chemical Industries (Osaka, Japan). Trolox (TX) and ascorbic acid (AsA) were purchased from Wako Pure Chemical Industries (Osaka, Japan) (Table 1).

Cell culture and treatments

Frozen normal human umbilical vein endothelial cells (HUVEC), purchased from Kurabo Industries Ltd., were used as the indicator cell line. Prior to exposure, the frozen cells were thawed and maintained by subculture for 5-7 days in a CO_2 incubator at 37℃ in a 5% CO_2 atmosphere. HuMedia-EG2 purchased from Kurabo Industries Ltd. was used as the culture medium.

The cells (7×10^5) were seeded on a tissue culture dish (60 mm in diameter containing 5 mL of medium) and incubated for 4 days. After replacement with 2.7 mL of fresh medium, 0.15 mL of test solution and 0.15 mL of ROS generation solution were added to the culture for 1 h. The 0.15mL aliquot of culture medium and vehicle were added to the untreated and solvent control cultures instead of the test solution, respectively. After exposure, the cells were washed twice with saline and analyzed immediately for DNA damage.

Single cell gel electrophoresis assay

DNA damage in individual cells was detected using the single cell gel electrophoresis assay (SCG assay) as DNA single strand breakage. The slides were prepared by the three-layer procedure. After exposure, the cells were resuspended at the concentration of 2.0×10^4 cells / 100 μL in 1.0% low-melting agarose in saline and immediately pipetted onto a slide precoated with a layer of regular agarose, and then a top layer of regular agarose was added. After the agarose had solidified, the slides were placed in ice-cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sarkosyl, 5% dimethyl sulfoxide, and 1% Triton X100 [pH 10.0]) for 60 min. Prior to elec-

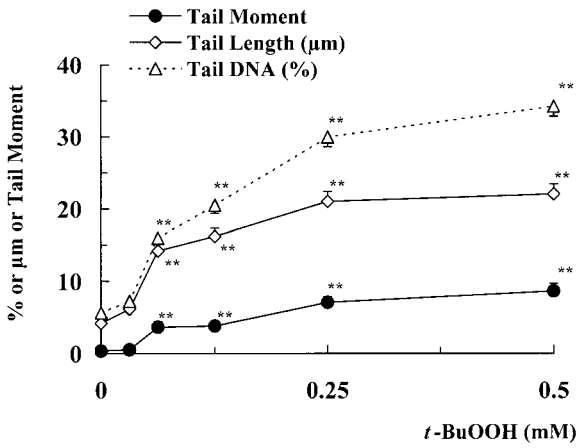


Fig. 2 Tail moment, tail length, and tail DNA of *t*-BuOOH-treated HUVEC using SCG assay.
The cells were treated with 0.0313-0.5 mM *t*-BuOOH for 60 min and immediately assayed for DNA damage with SCG assay. *t*-BuOOH, *t*-butylhydroperoxide. The data represent mean ± S.E. $^{**}p<0.01$ as compared with control (0 μM *t*-BuOOH).

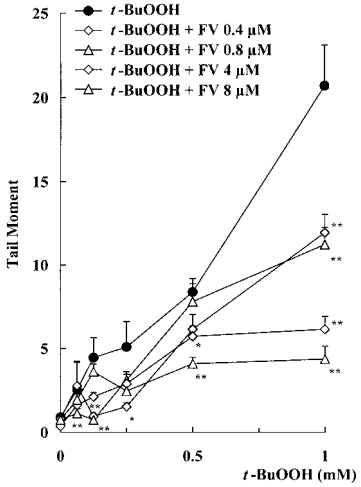


Fig. 3 The effect of FV on *t*-BuOOH-induced DNA damage.
The cells were treated with 0.0625-1 mM *t*-BuOOH for 60 min in the absence or presence of 0.4-8 μM FV, and immediately assayed for DNA damage with SCG assay. Results are expressed as the tail moment, and represent the mean of at least two separate experiments, each performed in duplicate. *t*-BuOOH, *t*-butylhydroperoxide; FV, fluvastatin. The data represent mean ± S.E. $^{*}p<0.05$, $^{**}p<0.01$ as compared with control (*t*-BuOOH only).

trophoresis, the slides were incubated in an alkaline buffer (300 mM NaOH and 1 mM EDTA; pH > 13) for 10 min to allow the DNA to unwind. After alkali unwinding, the single-stranded DNA in the gel was electrophoresed at 300 mA and 1.0 V/cm for 15 min in the same alkaline buffer maintained below 4℃. Following electrophoresis, the alkali in the gels was neutralized with Tris-HCl buffer (pH 7.4) for 10 min and rinsed with saline. The DNA was visualized by staining with ethidium bromide and observed using a Nikon Labophot fluorescence microscope. A specialised SCG analysis system (Komet 3.0, Kinetic Imaging Ltd., Liverpool, UK) was used to analyze the data. Tail length (μm), tail DNA (%), and tail moment were recorded for 50 cells per slide with 2 slides per variable investigated. The parameter used to describe the extent of DNA damage was the tail moment, which is defined as the product of the tail length and the fraction of DNA in the tail in this software (tail length × tail DNA / 100). The measurements from 100 randomly selected cells per treatment condition were averaged.

Effects on DNA Damage Induced by ROS in vitro

The protective effects against oxidative DNA damage induced by ROS were measured by the SCG assay. *t*-BuOOH and H_2O_2 were each used as the ROS generating systems. Well-known antioxidants AsA and TX were used. PB has been reported to have a highly lipophilic property and phenolic moieties, which show antioxidant properties partly due to their free radical scavenging effect (Valoti et al., 1989; Hiramatsu et al., 1994). Each reagent was dissolved in distilled water, except for TX and PB. TX was dissolved in 1M $NaHCO_3$ and PB in DMSO. FV was diluted to adjust the final concentrations to 0.4, 0.8, 4, and 8 μM, and the metabolites (M2, M3, and M4), AsA, TX, and PB were 0.8 and 8 μM in the reaction mixture. *t*-BuOOH was diluted to adjust the final concentrations to 0.0313, 0.0625, 0.125, 0.25, 0.5, and 1 mM in the reaction mixture and H_2O_2 was diluted to adjust the final concentrations to 0.5, 1, 2, 3, and 4 μM.

Duplicate plates were prepared and observed for each condition. The protocol was performed at least twice for reliability, and satisfactory results were obtained between repeated tests.

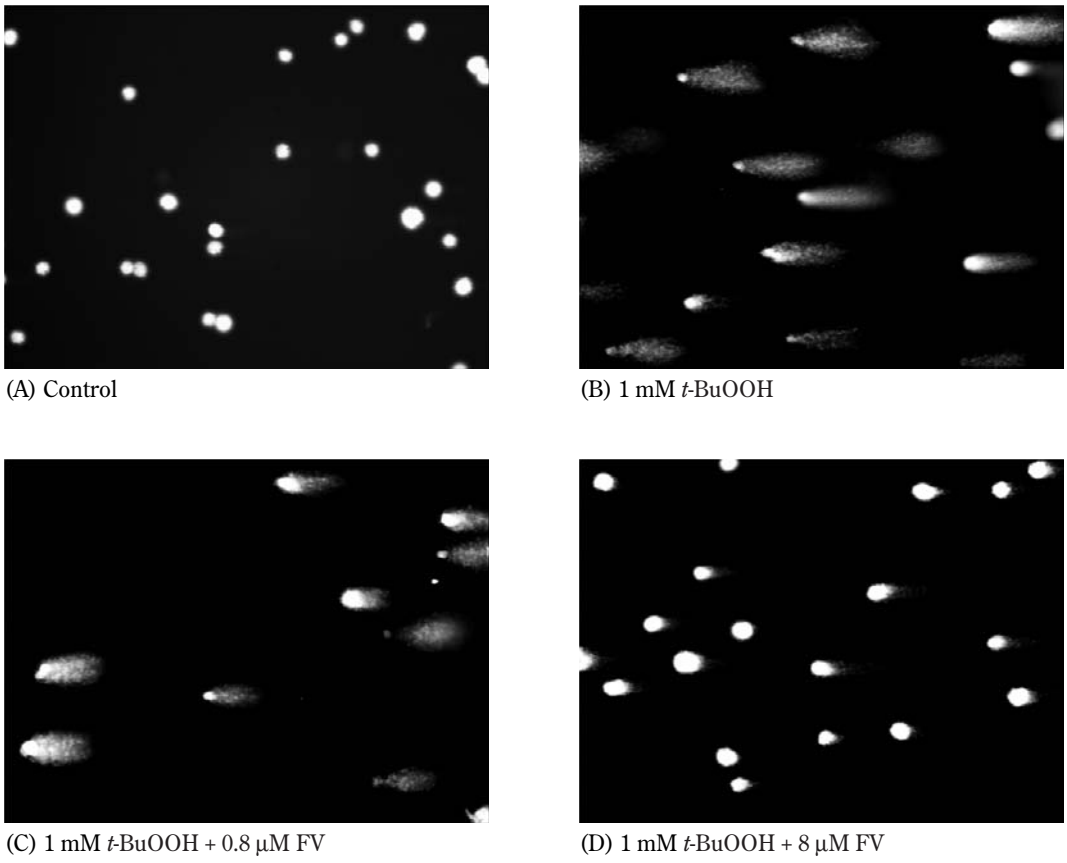
Statistical analysis

Statistical significance among the groups was analyzed by the Dunnett's multiple comparison test. The significance of differences was evaluated by the Dunnett's parametric test when variances were equal ($p \geqq 0.05$), by the Bartlett's test for homogeneity of variance, and the Dunnett's non-parametric test when variances were unequal ($p < 0.05$). Results were considered to be significant at a probability level of $p < 0.05$ or 0.01.

Results

Effects of FV on DNA Damage Induced by *t*-BuOOH in vitro

We assessed the sensitivity of the conventional SCG assay to detect the DNA damage of HUVEC. The cells were exposed to *t*-BuOOH at 37℃ at various concentra-



(A) Control (B) 1 mM *t*-BuOOH

(C) 1 mM *t*-BuOOH + 0.8 μM FV (D) 1 mM *t*-BuOOH + 8 μM FV

Fig. 4 Fluorescence photographs of HUVEC after exposure to 1 mM *t*-BuOOH in the SCG assay.
(A) (Control), nuclei of undamaged negative control cells, 200× magnification. (B) (1 mM *t*-BuOOH), severely damaged cell. The fragmented DNA has migrated out of the nucleus (comet head) and streamed out in a long tail towards the anode. Highly fragmented DNA has migrated away from the nuclear cage, leaving a small comet head. (C) (1 mM *t*-BuOOH + 0.8 μM FV) and (D) (1 mM *t*-BuOOH + 8 μM FV), FV inhibited the *t*-BuOOH-induced DNA damage in a dose-dependent manner. *t*-BuOOH, *t*-butylhydroperoxide; FV, fluvastatin.

tions. Fig. 2 shows a representative SCG assay of DNA damage as a function of *t*-BuOOH concentration exposed for 60 min. Significant increases in tail length, tail DNA, and tail moment were observed in HUVEC exposed to *t*-BuOOH showing a dose-dependent manner. The effect of FV on DNA damage produced by *t*-BuOOH was investigated. In these experiments, the cells were exposed to various concentrations of *t*-BuOOH for 60 min, in the absence or presence of FV, and the level of DNA damage was measured. The addition of FV significantly reduced the levels of tail moment induced by *t*-BuOOH in a dose-dependent manner (Fig. 3). Fig. 4 shows representative photomicrographs of the ethidium bromide-stained nuclei indicating that 0.8 and 8 μM FV virtually inhibited the extensive DNA cleavage caused by 1 mM *t*-BuOOH in a dose-dependent manner.

As shown in Fig. 5, the addition of 8 μM FV significantly reduced *t*-BuOOH-induced DNA damage; the results were comparable to those with AsA, TX, and PB. FV reduced the DNA damage to an extent similar to TX and PB. In AsA, the DNA damage was reduced significantly even at 0.8 μM. M2, M3, and M4 also inhibited *t*-BuOOH-induced DNA strand scission significantly even at 0.8 μM, and these effects showed dose-dependency. Meanwhile no protective effects of PV and SV were shown in this system.

Effects of FV on DNA Damage Induced by H_2O_2 in vitro

The cells were exposed to H_2O_2 at 37℃ in various concentrations. Fig. 6 shows a representative SCG assay of DNA damage as a function of H_2O_2 concentration exposed for 60 min. Significant increases in tail length, tail DNA, and tail moment were observed in HUVEC exposed to H_2O_2 in a dose-dependent manner.

The effect of FV on DNA damage produced by H_2O_2 was investigated. The cells were exposed to various concentrations of H_2O_2 for 60 min, with or without of FV, and the level of DNA damage was measured immediately after the exposure using the SCG assay. The concentrations of 0.8, 4, and 8 μM FV significantly inhibited the H_2O_2-induced tail moment (Fig. 7).

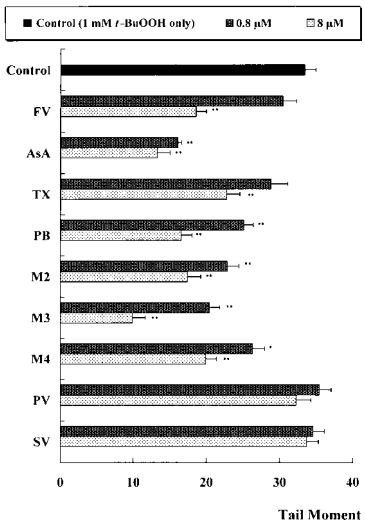


Fig. 5 The effect of FV, its metabolites (M2, M3, and M4), and antioxidants on 1 mM *t*-BuOOH-induced DNA damage.
The cells were treated with 1 mM *t*-BuOOH for 60 min in the absence or presence of 0.8-8 μM FV, its metabolites (M2, M3, and M4), and various antioxidants, and immediately assayed for DNA damage with SCG assay. Results are expressed as the tail moment, and represent the mean of at least two separate experiments. M2, M3, and M4 designate principal FV metabolites in humans. *t*-BuOOH, *t*-butylhydroperoxide; FV, fluvastatin; AsA, ascorbic acid; TX, trolox; PB, probucol; PV, pravastatin; SV, simvastatin. The data represent mean ± S.E. * $p<0.05$, ** $p<0.01$ as compared with control (t-BuOOH only).

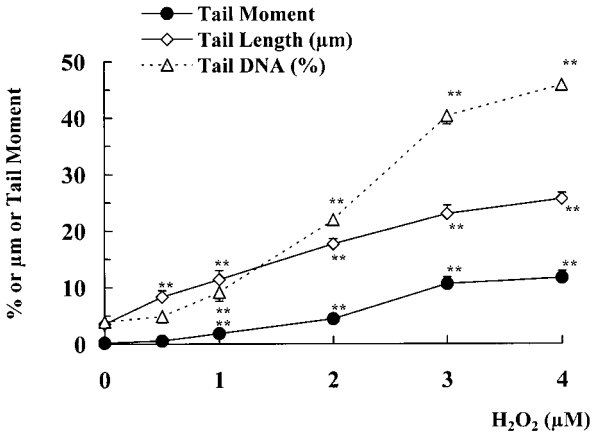


Fig. 6 Tail moment, tail length, and tail DNA of H_2O_2-treated HUVEC using SCG assay.
The cells were treated with 0.5-4 μM H_2O_2 for 60 min and immediately assayed for DNA damage with SCG assay. The tail moment is the product of the length of the tail and the amount of DNA present within the tail. The data represent mean ± S.E. ** $p<0.01$ as compared with control (0 μM H_2O_2).

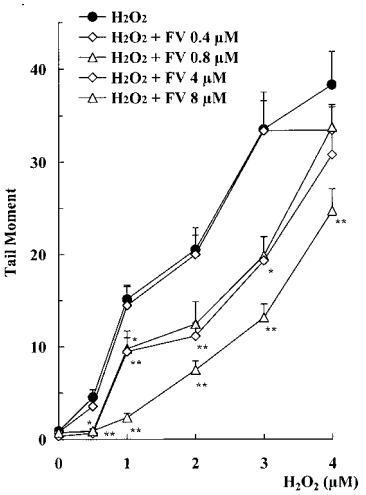


Fig. 7 The effect of FV on H_2O_2-induced DNA damage.
The cells were treated with 0.5-4 μM H_2O_2 for 60 min in the absence or presence of 0.4-8 μM FV, and immediately assayed for DNA damage with SCG assay. Results are expressed as the tail moment, and represents the mean of at least two separate experiments, each performed in duplicate. FV, fluvastatin. The data represent mean ± S.E. ** $p<0.01$ as compared with control (H_2O_2 only).

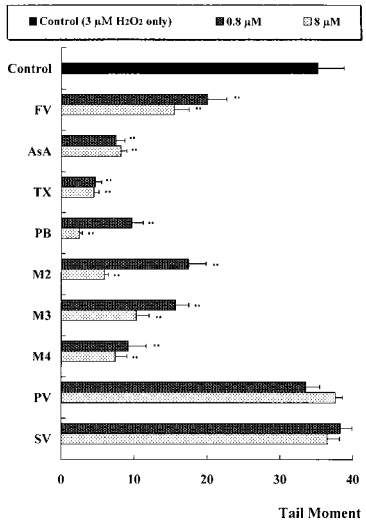


Fig. 8 The effect of FV, its metabolites (M2, M3, and M4) and antioxidants on 3 μM H_2O_2-induced DNA damage.
The cells were treated with 3 μM H_2O_2 for 60 min in the absence or presence of 0.8-8 μM FV, its metabolites (M2, M3, and M4), and various antioxidants, and immediately assayed for DNA damage with SCG assay. Results are expressed as the tail moment, and represent the mean of at least two separate experiments. M2, M3, and M4 designate principal FV metabolites in humans. FV, fluvastatin; AsA, ascorbic acid; TX, trolox; PB, probucol; PV, pravastatin; SV, simvastatin. The data represent mean ± S.E. ** $p<0.01$ as compared with control (H_2O_2 only).

As shown in Fig. 8, FV effectively reduced DNA damage caused by H_2O_2, and this effect was dose-dependent. The addition of 0.8 and 8 μM FV significantly reduced the DNA damage. AsA, TX, and PB reduced H_2O_2-induced DNA damage to a similar extent. M2, M3, and M4, metabolites of FV, also reduced the DNA damage to a similar extent, in a dose-dependent manner. The protective effects of FV against DNA damage were compared with those of PV or SV. FV exhibited protective effects against H_2O_2-induced breakage, but PV and SV did not.

Discussion

ROS may play a major role as endogenous initiators and promoters of DNA damage and in mutations that contribute to cancer, diabetes, heart disease, and other age-related diseases (Ames, 1989; Wiseman and Halliwell, 1996; Anderson et al., 1997). On the other hand, recent studies have suggested that antioxidant treatment reduced the risk of various diseases. Antioxidants may represent an important defense against cellular injury mediated by ROS, and many antioxidants (Smith, 1998; Blask et al., 1999; Ishino et al., 1999; Zhao et al., 1999; Owen et al., 2000).

We investigated the protective effects of FV and its human metabolites against oxidative DNA damage using HUVEC in vitro, and compared the effects with two other HMG-CoA reductase inhibitors. The protective effects on oxidative DNA damage induced by H_2O_2 and *t*-BuOOH were examined using the SCG assay. The alkaline (pH > 13) version of the SCG assay is known to be capable of detecting DNA single-strand breaks, alkali-labile sites, DNA-DNA/DNA-protein cross-linking, and DNA single-strand breaks associated with incomplete excision repair sites (Singh et al., 1988; McKelvey-Martin et al., 1993; Fairbairn et al., 1995; Hartmann and Spier, 1995; Tice et al., 2000).

FV showed protective effects against oxidative DNA damage of HUVEC induced by both H_2O_2 and *t*-BuOOH. FV and its metabolites caused a reduction in DNA damage induced by 1 mM *t*-BuOOH, and the protective potency was equal to that of AsA, TX, and PB, which are well-known antioxidants. FV and its metabolites also reduced 3 μM H_2O_2-induced DNA damage to the same extent as AsA, TX, and PB. In addition, only FV consistently showed the protective effect on the oxidative DNA damage in comparison with two other HMG-CoA reductase inhibitors, PV and SV. The previous study indicated that the chemical structure, including the fluorophenyl indole moiety, differs from that of PV and SV (Suzumura et al., 1999b), and the active site for the antioxidative ability of FV is the allylic carbon conjugated with the indole ring (Nakamura et al., 2000).

FV have been confirmed to chemically scavenge hydroxyl radical (HO•) using an ESR spectrometer (Yamamoto et al., 1998). FV exhibited protective effects against the oxidation of LDL (Mitani et al., 1996; Hussein et al., 1997; Suzumura et al., 1999c). It has been suggested that FV may help to reduce the risk of atherosclerosis by protecting LDL from oxidative modification (Suzumura et al., 1999c). We have reported on the protective effects of FV and its metabolites on oxidative DNA damage from ROS in vitro. The protective effects have been proved in terms of the DNA breakage rate using a single-stranded phage DNA system, and in the Ames test with *Salmonella typhimurium* TA102 and TA104 test strains (Imaeda et al., 2000; Imaeda et al., 2001). The antioxidative activities indicated in previous studies are consistent with the protective effects against oxidative DNA damage of HUVEC. These results suggested that FV and its metabolites protect against oxidative DNA damage. Metabolites provided even stronger protection against DNA damage of HUVEC than FV. The metabolites M2 and M3 having a phenolic hydroxyl group on the indole moiety showed more potent effects than FV itself, the fluorophenyl indole moiety was suggested to be important for the manifestation of the hydroxyl radical scavenging activity, and the phenolic hydroxyl group was considered to enhance this activity (Suzumura et al., 1999b).

In conclusion, FV and, to an even greater effect, its major human metabolites, may reduce the risk of various diseases associated with oxidative stress, such as atherosclerosis, cancer, diabetes, and other age-related diseases, by inhibiting the oxidation of LDL, the oxidative modification in human vascular endothelial cells, and other risk factors.

References

Anderson, D., B.J. Phillips, T.W. Yu, A.J. Edwards, R. Ayesh and K.R. Butterworth (1997) The effect of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with low or high cholesterol levels, Environ. Mol. Mutagen., 30, 161-174.

Ames, B.A. (1983) Dietary carcinogens and anticarcinogens, Science, 221, 1256-1264.

Ames, B.A. (1989) Endogeneous oxidative DNA damage, aging, and cancer, Free Radic. Res. Commun., 7, 121-128.

Araki, A. (1997) Oxidative stress and diabetes mellitus: a possible role of alpha-dicarbonyl compounds in free radical formation, Nippon Ronen Igakkai Zasshi, 34, 716-720.

Blask, D.E., L.A. Sauer, R.T. Dauchy, E.W. Holowachuk, M.S. Ruhoff and H.S. Kopff (1999) Melatonin inhibition of cancer growth in vivo involves suppression of tumor fatty acid metabolism via melatonin receptor-mediated signal transduction events, Cancer Res., 59, 4693-4701.

Dain, J.G., E Fu, J. Gorski, J. Nicoletti and T.J. Scallen (1993) Biotransformation of fluvastatin sodium in humans, Drug Metab. Dispos., 21, 567-572.

Fairbairn, D.W., P.L. Olive and K.L. O'Neill (1995) The comet assay: A comprehensive review, Mutat. Res., 339, 37-59.

Goldstein, J.L., Y.K. Ho and M.S. Brown (1979) Binding site on macrophages that mediates uptake and degradation of acetylated

low density lipoprotein, producing massive cholesterol deposition, Proc. Natl. Acad. Sci. USA, 76, 333-337.

Hartmann, A. and G. Spier (1995) Genotoxic effects of chemicals in the single cell gel (SCG) test with human blood cells in relation to induction of sister chromatid exchanges (SCE), Mutat. Res., 346, 49-56.

Hiramatsu, M., J. Liu, R. Edamatsu, S. Ohba, D. Kadowaki and A. Mori (1994) Probucol scavenged 1,1-diphenyl-2-picrylhydrazyl radicals and inhibited formation of tiobarbituric acid reactive substances, Free Radic. Biol. Med., 16, 201-206.

Hussein, O., S. Schlezinger, M. Rosenblat, S. Keidar and M. Aviram, (1997) Reduced susceptibility of low density lipoprotein (LDL) to lipid peroxidation after fluvastatin therapy is associated with the hypocholesterolemic effect of the drug and its binding to the LDL, Atherosclerosis, 128, 11-18.

Imaeda, A., T. Aoki, Y. Kondo and M. Hori (2000) Effectes of antioxidants on the mutagenicity of reactive oxygen species-generating systems towards Salmonella typhimurium TA102 and TA104 strains, Environ. Mutagen Res., 22, 1-7.

Imaeda, A., T. Aoki, Y. Kondo, M. Hori, M. Ogata, H. Obayashi, G. Hasegawa, N. Nakamura, K. Tokuda, H. Nishino, T. Yoshikawa and M. Kondo (2001) Protective effects of fluvastatin against reactive oxygen species induced DNA damage and mutagenesis, Free Rad. Res., 34, 33-44.

Ishino, A., S. Mita, S. Watanabe and H. Sakagami (1999) Effect of anticancer drugs, metals and antioxidants on cytotoxic activity of epigallocatechin gallate, Anticancer Res., 19, 4343-4348.

Leinonen J., V. Rantalaiho, T. Lehtimaki, T. Koivula, O. Wirta, A. Pasternack and H. Alho (1998) The association between the total antioxidant potential of plasma and the presence of coronary heart disease and renal dysfunction in patients with NIDDM, Free Radic. Res., 29, 273-281.

Leonhardt, W., T. Kurktschiev, D. Meissner, P. Lattke, C. Abletshauser, G. Weidinger, W. Jaross and M. Hanefeld (1997) Effects of fluvastatin therapy on lipids, antioxidants, oxidation of low density lipoproteins and trace metals, Eur. J. Clin. Pharmacol., 53, 65-69.

McKelvey-Martin, V.J., M.H.L. Green, P. Schmezer, B.L. Pool-Zobel, M.P. DeMeo and A. Collins (1993) The single cell gel electrophoresis assay (comet assay): A European review, Mutat. Res., 228, 47-63.

Mitani, H., T. Bandoh, J. Ishikawa, M. Kimura, T. Totsuka and S. Hayashi (1996) Inhibitory effects of fluvastatin, a new HMG-CoA reductase inhibitor, on the increase in vascular ACE activity in cholesterol-fed rabbits, Brit. J. Pharmacol., 119, 1269-1275.

Nakamura, T., H. Nishi, Y. Kokuseny, K. Hirota and Y. Miura (2000) Mechanism of antioxidative activity of fluvastatin-determination of the active position, Chem. Pharm. Bull., 48, 235-237.

Nakashima, A., M. Ohtawa, N. Masuda, H. Morikawa and T. Bando (1999) Antioxidative effects of fluvastatin, and its major metabolites, Yakugaku Zasshi, 119, 93-99.

Owen, R.W., A. Giacosa, W.E. Hull, R. Haubner, B. Spiegelhalder and H. Bartsch (2000) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil, Eur. J. Cancer, 36, 1235-1247.

Plosker, G.L. and A.J. Wagstaff (1996) Fluvastatin — a review of its pharmacology and use in the management of hypercholesterolaemia, Drugs, 51, 433-459.

Singh, N.P., M.T. McCoy, R.R. Tice and E.L. Schneider (1988) A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res., 175, 184-191.

Smith T.A. (1998) Carotenoids and cancer: prevention and potential therapy, Br. J. Biomed. Sci., 55, 268-275.

Suzumura, K., M. Yasuhara and H. Narita (1999a) Superoxide anion scavenging properties of fluvastatin and its metabolites, Chem. Pharm. Bull., 47, 1477-1480.

Suzumura, K., M. Yasuhara, K. Tanaka, A. Odawara, H. Narita and T. Suzuki (1999b) An in vitro study of the hydroxyl radical scavenging property of fluvastatin, an HMG-CoA reductase inhibitor, Chem. Pharm. Bull., 47, 1010-1012.

Suzumura, K., M. Yasuhara, K. Tanaka and T. Suzuki (1999c) Protective effect of fluvastatin sodium (XU-62-320), a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, on oxidative modification of human low-density lipoprotein in vitro, Biochem. Pharmacol., 57, 697-703.

Tice, R.R., E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.-C. Ryu and Y.F. Sasaki (2000) Single cell gel / comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen., 35, 206-221.

Valoti, M., H.J. Sipe, G. Sgaragli and R.P. Mason (1989) Free radical intermediates during peroxidation of 2-t-butyl-4-methoxyphenol, 2,6-di-t-butyl-4-methlphenol, and related phenol compounds, Arch. Biochem. Biophys., 269, 423-432.

Wiseman, H. and B. Halliwell (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer, Biochem. J., 313, 17-29.

Yamamoto, A., K. Hoshi and K. Ichihara (1998) Fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase, scavenges free radicals and inhibits lipid peroxidation in rat liver microsomes, Eur. J. Pharmacol., 361, 143-149.

Zhao, J., Y. Sharma and R. Agarwal (1999) Significant inhibition by the flavonoid antioxidant silymarin against 12-O-tetradecanoylphorbol 13-acetate-caused modulation of antioxidant and inflammatory enzymes, and cyclooxygenase 2 and interleukin-1alpha expression in SENCAR mouse epidermis: implications in the prevention of stage I tumor promotion, Mol. Carcinog., 26, 321-333.

Environ. Mutagen Res., 24 : 37-41 (2002) Original Article

Failure of stevioside to induce micronucleus formation in the rodent bone marrow cells

Temcharoen, P.[1*], S. Klongpanichpak[2], M. Suwannatrai[2,3], S. Apibal[4] and C. Toskulkao[2]

[1]Departments of Pathobiology, [2]Physiology and [3]Graduate Program in Toxicology, Faculty of Science, Mahidol University, [4]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama Ⅵ Road, Bangkok 10400, Thailand

Summary

Stevioside, a major constituent of *Stevia rebaudiana* Bertoni which is commonly used as a non-caloric sugar substitute in many countries, was evaluated for its chromosome damaging potential by the bone marrow micronucleus test in both sexes of hamsters, rats and mice. Experimental animals received an oral administration of 10 g stevioside/kg body weight (BW). Animals were killed 24, 30, 48 and 72 hours after the treatment. Bone marrow cells were collected and 2000 polychromatic erythrocytes (PCEs) per animal were analyzed for the presence of micronuclei. Four hundred erythrocytes were also scored for the PCEs: normochromatic erythrocytes (NCEs) ratio. Stevioside at the dose of 10 g/kg BW had no effect on the frequencies of micronucleus formation in the bone marrow erythrocytes of either sex of hamsters, rats and mice at any time point when compared to the negative control. Moreover, there were no apparent changes in the PCE:NCE ratio in either sexes of the tested species with an exception of male hamsters which exhibited significantly lower PCE:NCE ratio at 48 h after the treatment. These results suggest that stevioside at the given dosage had no chromosome damaging effect on the bone marrow cells of the tested rodent species. However the genotoxic risk of stevioside to humans is an issue that needs further investigation.

Keywords: stevioside, micronucleus test, rodent bone marrow cell

Introduction

Stevioside, a diterpene glycoside, is a major sweet component isolated from the leaves of the herbal plant *Stevia rebaudiana* Bertoni (Crammer and Ikan, 1986). It is one of the most important natural sweeteners and has been widely used to treat hypoglycemia in Paraguay for a number of years (Soejarto et al., 1983). Because stevioside is a white crystalline, odorless substance, approximately 300 times sweeter than sucrose (Kakayama and Isima, 1976), it is presently used as a non-nutritive sweetening agent in various kinds of food and food products in countries such as Japan, Brazil and People's Republic of China (Sasaki, 1983; Kinghorn and Soejarto, 1991). Stevioside has been subjected to various assessments for its toxic effect and no serious toxic effects have been reported (Yamada et al., 1985; Yodyingyuad and Bunyawong, 1991; Xili et al., 1992). For its genotoxic effect, stevioside and crude extracts of *Stevia rebaudiana* have been evaluated by several test systems including bacterial reversion test (Okumura et al., 1978; Matsui et al., 1996), Ames test (Suttajit et al., 1993; Klongpanichpak et al., 1997), Rec-assay (Okumura et al., 1978; Matsui et al., 1996), forward mutation assay in bacteria (Pezzuto et al., 1985; Matsui et al., 1996; Temcharoen et al., 1998), chromosomal aberration test (Ishidate et al., 1984; Suttajit et al., 1993; Matsui et al., 1996), host mediated assay (Tama Biochemical Co. Ltd., 1981) and dominant lethal test (Tama Biochemical Co. Ltd., 1981). In all of the tested systems stevioside showed negative results, while crude extracts (50% purity of stevioside) from the Stevia leaf showed a slight positive

* scptm@mahidol.ac.th
Received: February 18, 2002, accepted: April 12, 2002

result only in the in vitro chromosomal aberration test (Ishidate et al., 1984; Matsui et al., 1996). However, all of these tested systems were performed in vitro.

Therefore, there was a need for the genotoxicity of stevioside to be further assessed in animal models. In the present study, the micronucleus test was performed in both sexes of hamsters, rats and mice. The results showed that stevioside did not induce micronuclei in either sex of all tested animal species at various time points of treatment.

Materials and Methods

Adult male and female Syrian golden hamsters (80-100 g) were supplied by the Animal Production Center, Faculty of Science, Mahidol University, Bangkok, Thailand. Adult male and female Wistar rats (100-120 g) as well as Swiss albino mice (30-35 g) were obtained from the National Animal Center, Mahidol University, Salaya, Thailand. They were housed in a controlled condition at a room temperature of 25 ± 2°C, relative humidity of 60%, with an automatic light cycle period of 12 hours light/12 hours dark. The animals were kept in stainless steel cages with wire mesh cover and fed with CP rat diet (Pokapan Animal Food Co. Ltd.) and water *ad libitum*. Prior to each experiment, the animals were fasted overnight but allowed free access to water. Stevioside (approx. 96% purity) was extracted and purified from dried *Stevia rebaudiana* leaves as previously described by Adduci et al. (1987).

Groups of 20 male and female animals of each species were administered by gavage with 10 g of stevioside/kg BW (dissolved in distilled water to a concentration of 250 mg/ml). Positive control animals received an intraperitoneal administration of 80 mg cyclophosphamide/kg BW, while the negative control were administered by gavage with distilled water. Five animals of each sex were killed by cervical dislocation at 24, 30, 48 and 72 h after the treatment. Femoral marrow cells were flushed out with fetal bovine serum, smeared on clean glass slides, and fixed with methanol for 5 minutes before being air dried and double stained with May-Grunwald and Giemsa (Schmid, 1975). The slides were labeled and examined without any reference to the treatment. The frequencies of micronucleated polychromatic erythrocytes (MNPCEs) in 2000 PCEs per animal and the ratio of PCE to NCE in 400 erythrocytes were determined under light microscope.

The data for micronucleus induction were analyzed statistically by the Kastenbaum-Bowman method (Kastenbaum and Bowman, 1970). Data for PCE/NCE ratio were analyzed statistically by using Student's *t* test.

Results

The effects of stevioside on micronucleus formation in both male and female hamsters, rats and mice are shown in Tables 1-3, respectively. Stevioside at the dose of 10 g/kg BW did not significantly alter the frequencies of MNPCEs at any time point. Furthermore, there were no apparent changes in the ratio of PCE to NCE in either sex of all those species examined except the PCE:NCE ratio in the male hamsters at 48 h after the treatment, which was significantly lower than that of the negative control group. In the positive control animals, cyclophosphamide (80 mg/kg BW, i.p.), induced a significant increase in the frequency of MNPCEs in every treated animal species. These results suggested that stevioside did not induce micronucleus formation in bone marrow polychromatic erythrocytes of hamsters, rats and mice of either sex.

Discussion

The mutagenicity and clastogenicity of crude products, crude extracts of the leaves of *Stevia rebaudiana* and stevioside have been determined in a number test systems. Crude extracts of Stevia (18% stevioside) and stevioside (> 80%) gave negative results in bacterial reverse mutation test with *Escherichia coli* WP2 *uvrA*/pKM 101 and *Salmonella typhimurium* G46 (Okumura et al., 1978; Matsui et al., 1996), and Rec-assay with *Bacillus subtilis* H17 (Rec^+) and M45 (Rec^-) with or without rat liver S9 fractions (Okumura et al., 1978; Matsui et al., 1996). In Ames test, the results were negative for all components against *Salmonella typhimurium* TA1535, TA1537, TA1538, TA97, TA98, TA100, TA102 and TA104, even when the dose was as high as 50 mg/plate with or without liver S9 fractions from rat, mouse, hamster, guinea pig and rabbit (Suttajit et al., 1993; Klongpanichpak et al., 1997), with an exception of the crude product which showed a slight positive result in *S. typhimurium* TA100 with rat liver S9 fraction (Tama Biochemical Co. Ltd., 1981). Furthermore, the test with stevioside also yielded negative results in the forward mutation assay using *S. typhimurium* TM677, with or without liver S9 fraction from rat (Pezzuto et al., 1985; Matsui et al., 1996; Temcharoen et al., 1998), *umu* test in *S. typhimurium* TA1535/pSK1002 with or without liver S9 fraction (Matsui et al.,1996), host mediated assay via mouse (Tama Biochemical Co. Ltd., 1981), and mutation test using germinal cells of silk worm (oocyte) system (Tama Biochemical Co. Ltd., 1981). In the clastogenic study, stevioside showed negative results in chromosomal aberration test in cultured human lymphocyte (Suttajit et al., 1993), human fetal fibroblast (Tama Biochemical Co. Ltd., 1981), Chinese hamster lung fibroblast cell line (Ishidate et al., 1984; Matsui et al., 1996), rat medullary cells (Tama Biochemical Co. Ltd., 1981), and sister chromatid

Table 1 Micronucleus test in hamster bone marrow after oral administration of stevioside.

Compound	Dose	Time after	No. of MNPCEs / 1,000 PCEs[a]		PCE / NCE ratio[b]	
	(g/kg BW)	administration (hours)	male	female	male	female
Stevioside	0(water)	0	2.20 ± 0.58	2.00 ± 0.55	0.77 ± 0.04	0.71 ± 0.02
	10	24	0.80 ± 0.37	1.60 ± 0.40	0.68 ± 0.03	0.72 ± 0.04
		30	0.40 ± 0.24	0.60 ± 0.24	0.66 ± 0.03	0.67 ± 0.02
		48	2.00 ± 0.52	1.40 ± 0.93	0.54 ± 0.01[c]	0.65 ± 0.06
		72	1.25 ± 0.75	1.60 ± 0.51	0.74 ± 0.04	0.73 ± 0.03
Cyclophosphamide (i.p.)	0.08	30	32.6 ± 4.25[d]	34.0 ± 4.30[d]	0.65 ± 0.02[c]	0.56 ± 0.03[d]

[a]Mean ± SEM, n = 5 , 2000 PCEs scored per animal , [b]Mean ± SEM, n = 5, 400 erythrocytes (PCE + NCE) scored per animal.
Significant difference from control : $p < 0.05$[c], $p < 0.01$[d].

Table 2 Micronucleus test in rat bone marrow after oral administration of stevioside.

Compound	Dose	Time after	No. of MNPCEs / 1,000 PCEs[a]		PCE / NCE ratio[b]	
	(g/kg BW)	administration (hours)	male	female	male	female
Stevioside	0(water)	0	1.14 ± 0.14	1.45 ± 0.33	0.79 ± 0.01	0.69 ± 0.03
	10	24	2.00 ± 0.52	1.20 ± 0.58	0.76 ± 0.04	0.70 ± 0.04
		30	0.80 ± 0.31	2.80 ± 1.36	0.80 ± 0.03	0.81 ± 0.03
		48	1.80 ± 0.58	2.00 ± 1.31	0.80 ± 0.05	0.73 ± 0.02
		72	1.60 ± 0.75	0.80 ± 0.58	0.75 ± 0.03	0.79 ± 0.01
Cyclophosphamide (i.p.)	0.08	30	17.4 ± 2.04[d]	16.0 ± 2.43[d]	0.36 ± 0.04[d]	0.41 ± 0.03[c]

[a]Mean ± SEM, n = 5 , 2000 PCEs scored per animal , [b]Mean ± SEM, n = 5, 400 erythrocytes (PCE + NCE) scored per animal.
Significant difference from control : $p < 0.05$[c], $p < 0.01$[d].

Table 3 Micronucleus test in mouse bone marrow after oral administration of stevioside.

Compound	Dose	Time after	No. of MNPCEs / 1,000 PCEs[a]		PCE / NCE ratio[b]	
	(g/kg BW)	administration (hours)	male	female	male	female
Stevioside	0(water)	0	0.50 ± 0.27	1.00 ± 0.57	0.55 ± 0.04	0.73 ± 0.05
	10	24	1.40 ± 0.40	1.60 ± 0.49	0.60 ± 0.04	0.76 ± 0.05
		30	1.00 ± 0.50	2.40 ± 1.16	0.52 ± 0.01	0.80 ± 0.03
		48	0.40 ± 0.39	1.20 ± 0.65	0.56 ± 0.03	0.77 ± 0.07
		72	0.40 ± 0.30	0.80 ± 0.20	0.53 ± 0.03	0.75 ± 0.07
Cyclophosphamide (i.p.)	0.08	30	31.4 ± 7.07[d]	28.2 ± 5.88[d]	0.50 ± 0.03	0.39 ± 0.03[d]

[a]Mean ± SEM, n = 5 , 2000 PCEs scored per animal , [b]Mean ± SEM, n = 5, 400 erythrocytes (PCE + NCE) scored per animal.
Significant difference from control : $p < 0.05$[c], $p < 0.01$[d].

exchange in human fetal fibroblasts with or without rat liver S9 fraction (Tama Biochemical Co. Ltd., 1981). However, the crude product (50% purity of stevioside) yielded a slightly positive result when tested by the chromosomal aberration test in Chinese hamster lung fibroblast (Tama Biochemical Co. Ltd., 1981; Ishidate et al., 1984). In the dominant lethal test, crude product was administered orally at the doses of 10, 15 and 20 g/kg BW to 9-week-old male mice and only at the dose of 15 g/kg BW that it induced a slight but significant difference in the number of implantations. However, with no dose-response relationship, it was regarded as a negative result (Tama Biochemical Co. Ltd., 1981). The observed positive results of crude products and crude extracts in some test systems were probably caused by the impurities of the products. Previous studies of stevioside, all of which showed negative results, were performed only in the in vitro test systems. The present study was the first to assess the clastogenic effect of stevioside in the in vivo test system using micronucleus test in rodents including hamster, mouse and rat. It was found that stevioside at the dose as high as 10 g/kg BW had no clastogenic potential in either sex of all treated animals at various time intervals after an oral administration. These results were consistent with the previous in vitro studies confirming an absence of mutagenic and clastogenic potential of stevioside. The present results also showed no changed in the PCE:NCE ratio in most animals. The slight but significant decrease in PCE:NCE ratio observed in male hamsters after 48 h treatment (Table 1) was, however, in the acceptable normal range (Adler, 1984). Nevertheless, it indicated that stevioside at a certain dose could somehow reach the bone marrow to cause a slight cytotoxicity but did not exert clastogenic effect on the bone marrow cells.

Stevioside, given orally, is known to be converted by colon microflora bacteria to steviol which is subsequently completely absorbed (Wingard et al., 1980; Nakayama et al., 1986). Hamster was found to be more susceptible to the toxicities of stevioside and steviol when compared to the rat and mouse (Toskulkao et al., 1997). The liver S9 fraction from Aroclor pretreated hamsters exhibited higher efficiency in activating both the mutagenic and cytotoxic effects of steviol, when compared to other rodents (Temcharoen et al., 1998). In addition, steviol at the dose of 4 g/kg BW for hamsters, and at the higher dose of 8 g/kg BW for rats and mice exhibited a slight cytotoxicity to the bone marrow cells (Temcharoen et al., 2000). Base on the total conversion of stevioside to steviol in the colon and their molecular weights, 10 g stevioside/kg BW used in this study was equivalent to 3.96 g steviol/kg BW in case of completely converted by colon microflora bacteria. Therefore, it was possible that stevioside itself was not cytotoxic to the bone marrow cells, but after being converted to steviol and reabsorbed into the circulation. Steviol could reach the bone marrow and caused the observed slight cytotoxicity to the bone marrow cells.

In summary, the present results indicated that the observed slight cytotoxic effect on bone marrow cells may have resulted directly from stevioside or its metabolites. As for the assessment of the genotoxic risk of stevioside, it could be concluded that stevioside had no genotoxic potential. Moreover the average human consumption of 2 mg of stevioside/kg BW/day or the acceptable daily intake (ADI) of 7.938 mg of stevioside/kg BW/ day (Xili et al., 1992) was much lower than the tested dose in this study, stevioside should be not harmful for use as a sweetener for human. However, the genotoxic risk of stevioside need to be fully studied in human.

Acknowledgement

The authors are thankful to Assoc. Prof. Dr. Monthip Tiensuwan, Department of Mathematics, for her statistical assistance and also thanks to Mrs. Y. Sengsai for her typing. Stevioside was obtained from the Thai Research Institute of Pharmacognosy, Chiang Mai. Financial support was provided by the National Science and Technology Development Agency (NSTDA), the National Research Council and Mahidol University, Thailand.

References

Adduci, J., D. Buddhasukh and B. Ternai (1987) Improved isolation and purification of stevioside, J. Sci. Soc. Thailand, 13, 179-183.

Adler, I.D. (1984) Cytogenetic tests in mammals, In : S. Venitt and J.M. Parry (Eds), Mutagenicity testing, a Practical approach, IRL press, Oxford, Washington D.C., U.S.A., pp. 275-306.

Crammer, B. and R. Ikan (1986) Sweet glycosides from the Stevia plant, Chem. Br., 22, 915-918.

Ishidate, M. Jr., T. Sofuni, K. Yoshikawa, M. Hayashi, T. Nohmi, M. Sawada and A. Matsuoka (1984) Primary mutagenicity screening of food additives currently used in Japan, Fd. Chem. Toxicol., 22, 623-636.

Kakayama, O. and N. Isima (1976) Sensory evaluation of stevioside as a sweetener, Shokuhin Sogo Kenkyujo Kenkyu Hokoku, 31, 80-85.

Kastenbaum, M.A. and K.O. Bowman (1970) Tables for determining the statistical significance of mutation frequencies, Mutation Res., 9, 527-549.

Kinghorn, A.D. and D.D. Soejarto (1991) Stevioside. In L. O'Brien Nabors and R.C. Gelardi (Eds), " Alternative sweeteners," Marcel Dekker, New York, pp. 157-171.

Klongpanichpak, S., P. Temcharoen, C. Toskulkao, S. Apibal and T. Glinsukon (1997) Lack of mutagenicity of stevioside and steviol in *Salmonella typhimurium* TA98 and TA 100, J Med. Assoc. Thai. (Suppl.1), 80, S121-S128.

Matsui, M., K. Matsui, Y. Kawasaki, Y. Oda, T. Noguchi, Y. Kitagawa, M. Sawada, M. Hayashi, T. Nohmi, K. Yoshihira, M. Ishidate, Jr. and T. Sofuni (1996) Evaluation of the genotoxicity of stevioside and steviol using six in vitro and one in vivo mutagenicity assays, Mutagenesis, 11, 573-579.

Nakayama, K., D. Kasahara and F. Yamamoto (1986) Absorption, distribution, metabolism and excretion of stevioside in rats, J. Food Hyg. Soc. (Japan), 27, 1-8.

Okumura, M., Y. Fujita, M. Imamura and K. Aikawa (1978) Studies on the safety of stevioside with rec-assay and reversion test, J. Food. Hyg. Soc. (Japan), 19, 486-490.

Pezzuto, J.M., C.M. Compadre, S. M., Swanson, N.P.D. Nanayakkara and A.D. Kinghorn (1985) Metabolically activated steviol, the aglycone of stevioside, is mutagenic, Proc. Natl. Acad. Sci., 82, 2478-2482.

Sasaki, K. (1983) Use of stevioside sweeteners in drinks, New Food Ind. (Japan), 25, 38-43.

Schmid, W. (1975) The micronucleus test, Mutation Res., 31, 9-15.

Soejarto, D.D., C.M. Compadre, P.J. Medon, S.K. Kamath and A.D. Kinghorn (1983) Potential sweetening agents of plan origin. II. Field search for sweet-testing Stevia species, Econ. Bot., 37, 71-79.

Suttajit, M., U. Vinitketkaumnuen, U. Meevatee and D. Buddhasukh (1993) Mutagenicity and human chromosomal effect of stevioside, a sweetener from *Stevia rebaudiana* Bertoni, Environ. Health Perspect., (Suppl.3) 101, 53-56.

Tama Biochremical Co. Ltd. (1981) Safety of Stevia, 7-1 Nishishinjuku, 2-Chome, Shinjuku-Ku, Tokyo 160, Japan, 1-20.

Temcharoen. P., J. Pimbua, T. Glinsukon, W. Rojanapo and S. Apibal (1998) Mutagenic activity of steviol to *Salmonella typhimurium* TM 677 : Comparison of the activity of S9 liver fractions from five laboratory animal species, Bull. Health Sci.&Tech., 1, 38-45.

Temcharoen, P., M. Suwannatrai, S. Klongpanichpak, S. Apibal, T. Glinsukon and C. Toskulkao (2000) Evaluation of the effect of steviol on chromosomal damage using micronucleus test in three laboratory animal species, J. Med. Assoc. Thai., 83 (Suppl. 1) S101-S108.

Toskulkao, C., L. Chaturat, P. Temcharoen and T. Glinsukon (1997) Acute toxicity of stevioside, a natural sweetener, and its metabolite, steviol, in several animal species, Drug Chem. Toxicol., 20, 31-44.

Wingard, R.E., Jr, J.P. Brown, F.E. Enderlin, J.A. Dale, R.L. Hale and C.T. Seitz (1980) Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A, Experientia, 36, 519-520.

Xili, L., B. Chengjiany, X. Eryi, S. Reiming, W. Yuengming, S. Haodong and H. Zhiyian (1992) Chronic oral toxicity and carcinogenicity study of stevioside in rats, Fd. Chem. Toxicol., 30, 957-965.

Yamada, A., S. Ohgaki, T. Noda and M. Shimizu (1985) Chronic toxicity study of dietary Stevia extracts in F 344 rats, J. Food Hyg. Soc. (Japan), 26, 169-183.

Yodyingyuad, V. and S. Bunyawong (1991) Effect of stevioside on growth and reproduction, Human Reprod., 6, 158-165.

Environ. Mutagen Res., 24 ： 43-60（2002）

8th ICEMを終えて


黒田会員撮影

8th ICEMと「9月11日」

8th ICEM組織委員会委員長　早津彦哉

誰しもあの9月11日の事件にはショックを受けたにちがいない．私もたまたまテレビでこの実況を見てしまった．8th ICEMが間近になって，その準備に忙殺されていた中で，この惨事が起こり，これは大変だ，どうしようと思った一人である．これで科学が中心となると思っていた21世紀はいったいどうなっていくのか？科学の一分野としての環境変異現研究の位置づけは？など，心に浮かんでは消え，また浮かんでくるといった揺れ動く心境となった．

以後8th ICEMが開催された10月下旬までが組織委員会の最大に苦しい日々であったといえる．外国，特にアメリカ，カナダからは，主要な参加者達の苦悩や失意の言葉や，逆に，なにくそ負けるか頑張ろうといった決意表明などが寄せられた．われわれは，いつ戦争が始まるか，世界中の主要空港が閉鎖になる可能性まで考えながら，かたずをのんで成り行きを見守るしかなかった．この間の危機的状況を示すものとして，私が受信したe-mailの数がある．9月11日以降9月末日まで合計230通，10月には入ってからさらに増えて1日当たり平均25通となり，最大の受信数は10月15日の1日71通であった．

8th ICEMの初日10月21日の夜のレセプションが会の成否を占う鍵であると思っていたが，そこで見た会場にあふれんばかりの大勢の参加者達とその笑顔で，私はこれは大丈夫だとの確信を得た．用意されたスナック類はあっという間になくなってしまい，私自身殆ど何も口に入らなかったのも喜び以外の何物でもなかった．

会議の最中および終了後に参加された皆さんから寄せられた沢山の嬉しいメッセージからも，今回のICEMは成功であったといってよいと思う．学問的にも80％くらいは期待通りの成果があったと思うし，ソーシャルイベントは，ホームステイの成功ともあいまって，素晴らしかったと皆からほめられた．

会が終って3ヵ月を経た現在も，世界各国から8th ICEMは良かったとの言葉が寄せられ続けている．この会を盛り上げるのに大きな役目を果たしていただいたJEMS会員の皆様に深く感謝するとともに，8th ICEMが「9月11日」の悪夢を振り払って，環境変異原研究の21世紀のスタートダッシュの勢いをつけることに成功したことを皆さんとともに喜びたいと思う．

8th ICEM実行委員長　木苗直秀
（静岡県立大学食品栄養科学部）

第8回国際環境変異原学会（8th ICEM）は，平成13年10月21日（日）より26日（金）まで静岡県コンベンションアーツセンター「グランシップ」で日本学術会議と共同主催の形で開催されました．9月11日に米国で発生したテロ事件のため，およそ90名のキャンセルがありましたが，最終的には44ヵ国1地域より外国人が412名，日本人が409名の計821名が参加されました．4年毎に世界各国で開催されている本学会を21世紀初頭に日本（静岡）で開催できましたことは，大変意義のあることと考えております．今大会の特徴としては，20世紀に問題となった生活環境中の変異原・発がん物質について遺伝子への修飾様式の解析など基礎的研究とともにそれらのリスク評価，リスク管理，リスクコミュニケーションなど応用面にまでアカデミックな討論ができたこと，またそれら化学物質の作用を封じ込む抗変異原・抗発がん物質に関しても新しい知見が数多く発表され，参加者の関心が非常に高まったことなどが挙げられます．大会を通して基調講演3題，特別講演5題のほか，シンポジウム25，ワークショップ2，スペシャルセッション2，一般口頭発表160題，ポスター発表360題などとても実り多い学会となりました．さらに静岡県，静岡市の御協力を頂き，21世紀にこの分野を担う若き科学者達60名がホームステイできましたことは，国際交流にも貢献できたことを表すもので，国際学会の新しいモデルを提案できたものと考えております．

本大会の開催に際しまして，日本学術会議，国際環境変異原連盟，日本環境変異原学会をはじめ，地方自治体や企業の皆様の温かい御支援を賜りまして誠にありがとうございました．お陰をもちまして，本大会を無事且つ成功裡に終えることができました．ここに，本大会終了の御報告とともに，重ねて厚く御礼申し上げます．

産総研分子細胞　須藤鎮世

個人的なことですが，City of Hopeに留学する前に故大野乾先生からJeffreysの論文は重要だから読んでこいと指示された．親子鑑定などDNA診断の基礎となったマイクロサテライト多型に関する最初の論文である．今回，聴講の機会を得て嬉しく思う．大野先生は1928年2月1日生まれ，2000年1月13日に亡くなられた．“He was a great man”と早すぎる逝去を惜しまれた．

Jeffreysさんもgreat manで愛妻家，great menの条件は愛妻家のようだ．初来日ですが“My wife and I both fell in love with your country!”とのことであった．晩餐会では英人たちがノーベル賞をもらうかも知れないと噂，ノーベル委員会のGustafssonさん，頼みます．私が担当したDNA Technologyシンポジウムでは7演者中2名が欠席した．その1人はサテライト会議Functional Genomicsを要約するはずのAaronさんで，これに参加されたLohmanさんが急遽，Aaronさんからデータを入手，代役を果たされた．有難う．Aaronさんは座長でもあり，英国のPowellさんに代役をお願いした．が，なかなか返事が来ない．座長代役は会期中に確認した．後で知ったが，彼女は9月11日，San DiegoでのSAGE（serial analysis of gene expression）の学会に参加中で，空港閉鎖中は予定どおり親戚のところで過ごしたので問題なかったという．あの時期，幼い子供達を留守に2度海外に出してくれた家族の皆さんに謝意を表したい．彼女の職場は11月までの契約で，次の課題として蛋白構造の勉強を開始したという．成功を祈る．同じ座長の林崎さんはエジンバラでのマウスゲノム学会（10/21～24）からのとんぼ返りであった．行きのジャンボ機は乗客が2人とのことであった．困難な時に多数の方々の協力を得て成功裡に終わったことを嬉しく，誇りに思う．私はプログラム，行事接遇，展示会に参画したが，晩餐会の司会は良い経験になった．多々至らぬところはお詫びし，皆様のご協力に感謝します．特に静岡部会の皆様ご苦労さまでした．とてもよい学会であったという数多くの声を代表してSir Alec Jeffreysの言葉で締める．

“The meeting was excellent.”

国立医薬品食品衛生研究所変異遺伝部
能美健彦

2001年10月に静岡のグランシップで開かれた第8回国際環境変異原学会（8th ICEM）は盛会のうちに幕を閉じ，準備に関わった者の一人としてたいへんうれしく思っている．思い起こせば8th ICEMへの道は，その4年前フランス（ツールーズ）の7th ICEMにおいて，次の国際環境変異原学会が日本で開催されると決まったところから始まった．日本地図を配して次のICEMが日本で開かれることを知らせるビラが，ツールーズの会場のそこここに貼られていたことを今でも思い出す．それから3ヵ月，1997年12月に開かれた日本環境変異原学会（JEMS）の評議員会において，8th ICEMは早津委員長を中心に静岡，奈良，ソウル，上海で行うことが合意され，翌年4月には国立がんセンターで第1回目の準備会が開かれた．準備会では「質素で効率よく，かつat homeな雰囲気で会を運営する」というモットーが早津委員長から提案され，結局このモットーが学会運営全体の原則として最後まで生きていたように思う．その後，第2回（98年11月名古屋），第3回準備委員会（99年2月，静岡）を経て，99年11月には岐阜で第1回の組織委員会が開かれた．この時点で，長尾プログラム委員長から8th ICEMでのプログラム編成の概要が示された．最終的に8th ICEMでは，plenary sessionが8，シンポジウムが23，特別セッションが3，ワークショップが2，それぞれ開催され幅広い内容が深く論議されたように思う．特にplenary lectureは，いずれも素晴らしい講演ばかりであった．

私自身もTranslesion DNA synthesis，Transgenic rodents，Structural biologyという3つのシンポジウムのモデレーターを引き受けさせていただいた．「Translesion DNA synthesis」は，主にDNA損傷を乗り越えて複製を行う新しいタイプのDNAポリメラーゼ（YファミリーDNAポリメラーゼ）に関するセッションであり，この2年ほどの間にその重要性が認識されてきた課題である．前回のICEMでは，あまり論議されなかった内容であり，シンポジウムの当日（10月22日）は，はたしてどれくらいの人が聞きに来てくれるかと心配したが，200人近く入るC会場が満員になる盛況ぶりであった．「Transgenic rodents」は，遺伝子改変を行ったマウス，ラットを用いて変異と発がんの研究を行うセッションであり，米国，カナダ，オランダ，日本から講演があった．この研究は主に環境変異原分野で発展してきたmade in EMSの研究であり，これからも新しいマウス，ラットが作られ変異原，がん原研究に新風を吹き込むことが期待された．「Structural biology」は，やはり前回のICEMでは論議されなかった課題であるが，今回の演者の顔ぶれ（T.A. Kunkel, J.A. Tainer, W. Yang, D.B. Wigley, T. Shibata, K. Morikawa）はどこの学会にも負けない豪華なものであり，「Translesion DNA synthesis」に続いて多くの人が聞きに来てくれるかと期待したが，開催日が最終日に近い木曜の午前であったせいか聴衆が少なく，その点は残念であった．セッションの終了後に座長の一人であったT.A. Kunkel博士（NIEHS）から「自分が以前にGordon ConferenceでStructural biologyのセッションを開いた時にも，その意義が理解されずあまり人が集まらなかったが，今ではStructural biologyがいろいろな分野で主要な研究方法となっている．今にこの環境変異原研究の分野でもその重要性が理解されるだろう」と励まされた．

会場のグランシップは建物が大きくて新しく，議論を深め交流を図るには絶好の場所であった．会場の内外では，黄色いウインドブレーカーに身を包んだ担当者が，朝早くから夜遅くまで会の円滑な運営のために努力され，頭の下がる思いがした．木苗JEMS会長を中心にした静岡の組織委員の先生方の熱意と善意が会場全体を包んでいるような気がした．

2004年にサンフランシスコで開かれる9th ICEMでは，

はたしてどのようなセッションが新しく設けられ変異原研究を引っ張ってゆくのであろうか．それはこれから4年間の私たちの研究の進展にかかっている．

Reflections on the 8th International Conference on Environmental Mutagens (ICEM) and Satellite Meetings

Michael D. Waters[1]* and Paul H.M. Lohman[2]
([1]U.S. Environmental Protection Agency and National Institute of Environmental Health Sciences Research Triangle Park, NC USA, [2]Laboratory of Radiation Genetics and Chemical Mutagenesis Leiden University Medical Center Wassenaarseweg 72, 2333 AL Leiden The Netherlands)

The IAEMS was very pleased to join with the Environmental Mutagen Society of Japan (JEMS) and the Science Council of Japan in sponsoring the 8th ICEM in Shizuoka, Japan, on October 21-26, 2001. Satellite meetings organized in conjunction with the 8th ICEM included Seattle, USA, and Seoul, Korea, before the ICEM and Nara, Japan, and Shanghai, China, afterwards. In addition, the 5th International Symposium on Chromosome Aberrations (5th ISCA) was held on Awaji Island, Japan, immediately after the ICEM.

This was the second time in twenty years that the ICEM has been held in this beautiful country of Japan. The 3rd ICEM was organized in Tokyo, and its satellite meetings were held in Kyoto and Mishima. It has been an astounding twenty years for environmental mutagenesis research! In 1981, we were discussing fundamental mechanisms of mutagenesis and debating the merits of the Ames test. In 2001, we were exploring the implications of sequencing the human genome and applying toxicogenomics in groundbreaking research on global gene/protein expression.

In concert with its theme, "Research for the New Millennium", the 8th ICEM truly crossed the threshold into what is already being called the "Golden Age of Biology". The 8th ICEM was indeed a great scientific success. This important international meeting enabled exchange of views on current scientific research and served to transfer important new information worldwide. It undoubtedly catalyzed the establishment of many new international scientific collaborations. And, through the novel "home-stay program", it served to educate many international students and to introduce them to the wonderful Japanese culture.

The organization the 8th ICEM was impeccable, despite major challenges at every juncture including financial ones and even the terrorist events of September 11 in the US that threatened international travel. In spite of these challenges, the meeting and its satellites were held essentially as planned. The hard work for the meeting was performed by the 8th ICEM Organizing Committee under the capable leadership of its President, Hikoya Hayatsu; Vice President, Treasurer and Local Chairperson, Naohide Kinae; Vice President for Finance, Yoshinari Ohnishi, and Scientific Program Chairperson, Minako Nagao. Makoto Hayashi organized the IWGT (that unfortunately had to be cancelled), the IAEMS Internet course and the 5th ISCA. The organizers of the 8th ICEM and all satellite meetings truly deserve our recognition for staying the course and presenting an outstanding scientific conference.

The 8th ICEM also turned out beautifully in a social context. The people of Shizuoka Prefecture, the University of Shizuoka, and the city itself were extremely kind and hospitable. The new GranShip proved to be an exciting venue for the meeting. Professor Kinae together with his associates and student assistants were constantly available and helpful to all attendees. We were certainly treated as honored guests in Japan.

With the ICEM behind us we look forward to a new millennium of international research collaboration. The pleasant memories and new knowledge from Shizuoka, Nara, and Awaji Island will strengthen our continuing friendships and encourage us in our scientific pursuits in the future.

The 8th ICEM represented the completion our tenures as President and Secretary-General of the IAEMS. We want to sincerely thank all of the members of the JEMS for their constant support of our efforts over the past four years and for their lasting gifts to the IAEMS Member Societies-the 8th ICEM, the Nara satellite meeting, and the 5th ISCA. (20 December 2001)

8th ICEM に IAEMS - Fellow として参加して

静岡県立大学大学院生活健康科学研究科
食品栄養科学専攻　藤田理英子

今回の8th ICEMは私にとって初めて参加した国際学会でした．発表だけでなくスタッフとしても参加し，学会の表舞台と裏舞台を体験したわけですが，感想としては「とにかく忙しかった」「追われていた」，でも「やり遂げた」という満足感に浸ることができたと思います．始めは，慣れない英会語，接し方，習慣の違い等，戸惑うことば

かりで，日本にいながら，どこか違う国にでも来たような感じでした．それでも後半はなんとか雰囲気に馴染むことができ，身振り手振りも板につき，言葉を交わすことができるようになりました．ポスター発表では，演題数の多さに圧倒されました．私のところにも多くの方が見に来て下さり，自分とは異なった視点から鋭い指摘，質問を頂き，とても有意義な時間を過ごすことができました．特に，変異原の分野の最先端で活躍されている，論文上でしかお目にかかれない先生方と直接お話できたことは，とても貴重な経験でした．このような機会にめぐり会えたのも，国際学会ならではのできごとだったと思います．8th ICEMは，研究面だけでなく，学会運営の大切さを知る，本当に良い機会になりました．一生忘れない素晴らしい学会だったと思います．

最後に，8th ICEMに参加する機会を与えて下さいました木苗直秀教授に深く感謝致します．

サテライトミーティングを終えて

Functional Genomics印象記

（財）食品薬品安全センター秦野研究所
澁谷　徹

8th ICEMのSatellite Meetingである“Functional Genomics”に参加した．

会場はアメリカ西海岸のSeattle Tacoma空港のすぐ横のWyndham Hotelで，会期は10月16日から18日であった．直前におきたテロの影響があって，アメリカ国内からのspeaker数名のキャンセルがあった．日本からの事前登録者もすべて参加を断念されたようで，現地参加の私と阪大の三宅先生（JEMS非会員）の2名が参加したのみであった．私はWashington州立大学に行く用があり，同じSeattleでこの会が開催されていたので参加した．折からイチローのいるMarinersがWorld Series出場を争っていたので，ホテルのテレビを横目で見ながらの出席でもあった．会の全体は以下のようでした．

まずS.H. Taylor（UCSD）のKeynote Address“Structure, Function and Dynamics of PKA Signaling”があり，以下の5つのSessionsがあった．

1. Gene Function Impacts Human Populations Exposed to Xenobiotics

2. Determining Gene Function Depends on DNA Sequencing and Annotation

3. Expression of DNA Sequence Information is Integral to Function

4. SNP Analysis Leads to New Inferences about Disease, Therapy and Risk

5. Functional Genomic Approaches Using Whole Genomes and Comparative Genomics

6. Assessments of Genome Function through Proteonics

7. Emerging Technologies

講演はベンチャー企業からの最新のTechnologyとAcademiaからのbasicなLectureそれに製薬企業の開発段階でのToxicogenoimicsの実例などを織り交ぜたもので，アメリカでのこれに対する取り組みの一端をうかがうことが出来た．また，ポスターが30題程度発表されていたが，その内容は玉石混交といった感じであった．全体の印象は，テロ直後にもかかわらず，アメリカのAdventure Spritが充分に感じられるものであった．JEMSにとって，今後Toxicogenoimicsにどのように取り組むかが大きな課題であると感じられた．

この会の正式な報告は近く“Environmental and Molecular Mutagenesis”にChairを勤めたSid Aaron（会社の命令で日本のICEMに参加出来ずぼやいていた）によってまとめられるとのことですから，興味のある方はこちらを見ていただきたい．また，Abstractに興味のある方は小生までお問い合わせ下さい．

ICEMソウルサテライトミーティング参加報告

岐阜大学医学部　森　秀樹

第8回ICEMのソウルサテライトミーティングは10月17日～19日にYonsei大学のCentennial Hallにて開催された．

テーマは“Dietary and Medical Anti-mutagens and Anticarcinogens: Molecular Mecha-nisms and Chemoprevention Potential”であった．コーディネーターはソウル大学のYong-Joon Surth教授とYonsei大学のKwang Kyun Park教授である．11ヵ国から29名の招待演者が6のsessions（1. Antioxidative, Antigenotoxic and Antitumorigenic Compounds in the Diet, 2. Chemopreventive and Chemoprotective Potential of Dietary and Medicinal Plants: New Paradigm for Functional Foods, 3. Biochemical and Molecular Markers for Chemoprevention Studies, 4. Molecular Mechanisms of Chemopreventive or Chemoprotective Phytochemicals: Modulation of Intracellular Signaling Cascades, 5. Dietary Modulation of Formation, Metabolism, and Action of Carcinogens, 6. Effects of Edible Phytochemicals and Their Synthetic Derivatives on Carcinogenesis and Mutagenesis: From Basic Science to Clinical Applications）に分かれて講演した．この中には埼玉がん

センターの藤木先生と近畿大学の村上先生と私が含まれている．招待講演者でキャンセルしたのは米国の1名だけであった．別に91のポスター発表があったがこちらの殆どは韓国の研究者からのものであった．講演に際しての討論もかなり活発であったが，多くは招待演者間であった．Yonsei大学の会場は申し分無かった．Yonsei大学には羨ましい程のGuest Houseがあり，Welcome Receptionが行われた大学内のホールも立派なものであった．会期中に学生食堂や購買部も覗いてみたが，日本の大学生協とそっくりであった．Receptionもat homeで学生による音楽の演奏などがあり，結構楽しかった．いずれにせよ，この3日間の抗変異，抗発がん関係のサテライトミーティングは充分成果があったものと思われる．この会議が行われた前の週に別の会議で那覇へ行った．近場であるにも関わらず，私自身沖縄へ行くのも韓国へ行くのも初めてである．不思議なことになぜか那覇よりもソウルの街の方により日本的な雰囲気を感じた．所変われば品変わると思ったのはあちこちで白い紋様を有する小型のカラスの様な鳥を見た時ぐらいである．

「第5回国際染色体シンポジウム」開催報告

大会長　京都教育大学　生島隆治

第8回ICEMのサテライトミーティングとして，昨年10月26～28日に標記シンポジウムを兵庫県淡路島国際会議場で開催した．ドイツのエッセン大学で4年毎に開催されてきたもので，今回が初の日本開催となった．「21世紀における染色体異常研究の展望」という主題の下に，染色体の構造的・数的異常の基礎と応用の両面に関し，2題のkey lecture，16題の招待講演，20題のポスター発表が行われた．折からの国際テロ事件の影響を受け，2名の招待講演者が出席できないというハプニングもあったが，15ヵ国より100名近くの参加者を迎え，連日夜遅くまで熱気に満ちた討論で瞬く間に会期が終わってしまった．生命科学分野の目覚ましい発展を反映して，染色体異常の研究にも顕著な進展が多々見られ，そのすべてを国際的に共有するとともに近未来における更なる飛躍を展望できたことは最大の成果であった．日本丸による大阪湾クルージング・ディナーパーティーは外国人には殊の外好評で絶賛を博した．ともあれ，日本国発祥の地，淡路島の「夢舞台」（安藤忠雄設計）で大いに「夢」を見ようという当初の目論見は十二分に叶えられたと自己評価している．これも，偏に組織委員会メンバーをはじめ関係各位の温かい御援助の賜物と心より感謝申し上げる次第である．なお，本シンポジウムで発表された研究成果は，近くMutation Researchの特別号として出版されることになっている．

ICEM奈良サテライトミーティング

産業医科大学　葛西　宏

去る10月27，28日（土，日）奈良県新公会堂において「突然変異と発癌における活性酸素と活性窒素の役割」に関する8th ICEM奈良サテライト会議が開かれた．同時多発テロ事件後という悪条件にもかかわらず，海外からも多数の参加者があり，主催者の一人としてホッとした（招待者，海外14名，国内10名，ポスター発表49演題（うち海外から20），参加者総計146名）．Tannenbaumの基調講演を皮切りに，国内外の招待演者による，最先端の話が聞け，分野もDNA損傷，修復，シグナルトランスダクション，遺伝子発現など多岐に渡り，有意義な会となった．静岡から到着した人達からは，奈良という独特な雰囲気のため疲れを忘れ新たな気持ちで参加できた，という声が多く聞かれた．また早朝にもかかわらず「鹿寄せ」には多数の参加者があり笑いを誘っていた．この会が成功したのは，奈良医大に在籍されていた小西先生，中江先生のご尽力の賜であり，また多くの企業，組織からのご支援によるものである．この場を借りて深謝したい．

ICEM上海サテライトミーティング参加報告

国立医薬品食品衛生研究所　本間正充

上海サテライトミーティングと第9回アレクサンダーホランダーコースは10月30，31日に上海市にある復旦大学で開催された．中国環境変異原学会（CEMS）が主催し，翌日から始まる年次総会に先駆けて行われたものである．全体で約120名の参加のうち，招待者を含む外国人が10名程度で，ここでもやはりテロの影響で数名の外国人発表者のキャンセルがあり，プログラム内容の変更を余儀なくされた．テーマは“Human Population Monitoring for Cancer Prevention”で，2日目の午後には遺伝毒性モニタリングとしての小核試験と，コメット試験のワークショップが行われた．ワークショップではこの分野の世界的権威であるDr. Kirsh-Volder，Dr. Fenech，Dr. Tice等の熱心な教育講演と技術指導に，質問が相次ぎ，予定時間を大幅にオーバーし，夕方から行われる予定の懇親会が1時間以上も遅れる事態となった．中国での環境変異原研究はあまり知られていないが，CEMSは今年で10年目を迎え，総会での演題数も100を越える規模となっている．発表内容も国際的に十分通用するものも少なくない．今後の発展と，同じアジアの隣人であることを考えると，これまで欧米一辺倒であった我々の研究の方向を見直してもいいのではないかと思わせた．

3rd International Workshop on Genotoxicity Testing（8th ICEM Satellite Meeting）の開催延期の経緯について

祖父尼俊雄，林　真

International Workshop on Genotoxicity Testing（IWGT）は，1993年にメルボルンで，1999年にワシントンで開催され，第3回IWGTは8th ICEMのサテライトミーティングとして開催することが計画された（Organizers: D. Kirkland（UK），L. Müller（Switzer-land），M. Hayashi（Japan），T. Sofuni（Japan），J. MacGregor（USA），L. Schechtman（USA））．IWGTの目的は国際的に受け入れられる試験手法の確立にあり，今回は1）Mouse lymphoma assay，2）In vitro micronucleus assay，3）Transgenic genotoxicity models，4）p53 and Hras2 transgenic tumor models，5）Strategy and classificationを取り上げた．会場は静岡県立大学で，受け入れ態勢などの具体的な準備が着々と進められた最中に9月11日の事件が発生した．当初はこの事件がIWGT開催へどのように影響するかは掴みかねたが，しばらくすると海外旅行に対する懸念のメールが交換されるようになり，9月25日頃からは米国行政，企業などでの海外旅行の一時的禁止の情報が流れるようになった．一方，欧州側からは比較的冷静な反応が多く，予定通りの参加の意志を示した人々もいた．日本側から予定通りの開催のために参加を依頼したが，事態を深刻に受け止める流れは止められるものではなかった．

IWGTの目的が国際的な合意を得ることにあり，そのため各グループメンバーに不参加者がでると，国際的な合意を得たことにはならないことから，どれだけ不参加者がでるかは深刻な問題である．そのため，各グループメンバーについて参加の確認を行ったところ，時間の経緯に伴い不参加者が増加した．10月に入った時点で，71名の討論者のうち22名が不参加であった．また，開催か否かの決断のための時間的余裕もなくなってきた．サテライトミーティングの中止はICEM本会議に影響しかねないことから，組織委員，グループリーダーのみの議論でなく，IAEMSのDrs. M. Waters, P. Lohmanや8th ICEMの早津，木苗先生らとの意見交換など，最終判断は至難なものであった．IWGTの目的からみて開催する意義がなくなることと，本会議への影響が少ないことが予測されたことから，最終的に10月4日に3rd IWGTの延期を公式に発表した．

IWGTの延期によりこれに関連した本会議のシンポジウムが1つ中止になり，またIWGTの延期に伴い本会議への参加を取り止めた人もおり，全く本会議に影響がなかったとはいえない．しかし，本会議は盛会のうちに終了したことから，実質的な影響はきわめて少なかったといえる．現在，延期された3rd IWGTの開催計画が日本および英国で検討されている．静岡での3rd IWGTの開催に協力された皆様方に改めて深謝するとともに，引続きのご協力をお願いしたい．

8thICEM プログラム掲載にあたり

昨年9月11日に起きた同時多発テロの余波は遠い日本で行われた国際学会にも及びました．講演，発表のキャンセルもあり，プログラムや参加者リストの変更を余儀なくされ，不自由な中での開催となりました．そこで，環境変異原研究第24巻1号を8th ICEMの記念号とするにあたり，写真，回顧記事などのほかに，実際に行われた発表を記録として残すことは意義あることと考えました．演題番号と演者名，会場などを実際の発表順に並べただけの簡単なものですが，お手元のプログラムの演題番号と照らし合わせると，演題名はわかるようになっています．ご活用いただければ幸いです．

編集委員一同

21 October, 2001

Opening ceremony　17:00−18:30　Room A

Opening address	H Hayatsu（Japan）
Message	The Science Council of Japan
Welcome message	N Kinae（Japan）
Message	MD Waters（USA）
Message from the Prime Minister of Japan	（to be read on behalf of the minister）

President's lecture

PR-1	H Hayatsu（Japan）

Welcome Cocktail　18:30−20:30　Room C+D

22 October, 2001

Plenary session　8:30−12:00　RoomA

Keynote lecture

KL−1	T Sugimura（Japan）

Plenary lecture

PL−1	L Loeb（USA）

Plenary lecture

PL−2	C Barrett（USA）

Plenary lecture

PL−3	D MacPhee（Japan）

Lunch, Poster discussion 12:00−14:30 6F, Ocean（1F）

Lunch

Poster discussion P1−1 ～ P27−51（odd number posters）

Plenary session 14:30−15:15 Room A

Plenary lecture

PL−4 J−A Gustafsson（Sweden）

Symposia 1 and Special session 2 15:30−19:20 Room B ～ F

Symposium 1B: Dietary Mutagens and Carcinogens Room B

1B−2 M Nagao（Japan）
1B−3 R Sinha（USA）
1B−4 LR Ferguson（NewZealand）
1B−5 H Bartsch（Germany）
1B−7 JE Trosko（USA）

Symposium 1C: Translesion DNA Synthesis as a Mechanism of Mutagenesis

1C−1 GC Walker（USA） Room C
1C−2 RPP Fuchs（France）
1C−3 CW Lawrence（USA）
1C−4 F Hanaoka（Japan）
1C−5 R Woodgate（USA）
1C−6 M Moriya（USA）

Symposium 1D: Molecular Cytogenetic Approach to Gene Mapping and Function

1D−1 AT Natarajan（Netherlands） Room D
1D−2 J Inazawa（Japan）
1D−4 HG Weier（USA）
1D−5 M Ohimura（Japan）
1D−6 U Wintersberger（Austria）

Symposium 1E: Ecosustem and Human Health Room E

1E−1 WF Grant（Canada）
1E−2 HNB Gopalan（Kenya）
1E−3 GM Bohm（Brazil）
1E−4 G Cabrera（Mexico）
1E−6 S Knasmueller（Austria）
1E−7 TH Ma（USA）

Special session 2: The Future of the IAEMS Room F

1F−1 MD Waters（USA）
1F−3 PHM Lohman（Netherlands）
1F−5 DM DeMarini（USA）
1F−6, 7 WW Au（USA）
1F−8 LR Ribeiro（Brazil）
1F−9, 10 JM Gentile（USA）
open discussion

Plenary session 8:30−9:15 RoomA

Keynote lecture

KL−2	AJ Jeffreys（UK）

Symposia 2 9:30−12:30 Room A～F

Symposium 2A: Tea and Health

2A−1	N Kinae（Japan）
2A−2	C Ioannides（UK）
2A−3	Y Kuroda（Japan）
2A−4	Z Dong（USA）
2A−5	VE Steele（USA）
2A−6	H Mukhtar（USA）
discussion	

Symposium 2B: Mutagens and Carcinogens in Water, Air and Soil: Significance to Human Health

2B−1	K Wakabayashi（Japan）	Room B
2B−2	PA White（Canada）	
2B−3	T Watanabe（Japan）	
2B−4	J Lewtas（USA）	
2B−7	DM DeMarini（USA）	

Symposium 2C: Molecular Epidemiology with DNA Damage as Markers

2C−2	D Turner（Australia）	Room C
2C−4	T Kamataki（Japan）	
2C−5	SA Kyrtopoulos（Greece）	
2C−6	RJ Sram（Czech）	

Symposium 2D: Individual Difference in Human Metabolizing Capacity: Genetic and Environmental Influences

2D−2	H Autrup（Denmark）	Room D
2D−3	Y Fujii−Kuriyama（Japan）	
2D−5	Y Yamazoe（Japan）	
P27−48	M Waters（USA）	

Symposium 2E: Health and Aging Room E

2E−1	Y Furuichi（Japan）
2E−2	V Bohr（USA）
2E−3	SC Park（Korea）
2E−4	J Vijg（USA）
2E−5	T Ono（Japan）
O8−10	SV Vasilieve（Russia）

Symposium 2F: Radiation and DNA Repair−Protein Interactions Room F

2F−1	DJ Chan（USA）
2F−2	K Komatsu（Japan）
2F−3	A Pastink（Netherlands）
2F−4	K Miyagawa（Japan）

2F−5 J Thacker（UK）
2F−6 PC Hanawalt（USA）

Lunch, Poster discussion 12:00−14:30 6F, Ocean（1F）

Lunch
Poster discussion P1−2 ～ P27−52（even number posters）

Plenary session 12:30−14:30 Room A

Plenary lecture
PL−5 R Holliday（Australia）

Symposia 3, Workshop 1, and Special session 1a 15:30−19:20 Room A ～ F

Workshop 1: Validation of the in vitro micronucleus assay for safety evaluation of clastogenic／aneugenic compounds
3A−2 T Sofuni（Japan） Room A
3A−3 D Marzin（France）
3A−4 H Norppa（Finland）

Workshop 2: A SFTG International Collaborteive Study on in vitro Micronucleus Test, using human lymphocytes and CHO, CHL and L5178Y cell lines
3A−5 E Lorge（France）
3A−6 A Wakata（Japan）

Symposium 3B: Mechanisms of Antimutagenesis and Anticarcinogenesis
3B−1 RH Rdashwood（USA）
3B−2 MJ Plewa（USA） Room B
3B−3 YJ Surh（Korea）
3B−4 S De Flora（Italy）
3B−5 TG Rossman（USA）
3B−6 H Nishino（Japan）

Symposium 3C: Transgenic Rodents as a Tool for Modern Risk Assessment
3C−1 JA Healdle（Canada） Room C
3C−2 JG de Boer（Canada）
3C−3 H Van Steeg（Netherlands）
3C−4 T Nohmi（Japan）
3C−6 H Tsuda（Japan）
3C−7 JE French（USA）

Symposium 3D: Genomic Instability Room D
3D−1 H Ohmori（Japan）
O19−3 J−S Hoffman（France）
3D−3 H Nakagama（Japan）
3D−4 RH Schiestl（USA）
3D−5 MA Kadhim（UK）
3D−6 C Streffer（Germany）

Symposium 3E: Endogenous Mutagens and DNA Damage		Room E
3E−1	J Cadet (France)	
3E−2	S Loft (Denmark)	
3E−3	B Demple (USA)	
3E−4	J Laval (France)	
3E−5	I Rusyn (USA)	

Special session 1a: Science in Countries with Developing Environmental Mutagenesis Programs		
3F−1	JO Akerele (Nigeria)	Room F
3F−2	OA Badary (Egypt)	
3F−3	K Fahmy (Egypt)	
3F−4	RC Chaubey (India)	
3F−5	G Murin (Slovakia)	
5F−5	A Cebulska−Wasilewska (Poland)	
3F−7	KSA Mossanda (South Africa)	
3F−8	LS Hoyos G (Colombia)	
3F−9	M Abdel−Hamid (Egypt)	
3F−10	A Tagne (Cameroon)	

24 October, 2001

General oral sessions		8:30−11:45 Room B ～ H
Session 7: Antimutagens−Anticarcinogens		Room B
O7−14	U Vinitket−Kumnuen (Thailand)	
O7−1	E Elmore (USA)	
O7−2	CAM Krul (Netherlands)	
O7−3	J−K Lin (Taiwan)	
O7−4	JL Marnewidk (South Africa)	
O7−6	DM Simic (Yugoslavia)	
O7−7	S Arimoto−Kobayashi (Japan)	
O7−8	EO Farombi (Nigeria)	
O7−9	J−H Jang (Korea)	
O7−10	OJ Park (Korea)	
O7−11	IM Villaseor (Philippines)	
O7−12	U Rannug (Sweden)	

Session 2: Mutagens and Carcinogens in Water, Air and soil		Room C
O2−1	M Dybdahl (Denmark)	
O2−3	GM Bohm (Brazil)	
O2−4	D Pra (Brazil)	
O2−5	AC Basilio (Brazil)	
O2−6	N Sera (Japan)	
O2−7	G Bronzetti (Italy)	
O2−8	K−T Chung (USA)	
O2−9	S Wakuri (Japan)	
O2−10	M Machala (Czech)	
O2−11	BJ Majer (Austria)	
O2−12	P Joseph (USA)	

Session 6: Mehcanisms of Mutations — Room D

P19−5	I Kobayashi (Japan)
O6−1	K Negishi (Japan)
O6−2	E Sage (France)
O6−3	C Otsuka (Japan)
O6−4	J O−Wang (Japan)
O6−5	Y−N Yu (China)
O6−6	S Liu (China)
O6−7	K Felix (USA)

Session 18: Transgenerational Effects of Environmental Factors

O18−1	T Shibuya (Japan)

Session 14: Molecular Epidemiology — Room E

O14−1	C Bolognesi (Italy)
O14−2	G Speit (Germany)
O14−4	P Schmezer (Germany)
O14−5	A Horska (Slovakia)
O14−6	S Loft (Denmark)
O14−7	H Ohshima (France)
O14−8	K Nakachi (Japan)
O14−9	K Savela (Finland)

Session 9: Genetic Disease in DNA Repair

O9−1	P Mosesso (Italy)
O9−2	J Surralles (Spain)

Session 1: Mutagens and Carcinogens in Diet

O1−1	Y Totsuka (Japan)
O1−2	P−M Leong−Morgenthaler (Switzerland)
O1−3	P Moller (Denmark)
O1−4	K Hiramoto (Japan)
O1−5	JE Lampinen (Finland)
O1−6	R Hussein (Egypt)
O1−7	UB Vogel (Denmark)

Session 13: DNA Technology

O13−1	R Gealy (USA)
O13−2	C Furihata (Japan)
O13−3	MHL Green (UK)
O13−4	PB Veronique (Cameroon)

Session 3: Solar and Ionizing Radiation Mutagenesis — Room G

O3−1	K Kikugawa (Japan)
O3−2	A Czich (Germany)
O3−3	K Fujikawa (Japan)
O3−4	LA Vasilyeva (Russia)
O3−5	AN Osipov (Russia)
O3−6	A Masaoka (Japan)

Session 21: Comet Assay

O21−1	RC Chaubey (India)
O21−2	VJ McKelvey−Martin (UK)
O21−3	R Saran (India)
O21−4	A−H Tu (China)
O21−5	J−R Meunier (France)

Session 19: Genomic and Chromosomal Instability — Room H

O19−1	N Shima (USA)
O19−2	E Wintersberger (Austria)
1D−6	U Wintersberger (Austria)
O19−4	S Ban (Japan)
O19−5	SR Moore (USA)
O19−6	S Sutou (Japan)

Session 5: DNA Adducts

O5−2	E Eder (Germany)
O5−3	U Harju (Finland)
O5−4	PT Henderson (USA)
O5−5	A Umemoto (Japan)

Session 25: Genomics and Proteomics in Genetic Toxicology

O25−1	AJ Van der Ed (Netherlands)

Lunch, Excursion — 12:00 −

25 October, 2001

Plenary session — 8:30−9:15 — Room A

Keynote lecture

KL−3	EC Friedberg (USA)

Symposia 4 and Workshop 2

Workshop 2: The Comet Assay−Recent Advances and New Applications

4A−1	G Speit (Germany)	Room A
4A−2	D Anderson (UK)	
4A−3	YF Sasaki (Japan)	
4A−4	AR Collins (UK)	
4A−5	BL Pool−Zobel (Germany)	
4A−6	RR Tice (USA)	

Symposium 4B: Mechanistic Dynamics and Genetic Diseases in DNA Repair

4B−4	J Jiricny (Switzerland)	Room B
4B−2	T Tsuzuki (Japan)	
P8−2	PK Cooper (USA)	
4B−5	K Tanaka (Japan)	
4B−6	JHJ Hoeijmakers (Netherlands)	

Symposium 4C: Transgenetational Effects of Environmental Agents

4C−1	T Nomura (Japan)	Room C
4C−2	LM Anderson (USA)	
4C−4	K Mabuchi (USA)	
4C−5	WW Au (USA)	
4C−6	K Sankaranarayanan (Netherlands)	

Symposium 4D: Protective Mechanisms and the Concept of Genotoxic Activity

4D−1	J Rueff (Portugal)	Room D
4D−2	PD Josephy (Canada)	
4D−3	MJM Nivard (Netherlands)	
4D−4	I−D Adler (Germany)	
4D−5	S Fukushima (Japan)	
4D−6	JM Parry (UK)	

Symposium 4E: Epidemiology and Environmental Mutagens−Relevance and Limitations−Epidemiology is the touchstone of our science. How reliable is it ?

Introduction	BA Bridges (UK)
4E−2	P Vineis (Italy)
4E−1	BA Bridges (UK)
4E−3	O Hino (Japan)
Introduction to Discussion	DG MacPhee (Japan)
Discussion	

Symposium 4F: Structural Biology on DNA Replication and Its Relevance to Mutation Research

4F−1	TA Kunkel (USA)	Room F
4F−2	W Yang (USA)	
4F−3	DB Wigley (UK)	
4F−4	T Shibata (Japan)	
4F−5	JA Tainer (USA)	
4F−6	K Morikawa (Japan)	

Lunch, Lunch time seminar 12:30−14:30 6F／Room C

Symposia 5 and Special session 1b

Symposium 5A: Micronucleus Assay in Human Monitoring and the HUMN Project

5A−1	E Zeiger (USA)	Room A
5A−2	S Bonassi (Italy)	
5A−3	M Fenech (Australia)	
5A−4	NT Holland (USA)	
5A−5	M Kirsch−Volders (Belgium)	
5A−6	WP Chang (Taiwan)	
5A−7	D Scott (UK)	

Symposium 5B: Chemical and Biological Properties of DNA Adducts, Relevance to Mutagenesis and Carcinogenesis

5B−2	PB Farmer (UK)	Room B
5B−3	DH Phillips (UK)	

5B−4	S Shibutani (USA)	
5B−5	E Ohtsuka (Japan)	

Symposium 5C: Epigenetic Changes Related to Human Health		Room C
5C−2	M Costa (USA)	
5C−4	GP Pfeifer (USA)	
5C−5	T Sekiya (Japan)	
5C−6	T Ushijima (Japan)	

Symposium 5D: Solar Radiation and Mutagenesis		Room D
5D−1	RM Schaaper (USA)	
5D−2	L Mullenders (Netherlands)	
5D−3	T Ishikawa (Japan)	
5D−4	A Sarasin (France)	
5D−5	T Negishi (Japan)	
5D−6	RC von Borstel (Canada)	
5D−7	MHL Green (UK)	

Special session 1b: Science in Countries with Developing Environmental Mutagenesis Programs		
5F−1	R Aroutiounian (Armenia)	Room F
5F−3	RI Bersimbaev (Kazakhstan)	
P9−1	AR Muotri (Brazil)	
5F−6	H Groot−Restrepo (Colombia)	
5F−7	E Mirkova (Bulgaria)	
5F−8	RD Montero (Mexico)	
5F−9	AK Giri (India)	
5F−10	S Kietthubthew (Thailand)	

Banquet 18:00−21:30 Ocean

26 October, 2001

Symposium 6 8:30−12:00 Room A

Symposium 6A: DNA Techonology	
6A−1	M Miwa (Japan)
6A−2	J Powell (UK)
6A−3	TS Tanaka (USA)
6A−4	Y Suh (Korea)
6A−6	Y Hayashizaki (Japan)
6A−7	PH Lohman (Netherlands)

General oral sessions 8:30−12:00 Room B ～ H

Session 7: Antimutagens−Anticarcinogens		Room B
O7−13	H Steinkellner (Austria)	
O7−16	L Ao (China)	
O7−17	AM Aboul−Enein (Egypt)	
O7−18	E Lansky (Israel)	

Session 11: Transgenic Animals in Genetic Toxicology

O11-1	K Amanuma (Japan)
O11-4	T Chen (USA)
O11-5	H-J Martus (Switzerland)

Session 20: Micronuclei in vitro and in vivo — Room C

O20-1	L Abramsson-Zetterberg (Sweden)
O20-2	ME Gonsebatt (Mexico)
O20-3	S-H Kim (Japan)
O20-4	YG Izyumov (Russia)

Session 22: Germline Mutations

O22-2	CL Yauk (UK)

Session 2: Mutagens and Carcinogens in Water, Air and Soil

O2-13	T Grummt (Germany)
O2-14	Y Yamazaki (Japan)
O2-15	J-H Kwon (Korea)
O2-16	S Kira (Japan)
O2-17	T Takamura-Enya (Japan)

Session 8: DNA Repair and its Mechanisms — Room D

O8-1	CS Griffin (UK)
O8-3	M Frankenberg-Schwager (Germany)
O8-4	TP Nouspikel (USA)
O8-5	M Satoh (Canada)
O8-7	I Rusyn (USA)
O8-8	H Ide (Japan)
O8-9	B Tudek (Poland)
O8-12	B Kaina (Germany)

Session 16: Individual Differnces in Mutagenesis and Carcinogenesis

O16-1	RK Elespuru (USA)	Room E
O16-3	S-M Hou (Sweden)	
O16-4	A Rannug (Sweden)	
O16-5	H Tokiwa (Japan)	
O16-6	AJ Van der Eb (Netherlands)	
O16-8	C Keshava (USA)	

Session 4: Endogenous Mutagens

O4-2	M Kruszewski (Poland)
O4-3	T Nunoshiba (Japan)
O4-4	T Tsutsui (Japan)

Session 17: Molecular Cytogenetics, Chromosomal Aberraions — Room F

O17-1	J Boei (Netherlands)
O17-2	ET Sakamoto-Hojo (Brazil)
O17-3	F Darroudi (Netherlands)
O17-4	IP Aranha (Brazil)
O17-5	Y Ishii (Japan)
O17-6	M Dusinska (Slovakia)

	O17−7	J Nath (USA)		
	O17−8	VA Ratner (Russia)		
	Session 12: Epigenetic Changes			
	O12−1	M Suganuma (Japan)		
	O12−2	BM Thomson (New Zealand)		
	Session 10: Metabolisms of Genotoxic Agents			Room G
	O10−1	K Fujita (Japan)		
	O10−2	M Miyazaki (Japan)		
	O10−3	WP Watson (UK)		
	O10−4	A Koide (Japan)		
	O10−7	C Fred (Sweden)		
	Session 15: Health and Aging			
	O15−1	C Dellacroce (Italy)		
	P15−6	S Kawanishi (Japan)		
	Session 23: Thresholds in Genetic Toxicology			
	O23−1	AM Lynch (UK)		
	Session 25: Ecosystem and Human Health			
	O26−1	RI Bersimbaev (Kazakhstan)		
	Session 27: Others			Room H
	O27−1	M Honma (Japan)		
	O27−2	J−R Meunier (France)		
	O27−3	P Rettberg (Germany)		
	O27−4	RA Sidorov (Russia)		
	O27−5	JEE Haglund (Sweden)		
	O27−6	H Lu (China)		
	O27−7	L Chen (China)		
	O27−8	A Matsuoka (Japan)		
	O27−9	MA Ghoneim (Egypt)		
	O27−10	M Tornqvist (Sweden)		
	O27−11	S Tokudome (Japan)		
	O27−12	RWL Godschalk (Netherlands)		
Closing session	H Hayatsu N Kinae PC Hanawalt		12:00−12:15	Room A

Schedule of 8th ICEM

	21 (Sun)	22 (Mon)	23 (Tue)	24 (Wed)	25 (Thur)	26 (Fri)
8:00		**Registration**	**Registration**	**Registration**	**Registration**	**Registration**
9:00	JEMS registration	Keynote: *T Sugimura*	Keynote: *AJ Jeffreys*	General oral sessions (B ~ H)	Keynote: *EC Friedberg*	6A: DNA technology
10:00	JEMS annual meeting	Plenary: *L Loeb*	*Break*		*Break*	General oral sessions (B ~ H)
11:00		*Break*	2A: Tea and health		4A: Workshop 2 Comet	
12:00		Plenary: *C Barrett*	2B: Water, air and soil		4B: Disease and repair	
13:00		Plenary: *D MacPhee*	2C: Molecular epidemiology		4C: Transgenerational effects	
14:00		*Lunch* Posters	2D: Human metabolisms		4D: Thresholds,	
15:00	**8th ICEM registration**	Plenary: *J-A Gustafsson*	2E: Health & aging	**Excursion**	4E: Epidem and environmental	
16:00		*Break*	2F: Radiation and repair		4F: Structural biology	**Closing session**
17:00	**Opening ceremony** Room A	1B: Dietary mutagens	*Lunch* Posters		*Lunch* Lunch time seminar	
18:00	President's lecture: *H Hayatsu* MRS Award	1C: Transleion DNA synthesis	Plenary: *R Holliday*		5A: HUMN project	
19:00	**Welcome cocktail** Room C+D	1D: Molecular cytogenetics	*Break*		5B: DNA adducts	
20:00	20:30	1E: Ecosystem	3A: Workshop 1 *In vitro* MN		5C: Epigenetic changes	
		1F: Special session 2 Future of IAEMS	3B: Antimuta, anticar		5D: Solar radiation	
			3C: Transgenic rodents		5F: Special Session 1b Science in countries	
			3D: Genomic instability		**Banquet** (Ocean)	
			3E: Endogenous mutagens		21:30	
			3F: Special session 1a Science in countries			

Environ. Mutagen Res., 24 : 61(2002)

書　評

Food Borne Carcinogens－Heterocyclic Amines

M. Nagao and T. Sugimura(Eds.)
John Wiley & Sons, Ltd(2000)
373 pages, Price £90, ISBN 0-471-98399-3

人間の健康に最も関係の深い「環境」といえば，まず，日常の食べ物がある．わが国における環境変異原研究は，その歴史の初めから食べ物に関係していた．すなわち，食品保存料として使われていたAF2に強い変異原性が発見され，次いでそれに発がん性があることがわかって使用禁止になったことが，日本環境変異原学会のスタートのきっかけになったといえよう．1977年に国立がんセンター研究所の杉村隆，長尾美奈子両博士のグループが魚や肉の焦げに強い変異原性があることを見出し，その本体がヘテロサイクリックアミンであることを突きとめて以来，日本の変異原研究は，その成果を中心に多くの研究者が集まり，今日の隆盛をもたらしたといってよい．世界的にみても，この発見のインパクトは大きく，この20年間で，アメリカ，ヨーロッパをはじめ各国の優秀な科学者が競って「食べ物による発がんとヘテロサイクリックアミンの因果関係」を究明しようと，猛烈な勢いで研究を進めてきた．2000年という区切りの年度を迎えて，今回この分野の創始者であるお二人が編集して，本書を刊行されたことはまことに時宜を得たものといえよう．

本書は，12章30名の著者（日本10名，外国20名）の分担執筆により，ヘテロサイクリックアミン化合物群のすべてについて，それらの多岐にわたる生物への作用を「発がん性」に焦点をしぼって網羅的に解説したものである．まずSugimuraの"Preface"によって発見の端緒が語られ，第1章でさらに拡大して発がんの現象全体をカバーしつつ論じられた後，「各論」がスペシャリスト達によって詳細に記述されている．すなわち，合成法，食べ物，飲料，タバコ煙の中の含量；代謝；細胞成分との反応について解説があったのちに，Nagaoによる変異原性の詳細なレビューと考察がなされ，次いで発がんのデータが動物別，標的臓器別に紹介されている．また，がん以外の病変についての一章があり，さらに抗変異原性，抗がん性物質についての詳しい解説と考察がDashwoodによってなされている．各論の最後は，疫学者によるヘテロサイクリックアミンのヒト発がん物質としての位置づけがなされ，半肯定的な議論が展開されている．本書の締めくくりは，Sugimuraグループによる2つの章で，「結論と展望」が述べられている．

私が通読して感心したのは，資料として有用な章の中にあって，読み物としても面白く，同じ分野の研究者としてスリルをも味わえるような章がいくつもあることである．ところどころに眼を開かせるようなヒントが散りばめられたこれらのエッセイを読むことは，単にヘテロサイクリックアミン研究者のみでなく，変異原－発がん研究者の誰でもが感銘するところであろう．特に力作は，序章と結論の3つの章であり，ここに著者らの研究の哲学が込められている．「肉や魚の焼けこげが，人間の発がんの原因であることをきちんと立証するにはまだ時間がかかるであろうが，これまでの研究結果からこの可能性が高いこと，従って焼けこげの摂取は控えよう」という著者らの結論は，本書全体を読んで十分納得できる．

引用文献は延べ1300を越え，1999年までカバーしている（2000年の分もわずかにある）．ヘテロサイクリックアミンについてのほぼ全貌を網羅したこの本は，20世紀終期の科学の新発展を記録したものとして，大きな価値がある．

（早津彦哉・就実学園　岡山市西川原1−6−1）

日本環境変異原学会会則

第1章　名　　称

第1条　本会は日本環境変異原学会と称する．

第2条　本会の英語の名称はThe Japanese Environmental Mutagen Societyと称し，JEMSと略称する．

第3条　本会は事務所を〒170-0003 東京都豊島区駒込1－43－9　駒込TSビル　財団法人口腔保健協会内に置く．

第2章　目　　的

第4条　本会は人間・生物・地球環境における変異原，とくに公衆の健康に重大な関係を有する変異原とこれに関連する基礎研究の推進，ならびに関連情報・技術の伝達を目的とする．

第3章　事　　業

第5条　本会は前章の目的を達成するために，次の事業を行う．

1. 年1回大会を開催し，総会，ならびに学術上の研究成果の発表および知識・情報の交換を行う．
2. 学会誌「環境変異原研究」および会報「Jems News」を発行し，会員に配布する．
3. 学会賞等（学会賞，研究奨励賞，功労賞）を設け，環境変異原の分野ですぐれた研究を行った会員および将来の成果が期待される会員（原則として個人）に授与する．
4. 国際環境変異原学会連合に加入し，国際協力に必要な活動を行う．
5. Mutation Research等の英文誌をまとめて購入配付する．
6. その他公開シンポジウムの開催，研究会の支援など本会の目的を達成するために必要な活動を行う．

第4章　会　　員

第6条　本会の会員は，正会員，学生会員，賛助会員，購読会員および名誉会員とする．

第7条　正会員は，本会の目的に賛同し，環境変異原の研究に必要な知識と経験を有し，定められた会費を納入した者とする．

第8条　学生会員は，大学または大学院等に在籍し，毎年所定の手続きを経て，定められた会費を納入した者とする．

第9条　賛助会員は，本会の目的に賛同し，本会の事業を後援するために，定められた会費を納入した個人または法人とする．

第10条　購読会員は，学会誌「環境変異原研究」および会報「Jems News」の購読のみを行う個人または法人とする．

第11条　名誉会員は，変異原の研究または本会の発展に特に功績のあった者で，理事会が推薦し，評議員会の承認を得た者とする．また，名誉会員は会費の納入を免除される．

第12条　本会に入会を希望するものは，正会員1名の推薦書付きの所定の申込書に記入の上，年会費の納入とともに，本会事務所に申込むものとする．正式の入会の可否は，理事会および評議員会において決定する．

第13条　会員は毎年年会費を納入しなければならない．次年度の年会費の額は理事会において審議し，評議員会において定める．

第14条　会員は次の事由によって会員，役員および評議員の資格を喪失する．

1. 退会の届け出をしたとき．
2. 会費を滞納し，かつ催促に応じないとき．
3. 死亡，または法人が解散したとき．
4. 本会の名誉及び信用を甚だしく傷つけ，あるいは本会則に違反し，評議員会で除名の決議がなされたとき．

第5章　役員および評議員

第15条　本会には次の役員（理事および監事）および評議員を置く．

1. 理事11名（うち，会長1名，会長指名理事2名）
2. 監事2名
3. 評議員40名以上

第16条　評議員のうち30名は正会員の選挙により，正会員から選出する．それ以外の評議員（推薦評議員と称す）は，正会員3名以上，評議員，または理事の推薦により，理事会で正会員歴ならび

に学会活動歴を審査の上，評議員会で承認を得て選出される．

第17条　理事のうち9名および監事2名は選挙で選出された評議員（推薦評議員は除く）の選挙により，正会員から選出する．会長は理事（会長指名理事）2名を指名する．

第18条　会長は選出された理事9名の互選によって定める．

第19条　会長は本会を代表し，会務を掌握し，理事会，評議員会および総会を招集する．また，評議員会および総会において主たる会務について報告をしなければならない．

第20条　会長および理事は理事会を構成し，会務を執行する．会務執行のために理事会には，総務，会計，広報，国際協力，企画，編集，表彰人事，および書記担当理事を置く．

第21条　監事は本会の財産の状況，および理事の業務執行の状況を監査し，不整の廉あることを発見したときにはこれを評議員会および総会において報告する．また，監事は理事会，および評議員会に出席して意見を述べることができる．ただし，理事，各種委員会委員を兼ねることはできない．

第22条　評議員は評議員会を構成し，会務を審議する．

第23条　役員および評議員の任期は選出された年の翌年の1月1日から2年間とする．ただし，補欠または増員により選任された役員および評議員の任期は，補欠の場合は前任者の残任期間とし，増員の場合は現任者の残任期間とする．

第24条　役員および評議員は，再任されることができる．ただし，会長は原則として1期をもって限度とする．また，選挙で，得票数上位9名に入った前理事は5名以内まで，第25条の限度内で再任することができる．

第25条　理事および監事は連続2期，生涯4期をもって限度とする．ただし，会長指名理事は生涯1期しか指名されず，この1期は生涯4期に含まれる．

第26条　会長は必要に応じ，理事会の承認を得て，会長または担当理事を含む委員会を設けることができる．委員は理事会の承認を得て会長が委嘱する．委員の任期は会長の任期に合わせて2年とし，再任は妨げない．委員会委員長には，会長が就任するか，または会長が担当理事か適当な委員に委嘱する．

第27条　大会会長は理事会の推薦に基づき評議員会の承認を得て選出される．

第28条　大会会長は大会を主宰し，総会の議長となる．

第6章　会　　議

第29条　本会の会議は，総会，評議員会，および理事会とする．

第30条　総会は，正会員をもって構成し，大会開催時に年1回開催される．

第31条　総会において会則の改廃制定，予算・決算の承認，その他評議員会において審議した重要事項の承認を行う．

第32条　評議員会は原則として年2回開催する．評議員会（臨時評議員会を含む）は評議員総数の過半数（委任状を含む）をもって成立し，出席者の過半数の賛否をもって議決する．評議員会の議長は，会長または会長が指名した者が務める．理事は評議員会に出席できるが，議決には参加できない．

第33条　会長は総数の1/3以上の評議員の要請があるときは臨時評議員会を開催しなければならない．

第34条　理事会は理事の過半数の出席をもって成立し，出席者の過半数の賛否をもって議決する．

第7章　会　　務

第35条　総務担当理事の担当事項は次の通りとする．

1. 会員の入退会に関すること．
2. 会則等制度，規則に関すること．
3. 総会，評議員会，および理事会に関すること．
4. 役員および評議員の選挙に関すること．
5. 事務所との連絡．
6. 研究会等関連事業全般にわたること．
7. 関係委員会に関すること．
8. その他，他の理事担当事項に入らない事項．

第36条　会計担当理事の担当事項は次の通りとする．

1. 予算，決算に関すること．
2. 旅費の算出．
3. Mutation Research 等英文誌購入，配布に関すること．
4. 関係委員会に関すること．

第37条　広報担当理事の担当事項は次の通りとする．

1. インターネット「ホームページ」の開設等広報に関すること．
2. 各種団体との連絡調整に関すること．
3. 学会誌の広告に関すること．
4. 名簿の作成，配布に関すること．
5. 会員数の増強に関すること．
6. 関係委員会に関すること．

第38条　国際協力担当理事の担当事項は次の通りとする．

1. 国際環境変異原学会連合および国際会議事務局との連絡に関すること．
2. 関係委員会に関すること．

第39条　企画担当理事の担当事項は次の通りとする．

1. 公開シンポジウムの企画，開催に関すること．
2. 本会の事業全般の企画に関すること．
3. 関係委員会に関すること．

第40条　編集担当理事の担当事項は次の通りとする．

1. 学会誌等の企画，編集，出版および配布に関すること．
2. 著作権に関すること．
3. 関係委員会に関すること．

第41条　表彰人事担当理事の担当事項は次の通りとする．

1. 学会賞等および学会の推薦を必要とする研究助成金または賞等の候補者の公募，審査，選考，推薦に関すること．
2. 名誉会員の選考，その他表彰に関すること．
3. 関係委員会に関すること．

第42条　書記担当理事の担当事項は次の通りとする．

1. 理事会の議事を記録し，会長および理事の承認後，公表すること．
2. 評議員会および総会の議事を記録し，公表すること．
3. 関係委員会に関すること．

第8章　会　　計

第43条　本会の会計年度は1月1日に始まり，12月31日に終わる．

第44条　本会の経費は，本会会費，各種補助金，寄付金，事業に伴う収入，財産から生ずる収入等をもって充てる．

第45条　収支の予算および決算は，評議員会および総会の承認を得なければならない．

付　　則

1. 本会則は平成12年1月1日より施行した．
2. 第3章第5条の3，第41条の1については，平成13年10月21日に改定した．
3. 正会員，学生会員，賛助会員，および購読会員の会費は，それぞれ年額7,000円，5,000円，50,000円，および10,000円とする．ただし，Mutation Research等の英文誌の配布を希望するものは，会費の他に別途定める購読料を本会へ前納するものとする．

日本環境変異原学会細則

第1章　総　　則

第1条　日本環境変異原学会細則（以下細則という）は日本環境変異原学会会則（以下会則という）の目的を遂行するために必要な細目を定める．

第2条　細則の改廃制定は理事会で審議・議決し，評議員会の承認を得るものとする．

第2章　会員資格の喪失

第3条　会則第14条第2項は下記のように適用する．会費を2年間滞納した会員は学会誌等を受け取る資格を失い，3年間未納の場合は会員資格を喪失する．

第3章　選　　挙

第4条　会則に基づく選挙に関する事務は，会則第26条によって決められた選挙管理委員会委員（総務担当理事を含む）4名が行う．開票には当該委員3名以上と監事1名以上が立ち会う．

第5条　まず評議員の選挙を行い，30名を選出し，新評議員によって原則として先ず理事を選出し，次に監事を選出する．

第6条　会長，理事，監事には会則第24，25条により就任任期数に制限があるが，任期途中に，やむをえず辞任したり，補充就任した場合，その期間は1期と計算しない．

第4章　評議員の選出

第7条　評議員の選挙に関する事務は選挙管理委員会が行う．

第8条　選挙管理委員会は選挙人（正会員）名簿および被選挙人（正会員）名簿を作成し，公表しなければならない．

第9条　投票は，被選挙人の中から6名，または6名以下を連記し，無記名小封筒に入れ，さらに記名した封筒で郵送することによって行う．

第10条　当選は得票数順に30名とし，得票同数のときは選挙管理委員による抽選により決定する．

第11条　当選者はやむをえない理由のある場合，選挙管理委員会宛にその旨を書面に付して提出し，辞退することができる．辞退の申し出は告示を受けてから1週間以内にしなければならない．

第12条　当選者が辞退した場合は次点者を繰り上げて当選とする．

第13条　選挙で選出された評議員の定数に欠員が生じた場合には任期途中に補充はしない．ただし，推薦評議員を加えて合計40名未満になったときには，推薦評議員を追加することにより40名以上にする．

第5章　理事の選出

第14条　理事の選挙に関する事務は選挙管理委員会が行う．

第15条　投票は，選挙で選出された30名の評議員が，正会員の中から3名連記無記名で行う．

第16条　当選は得票数順に9名とし，得票同数のときは選挙管理委員による抽選により決定する．

第17条　当選者が辞退した場合は次点者を繰り上げて当選とする．次点者が2名以上のときは選挙管理委員による抽選により決定する．

第18条　理事の定数に欠員が生じた場合には，会長の指名により補充する．

第6章　監事の選出

第19条　監事の選挙に関する事務は選挙管理委員会が行う．

第20条　投票は，選挙で選出された30名の評議員が，正会員の中から単記無記名で行う．

第21条　当選は得票数順に2名とし，得票同数のときは選挙管理委員による抽選により決定する．

第22条　当選者が辞退した場合は次点者を繰り上げて当選とする．

第23条　監事の定数に欠員が生じた場合には，評議員による投票により補充する．

第7章　会長の選出

第24条　会長は選出された理事9名の互選によって決定するが，原則として，単記無記名の投票によって過半数を得たものが就任する．過半数を得たものがいない場合には上位2名の決選投票を行い，最高得票者が就任する．ただし，上位3名以上が同数の場合は，同数得票者の投票を繰り返し，過半数得票者が出れば決定とするが，過半数を得たものがいない場合には上位2名の決選投票を行う．

第8章　学会賞等の規定

第25条　日本環境変異原学会学会賞

1. 日本環境変異原学会学会賞(以下，学会賞と略)は，学会員のうち，環境変異原研究分野における業績がきわめて顕著であり，かつ本学会の進歩発展に多大な寄与をした者(原則として個人)に対して授与される．
2. 学会賞受賞候補者推薦募集要項は，会報誌（Jems News）に掲載するものとし，学会正会員によって推薦された受賞候補者は，学会所定の推薦申請書用紙を使用し，必要事項を記入の上，推薦者を経て指定期日以内に学会長宛申請するものとする．
3. 学会賞受賞候補者は，5年以上の会員歴を持つ学会員に限られる．
4. 学会賞の授賞件数は，毎年1件以内とし，選考に関しては表彰人事委員会がこれにあたり，学会賞の選考の結果に関しては，理事会と評議員会の承認を得るものとする．
5. 受賞者には賞状ならびに副賞を贈呈し，総会の席上で表彰するものとする．

第26条　日本環境変異原学会研究奨励賞

1. 日本環境変異原学会研究奨励賞(以下，研究奨励賞と略)は，学会員のうち，環境変異原研究分野において顕著な寄与をする発表を行い，かつ将来の研究の発展を期待し得る者(原則として個人)に対して授与される．
2. 研究奨励賞受賞候補者推薦募集要項は，会報誌（Jems News）に掲載するものとし，学会正会員によって推薦された候補者は，学会所定の申請用紙(推薦書ならびに申請書)を使用し，必要事項を記入のうえ，推薦者を経て指定期日以内に学会長宛申請するものとする．
3. 研究奨励賞受賞候補者は，3年以上の会員歴を持ち，かつ募集締め切り日において，満45才以下の学会員に限られる．
4. 研究奨励賞の授賞件数は，毎年2件程度とし，選考に関しては表彰人事委員会がこれにあたり，研究奨励賞の選考の結果に関しては，理事会と評議員会の承認を得るものとする．
5. 受賞者には賞状ならびに副賞を贈呈し，総

会の席上で表彰するものとする．

第27条　日本環境変異原学会功労賞

1. 日本環境変異原学会功労賞（以下，功労賞と略）は，学会員のうち，環境変異原研究分野における応用研究，変異原研究を通じた社会貢献および学会の運営への寄与などを通じ学会の進歩発展に対する総合的な貢献が顕著なもの（原則として個人）に対して授与される．
2. 功労賞受賞候補者推薦募集要項は，会報誌（Jems News）に掲載するものとし，学会正会員によって推薦された候補者は，学会所定の申請用紙(推薦書ならびに申請書)を使用し，必要事項を記入のうえ，推薦者を経て指定期日以内に学会長宛申請するものとする．
3. 功労賞受賞候補者は，10年以上の会員歴を持つ学会員に限られる．
4. 功労賞の授賞件数は，毎年1件程度とし，選考に関しては表彰人事委員会がこれにあたり，功労賞の選考の結果に関しては，理事会と評議員会の承認を得るものとする．
5. 受賞者には賞状ならびに副賞を贈呈し，総会の席上で表彰するものとする．

第9章　委員会の運営

第28条　委員および委員長は会則第26条によって会長が委嘱する．委員会委員長は委員会を招集，主催する．

第29条　委員長は委員会開催通知を委員全員，会長，総務担当理事，および会計担当理事に送付する．

第30条　委員長は委員会開催に必要な最小の経費を会計担当理事に要求することができるが，その採否は会計担当理事により，本学会の予算の範囲内とする．

第10章　表彰人事委員会の運営

第31条　委員会は下記の事項につき担当する．

1. 学会賞等，他団体の助成金または賞等，名誉会員の推薦に関する書類の提出日を定め，編集委員会委員長にJems Newsへの掲載を依頼する．
2. 表彰人事委員会委員に関係書類を配布する．
3. 表彰人事委員会を開催し受賞者を選考する．
4. 選考結果を評議員会・理事会へ報告し承認を得る．

第32条　表彰人事委員会委員長は原則として表彰人事担当理事が就任し，表彰人事委員会を召集，主催する．表彰人事担当理事を除いた委員は6名とする．

第33条　委員会は委員長を含む委員5名以上の出席によって成立する．

第34条　委員会における議決

1. 重要とみなされる審議事項については，無記名投票により賛否を問うものとする．
2. 投票による審議事項は，出席員の過半数の賛成により可決される．但し，この際白票は投票総数に参入しない．
3. 委員会に出席できない委員は，書面を以て審議事項に対し意見を述べることができるが，投票に参加することはできない．
4. 会長は本委員会に出席し，意見を述べることができるが，議決には参加できない．

第35条　学会賞等受賞者の選考

本委員会委員は学会賞等の推薦者となることはできず，受賞候補者として推薦された場合は，当該賞の選考に参加することはできない．学会賞，研究奨励賞，功労賞候補者の会員歴の適合性を確認した後に，投票による最終選考に先立って，各候補者につき，予め配布された資料に基づき，下記に従い研究内容等に関する討議を行う．

1. 学会賞受賞候補者について
   研究の独創性
   研究の高度性
   研究の普遍性
   学会の進歩発展に対する貢献
2. 研究奨励賞受賞候補者について
   研究の独創性
   研究の高度性
   研究の将来性
   学会における活動状況
3. 功労賞受賞候補者について
   研究の応用性
   学会における研究発表などの活動状況
   変異原研究を通じた社会貢献
   学会に対する貢献（評議員・理事・年会長などとしての貢献）

第36条　学会賞等受賞者の選出

学会賞等の受賞候補者について，「信任投票」(可または否を記入)を行い，有効投票数の過半数を獲得したものを信任された候補者とみな

し，これらの者についてのみ以下の手続きに従って受賞者を決定する．

1. 学会賞，功労賞

(1) 信任された候補者が1名の場合，この者を受賞者とする．

(2) 信任された候補者が2名の場合，「単記名方式投票」による上位得票者を受賞者とする．

(3) 候補者が3名以上の場合

「序列記入方式投票」または，これと「単記名方式投票」の併用により受賞者を下記の方法に従い選出する．

「序列記入方式投票」においては，候補者名を連記した投票用紙の各候補者に対して序列を記入する．但し，複数の候補者に対して同一序列を記入してはならない．序列は点数化し，例えば候補者が3名のとき，最高序列点を3，次いで2，1点とし，有効票について，各候補者の得点を集計する．

「序列記入方式投票」において，最多序列得点者が1名の場合，この者を受賞者とする．

最多序列得点者が2名の場合，これらの者について「単記名方式投票」を行い，上位得点者を受賞者とする．

2. 研究奨励賞

(1) 信任された候補者が2名以下の場合，この者またはこれらの者を受賞者とする．

(2) 信任された候補者が3名以上の場合

「序列記入方式投票」を基本とする下記の方法に従い選出する．

最多序列得点者が1名，および2位の得点者が1名の場合，または最多序列得点者が2名の場合，これらの者を受賞者とする．

最多序列得点者が1名で，2位の者が2名の場合，最多序列得点者を受賞者とし，これら2位の者に対しては，「単記名方式投票」を行い，上位者を受賞者とする．

第37条　学会賞等の英文名は学会賞をJEMS Award，研究奨励賞をJEMS Achievement Award，功労賞JEMS Service Awardとする．必要に応じ，JEMSのあとに（Japanese Environmental Mutagen Society），またAwardのあとに（西暦年号）を付すことができる．

第11章　編集委員会の運営

第38条　編集委員会委員長は原則として編集担当理事が就任し，編集委員会を召集，主催する．

第39条　編集委員会は委員長および6名の委員より構成される．委員は委員長の意見を参考にし，理事会の承認を得て会長が委嘱する．委員の任期は1期2年とし，原則として連続2期とする．

付　則

1. 本細則の第4−7章は新会則のもとでの選挙のため平成11年6月1日より施行した．
2. 第1章，第3章，第9章，第11章は平成12年1月1日より施行した．
3. 第2章，第8章，第10章は平成14年1月1日より施行した．

日本環境変異原学会

役員名簿（平成14年度）

会　長	林　　真	
理　事		
総務担当	田中憲穂	
会計担当	若林敬二	
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企画担当	木苗直秀	
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	林　　真	
	兵庫淳志	
	森田　健	
	若林敬二	
	田辺健一郎	

評議員名簿（平成14～15年度）

赤沼三恵	荒木明宏	宇野芳文
大澤浩一	太田敏博	大塚雅則
大西克成	葛西　宏	鎌滝哲也
川西正祐	木苗直秀	後藤純雄
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能美健彦	林　　真	早津彦哉
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松元郷六	宮川　誠	望月正隆
森　秀樹	森　幸雄	森田　健
矢嶋信浩	山添　康	吉川邦衛
若田明裕	若林敬二	

（五十音順）

入会手続きのご案内

入会申込書に必要事項をご記入の上，年会費とともに現金書留にて事務局宛てお送りください．

年 会 費　7,000円（正会員）
　　5,000円（学生会員）
事業年度　（1月1日～12月31日）

（お申し込みについて）

1. 入会申込書に必要事項を楷書でご記入の上，年会費とともに現金書留にて事務局までお送りください．
2. 正会員は評議員の推薦を必要とします．推薦者がおられない場合は，事務局にご相談ください．
3. 学生会員は指導教官の推薦と在学証明書を必要とします．翌年の3月31日まで会員として登録されます．
4. 年度末に入会申し込みをされる場合で，翌年度から入会希望の場合は，その旨お知らせください．
5. 学会誌は入会後に発行した号からお送りしますが，当該年度で入会前の学会誌をご希望の場合は事務局までご連絡ください．
6. 住所変更（学会誌送付先の変更）の際は，会員番号（学会誌等送付する際，宛名ラベルに印刷されています），氏名，新・旧住所をご記入の上，事務局宛，書面にてご連絡ください．

日本環境変異原学会
〒170-0003　東京都豊島区駒込1-43-9
駒込TSビル3F　財団法人　口腔保健協会内
電話　03-3947-8891（代）　FAX　03-3947-8341

日本環境変異原学会入会申込書

年　　月　　日

日本環境変異原学会長　殿

貴学会に入会いたしたく評議員の署名を添えて申し込みます．

フリガナ：
氏　　名：　　㊞
Name（ローマ字つづり）
生年月日（性別）　　年　月　日　　（男・女）
所属機関名：
住　　所：〒
TEL：　　FAX：
電子メイル：
Affiliation Address Belong
自宅住所： 電　　話：
Home address
学会誌送付先：　　①所属機関　　②自　宅
学　　位：　　年取得
研究領域（複数可）
加入学会名：

＿＿＿＿＿の本学会への入会を推薦致します．

日本環境変異原学会評議員

（署名）　　印

日付

入会申込書の送付先：〒170-0003　東京都豊島区駒込1-43-9　駒込TSビル
㈶口腔保健協会内　日本環境変異原学会事務局
TEL 03-3947-8891　FAX 03-3947-8341

キリトリ線

日本環境変異原学会 学生会員申込書

［1年間（翌年の3月31日まで）のみ有効です］

年　　月　　日

日本環境変異原学会長　殿

貴学会に学生会員として入会いたしたく貴学会員である指導教官の署名および在学証明書（裏面に添付）を添えて申し込みます．

フリガナ：
氏　　名：　　㊞
Name（ローマ字つづり）
生年月日（性別）　　19　年　月　日　　（男・女）
校名／学部：
住　　所：〒
TEL：　　FAX：
電子メイル：
Affiliation Address Belong
自宅住所： 電　　話：
Home address
学会誌送付先：　　①大　学　　②自　宅
研究領域（複数可）
指導教官名： 連　絡　先：

＿＿＿＿＿の本学会への学生会員としての入会を推薦致します．

指導教官

（署名）　　印

日付

入会申込書の送付先：〒170-0003　東京都豊島区駒込1-43-9　駒込TSビル
㈶口腔保健協会内　日本環境変異原学会事務局
TEL03-3947-8891　FAX03-3947-8341

キリトリ線

環境変異原研究 投稿規定

1. 掲載論文

環境変異原研究に関する未発表の「総説」,「一般論文」,「短報」, および「特別企画（受賞講演）」,「論説」,「資料・情報」などを掲載する．なお，投稿論文の採否は編集委員会の審査により決定する．

「総説」 は，一つのテーマに関連する多くの研究論文の総括，評価，解説などである．原則として編集委員会より寄稿を依頼する．

「一般論文」 は，変異原に関する独創的研究の原著論文で，それ自身独立して価値ある結論あるいは事実を含むものとする．陰性データも受け付ける．

「短報」 は，新しい技術の紹介や価値あるデータを含む短い報告とする．陰性データも受け付ける．

「論説」 は，一つのテーマに関連する多くの研究論文の総括，評価，解説などで，会員からの投稿によるものとする．

「資料・情報」は，環境変異原に関する調査の結果などをまとめたもの，および公開シンポジウム，研究会の要旨などとする．

（Letter to Editor も受け付ける．）

2. 投稿資格

共著者のうちの 1 人は日本環境変異原学会会員でなければならない．ただし，招待寄稿の場合にはこの限りではない．

3. 論文原稿の書き方

論文原稿の用語は英語または日本語とし，最新の執筆規定に従い簡潔にわかりやすく書く．総説，一般論文，論説は，写真・図表を含めて刷り上がり 8 頁以内．短報は 4 頁以内とする．ただし規定の頁数を超えることが明らかな場合は，事前に編集委員に相談する．

4. 論文原稿の送付

論文原稿は 5 部と本誌に綴じ込みの「投稿論文チェックリスト」を，下記に（簡易）書留便または宅配便で送付する．

〒 170‐0003
東京都豊島区駒込 1‐43‐9　駒込 TS ビル
インテルナ出版
日本環境変異原学会誌編集係
Tel．03‐3944‐2591
Fax．03‐3947‐8073

5. 著作権

本誌に掲載された記事，論文などの著作権は日本環境変異原学会に帰属するものとする．従って，本会が必要と認めた場合は転載し，また外部から引用の申請があった場合には，編集委員会において検討の上許可することがある．ただし，著作者自身が自分の記事，論文などの一部の複製，翻訳などの形で利用することを妨げるものではない．しかし，著作者自身であっても，全文を複製の形で他の著作物に利用する場合には，事前に文書にて学会長に申し出を行い，許諾を求めなければならない．

6. 校正

著者校正は原則として原稿に対する誤植の訂正に限る．原稿にない加筆・変更はしないこと．

7. 著者負担金

1） 投稿料は無料とする．ただし頁数の超過分や多額の経費を要する図表の実費は著者負担とする場合がある．

2） カラー印刷等の特殊印刷のため付加的に発生する費用は著者負担とする．

3） 別刷りはすべて著者負担とする．別刷り希望者は著者校正時に添付する申込書に 50 部単位で申し込む．

編集委員会からのお願い

「環境変異原研究 Environmental Mutagen Research」への投稿論文の，投稿から印刷までの時間の短縮を目指し，投稿論文の査読を評議員にお願いすることになりました．それに伴い，投稿規定の見直しも行い，今後は年大会での発表と同様に，投稿論文の分野を著者に申告していただき，同一分野を登録していただいている評議員に査読していただくことになります．**今回投稿時のチェックリストをお届けしますので，投稿論文と一緒に必ずお送り下さい．**

現在 7 名の編集委員が分担して学会誌，*Jems News* の編集作業を行っていますが，それぞれ自分の仕事を抱えボランティアとして活動しています．そこで，会員の皆様の積極的な雑誌作りへの協力として，年大会で発表されたものを一報でも多く論文にまとめ，本学会誌に投稿していただきますようお願い致します．また，*Jems News* への投稿，情報，意見，公募等もお願いします．

本誌の将来像に関する議論を進めております．英文誌化の話も出ており，編集委員を中心に検討を始めたところですが，皆様のご意見を是非お聞かせいただきたく思います．どんな些細なことでも結構ですから，学会誌に関することでコメント，提案等がありましたら，編集委員まで E‐mail にてお知らせください．編集委員一同

環境変異原研究 執筆規定

1. 用語は日本語または英語とする．
2. 原稿は原則としてワープロを用いて作成する．
   日本文の原稿：
   原稿は A4 判用紙に 1 行約 40 字，1 頁 30 ～ 31 行で印字する（刷り上がりの約 1/2 頁に相当する）．ただし，要約は英文（300 語以内）とする．また，別に題名，著者名（フルネーム），所属機関名ならびに所在地を英文で付ける．
   英文の原稿：
   原稿は A4 判用紙にダブルスペースで印字（12 ポイント）する．1 頁 25 ～ 27 行を標準とする．原稿は著者の責任において英語の添削訂正を受けたものに限る．
3. 論文の記述は，第 1 頁は表題，著者名，所属および所在地，第 2 頁は英文の要約（Summary）およびキーワード（英文 5 語以内，固有名詞や遺伝子名などで大文字の使用が必要な場合を除き，原則として小文字表記とする），第 3 頁以下，緒言（Introduction），実験材料および方法（Materials and Methods），結果（Results），考察（Discussion）または結果および考察，結語（Conclusion），謝辞（Acknowledgments），参考文献（References），表，図の説明および図の順序とする．ただし，総説の記述は，第 3 頁以下，緒言，1. ……，2. ……，結語，謝辞，参考文献，表，図の説明および図の順序とする．なお図と表の説明文はすべて英文とする．
4. 学名，遺伝子記号などはイタリックとし，その他まぎらわしい記号については原稿に適宜指示を与える．
5. 化学物質名は原則として英語とし，一般名を用いる．また，化学物質の CAS 登録番号を記載する．文中に用いる英語の単語あるいは句は固有名詞を除いて小文字で書きはじめる（文頭の場合は大文字）．
6. 数字は算用数字を用い，単位は慣用による省略記号を用いる．
7. 略語を使用するときは，論文中にはじめて使用するときに完全な語とその略語を括弧内に示す．
8. 句読点はカンマ（,）およびピリオド（.）とする．
9. 表，図は本文と別にし，それらの挿入箇所を本文の右余白に明示する．グラフ，写真，線画等はすべて図とし，一連の番号 Fig. 1, 2, …を付し，説明文（英文）を別紙に添える．
10. 図と写真は原図またはキャビネ大の光沢写真版とし，裏面に Fig. 1, 2, …および上下を鉛筆書きする．
11. 表は上部に一連の番号 Table 1, 2, …と英文の表題を記入する（文頭のみ大文字）．脚注を要するときに表示の語句の右肩に a，b，c，…を付記し，表の下欄外にそれぞれの説明を記す．
12. 本文中の文献引用は著者名および年号をもってする．
13. 参考文献は筆頭著者名のアルファベット順に配列し，雑誌の省略名は Chemical Abstracts の記載方法に従う．記載順序は著者氏名，（年），題名，雑誌名，巻，頁（単行本の一部引用の場合は著者氏名，（年），題名，編者名，書名，発行所，発行地，頁）の順とする．単行本そのものを引用する場合は，編者名あるいは著者名，（年），書名，発行所，発行地の順とする．文献の記載方法は下記の例に従う．

Ames, B.N., J. MaCann and E. Yamasaki（1975）Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutat. Res., 31, 347-364.

Ashby, J., F.J. de Serres, M. Draper, M. Ishidate Jr., B.H. Margolin, B. Matter and M.D. Shelby（1985）Overview and conclusion of the IPCS collaborative study on in vitro assay systems, In ： J. Ashby, F.J. de Serres et al.（Eds）, Evaluation of Short-Term Tests for Carcinogens, Elsevier, Amsterdam, pp. 117-174.

Friedberg, E.C., G.C. Walker and W. Siede（1995）DNA Repair and Mutagenesis, ASM Press, Washington, D.C.

藤川和男，梁　治子，近藤宗平（1984）ハエの翅毛スポットテスト―近ごろ注目されている短期試験法，環境変異原研究，6，107-113.

佐々木正夫（1983）環境変異原と染色体異常，外村晶（編），染色体異常，朝倉書店，東京，pp. 107-113.

松影昭夫（編）（1996）DNA 複製，修復と発癌，羊土社，東京．

（改訂　2000 年 12 月）

投稿論文チェックリスト

論文ご投稿の際，この用紙に必要事項をご記入のうえ，添付してください．

論文名（日本語）：
（英　語）：

窓口著者名：

連絡先（住所）

（TEL）
（FAX）
（E-mail）

分類（該当するものに印をつけてください）

- □ 突然変異のメカニズム
- □ 変異原の検出
- □ 環境汚染物質
- □ 抗変異原
- □ DNA 損傷（付加体）
- □ 変異原の代謝
- □ 変異原の修飾，発現
- □ 試験法の開発，改良
- □ その他（　　）

送付前のチェック項目

- □ 投稿規定，執筆規定に沿っていますか（特に引用文献）
- □ 英文校閲はお済みですか
- □ 投稿論文は 5 部ありますか

キリトリ線

編集後記

今さらのようですが，環境変異原学会の編集委員は専属ではありません．本業の傍ら，インテルナ出版の方々に気を揉んでいただきながらの作業です．いつも感心するのは，インテルナの方が連絡を下さるタイミングです．ぎりぎりまで待って，かつ，言われてから手をつけても間に合う…そんな時期です．今号に「特別企画」と銘打って登場した8thICEMの記事はJems Newsから移ってきたものです．どうぞお楽しみください．各編集委員のノルマはJems Newsと環境変異原研究各2号です．今号は私の記念すべき卒業作品となりました．雑誌編集に興味のある方，私の任期満了の折にはぜひ！交代してください．(M.Y.)

担当編集委員　山田　雅巳

編　集　委　員

評　議　員

環境変異原研究　第24巻　第1号　2002年

平成14年5月24日　印　刷
平成14年5月31日　発　行

発　行　者　　日本環境変異原学会
発行責任者　　林　　真
製　　作　　インテルナ出版

環境変異原研究
Environmental Mutagen Research Vol. 24 No. 1 2002

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入会申込書
学生会員申込書

環境変異原研究　投稿規定
執筆規定