J Pharmacol Sci **97**, 146 – 152 (2005)

Journal of Pharmacological Sciences

Full Paper

Ability of Fourteen Chemical Agents Used in Dental Practice to Induce Chromosome Aberrations in Syrian Hamster Embryo Cells

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Received September 24, 2004; Accepted November 25, 2004

Abstract. To assess the genotoxicity of 14 chemical agents used in dental practice, the ability of these agents to induce chromosome aberrations was examined using Syrian hamster embryo (SHE) cells. Statistically significant increases in the frequencies of chromosome aberrations were induced in SHE cells treated with 7 of 10 chemical agents used as endodontic medicaments, that is, carbol camphor, *m*-cresol, eugenol, guaiacol, zinc oxide, hydrogen peroxide, and formaldehyde. The other 3 chemical agents, that is, thymol, glutaraldehyde, and iodoform, did not increase the levels of chromosome aberrations. Of the 4 chemical agents that are used as an antiseptic on the oral mucosa, chromosome aberrations were induced by iodine, but not by the other 3 antiseptics, benzalkonium chloride, benzethonium chloride, and chlorhexidine. Among the 6 chemical agents exhibiting a negative response in the assay, only thymol induced chromosome aberrations in the presence of exogenous metabolic activation. Our results indicate that chemical agents having a positive response in the present study are potentially genotoxic to mammalian cells and need to be studied further in detail.

Keywords: chromosome aberration, chemical agents used in dentistry, Syrian hamster embryo cell

Introduction

Numerous and varied chemical agents are used as topical antiseptics in dental practice. As they are administered directly to the oral cavity, these agents should have a low toxicity and high safety. One approach to the assessment of safety is to assay the genotoxicity of such agents using cultured mammalian cells. One of a battery of genotoxicity tests is chromosome aberrations which have been used as a possible screen for mutagens /carcinogens (1).

In this report, we studied the ability of 14 chemical agents used in dental practice to induce chromosome aberrations using Syrian hamster embryo (SHE) cells. SHE cells have been utilized for a number of years to screen mutagens/carcinogens and to study the mechanism of carcinogenesis (2, 3). Ten chemical agents are used as endodontic medicaments: carbol camphor, eugenol, guaiacol, and thymol are used as pulp sedatives and antiseptics for caries cavities or root canals; zinc oxide is used as a pulp capping agent and a root canal antiseptic; hydrogen peroxide is used as a root canal irrigant; *m*-cresol, formaldehyde, and glutaraldehyde are used as root canal antiseptics; and iodoform is used as a root canal filling agent. The remaining 4 chemical agents, benzalkonium chloride, benzethonium chloride, chlorhexidine, and iodine, are used as antiseptics that are applied to the oral mucosa.

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Materials and Methods

Cells and chemical agents

SHE cell cultures were grown as described previously (2, 3). Carbol camphor (CC) was prepared by mixing phenol (Wako Pure Chemical, Osaka) and DL-camphor (Kanto Chemical, Tokyo) at the molar ratio 3:1. Eugenol (>95% pure) (Tokyo Kasei Kogyo, Tokyo), thymol (>98% pure) (Iwaki Seiyaku, Tokyo), and zinc oxide

(>99% pure) (Kanto Chemical) were obtained from the indicated sources. *m*-Cresol (>98% pure), formaldehyde solution (37% formaldehyde with 7 – 13% methanol), guaiacol (>99% pure), hydrogen peroxide solution (30w/v% hydrogen peroxide), iodine (>99.9% pure), and iodoform (>97% pure) were purchased from Wako Pure Chemical. Benzalkonium chloride solution (10w/v% benzalkonium chloride) (Takeda Chemical, Osaka), benzethonium chloride solution (10w/v% benzethonium chloride) (Sankyo, Tokyo), chlorhexidine gluconate solution (chlorhexidine) (5w/v%) (Sumitomo Seiyaku, Osaka), and glutaraldehyde solution (24.8 w/v% glutaraldehyde) (Taab Lab., Reading, UK) were purchased from the indicated sources. CC was diluted with culture medium. The concentration of CC was expressed as the molar concentration of phenol in CC. Eugenol was diluted with dimethyl sulfoxide (DMSO) at 400 mM. *m*-Cresol and guaiacol were diluted with culture medium at 10 mM. Iodoform and thymol were dissolved in DMSO at 500 mM. Zinc oxide was dissolved in 0.1 N HCl at 50 mM and filter-sterilized. Formaldehyde, glutaraldehyde, and hydrogen peroxide were diluted with Ca^{2+} and Mg^{2+}-free phosphate-buffered saline [PBS(−), pH 7.4] at 300 mM and filter-sterilized. Benzalkonium chloride, benzethonium chloride, and chlorhexidine were diluted with deionized water at 10 mg/ml and filter-sterilized. Iodine was dissolved in DMSO at 60 mg/ml. All these solutions were diluted with culture medium to the desired concentrations and applied to SHE cells.

Cytotoxicity

Cytotoxicity of the chemical agents tested was determined by the colony-forming efficiencies of SHE cells treated with these agents. SHE cells (5×10^5) in tertiary culture were plated into 75-cm^2 flasks (Costar, Cambridge, MA, USA), incubated overnight, and treated with each of 14 chemical agents at varying concentrations for 24 h. After harvesting with 0.1% trypsin, the cells were replated in triplicate onto 100-mm dishes (Costar) at 2000 cells/dish and incubated for 7 days for colony formation. The relative colony-forming efficiency was expressed as the number of colonies in the treated dishes divided by the number in the control dishes × 100. Actual colony-forming efficiency of control cells was 13.0 ± 0.7% (S.D.).

Chromosome aberrations

SHE cells (5×10^5) in tertiary culture were plated into 75-cm^2 flasks, incubated overnight, and treated with each of 14 chemical agents at varying concentrations for 24 h. SHE cells have the doubling time of 16 h and the treatment time is appropriate for detecting chromosome aberrations by diverse chemical carcinogens (2, 3). The cells were harvested with 0.1% trypsin for chromosome preparation. Three hours before harvest, Colcemid (GIBCO, Grand Island, NY, USA) was administered at 0.2 μg/ml and metaphase chromosomes were prepared as described previously (2, 3). For determination of chromosome aberrations, 100 metaphases were scored per experimental group. Achromatic lesions greater than the width of the chromatid were scored as gaps unless there was displacement of the broken piece of chromatid. If there was displacement, they were scored as breaks. In some experiments, the chromosome aberration assay was carried out in the presence of exogenous metabolic activation with rat liver post-mitochondrial supernatant (PMS), as described previously (4). Cells (5×10^5) were plated on 100-mm dishes and after overnight incubation, they were treated with chemical agents for 3 h in a 5% PMS mixture. This treatment condition is optimal for inducing gene mutations in SHE cells treated with indirectly acting chemical carcinogens (4). Cells were washed twice with 5 ml PBS(−) and incubated with fresh medium for 18 h followed by chromosome preparations.

Results

The abilities of 14 chemical agents to induce chromosome aberrations in SHE cells were examined (Table 1). Cytotoxicities determined by the colony-forming efficiencies of SHE cells treated with most of these chemical agents were increased with increasing concentrations of these agents. Treatment with 7 of 10 chemical agents used as endodontic medicaments, that is, carbol camphor (CC), *m*-cresol, eugenol, formaldehyde, guaiacol, hydrogen peroxide, and zinc oxide, induced statistically significant increases in the levels of chromosome aberrations in SHE cells. The other 3 endodontic medicaments, that is, glutaraldehyde, iodoform, and thymol, failed to enhance the levels of chromosome aberrations (Table 1). Among the 4 chemical agents that are applied to the oral mucosa as antiseptics, iodine caused significant increases in the levels of chromosome aberrations. The remaining 3 chemical agents, that is, benzalkonium chloride, benzethonium chloride, and chlorhexidine, did not induce chromosome aberrations (Table 1).

The clastogenic activity of the 6 chemical agents that exhibited a negative response in the chromosome aberration assay was also examined using SHE cells in the presence of exogenous metabolic activation with a 5% rat liver PMS. This experimental condition enhanced the ability of *m*-cresol and eugenol to induce chromosome aberrations in SHE cells compared with that in the absence of exogenous metabolic activation (Tables 1

Table 1. The ability of 14 chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells

Classification	Chemical agent	Concentration (μM)	Relative colony-forming efficiency (%)	Number of metaphases scored	Type of aberrations (%)								Aberrant metaphases (%)
					CG	ICG	CB	ICB	E	O	D	F	
Endodontic medicament	Carbol camphor (CC)	0	100	100	1	0	0	0	0	0	0	0	1.0
		2100	82	100	19	3	8	0	3	0	0	0	25.0**
		4200	77	100	16	3	9	0	8	1	0	0	30.0**
	m-Cresol	0	100	100	0	0	0	0	0	0	0	0	0
		200	102	100	0	0	0	0	0	0	0	0	2.0
		400	90	100	4	2	0	0	0	0	0	0	6.0*
		800	77	83	4.8	4.8	2.4	0	0	0	0	0	10.8**
		1000	77	100	7	1	0	0	0	0	0	0	7.0*
	Eugenol	0	100	100	0	0	0	0	0	0	0	0	0
		65	82	100	2	1	1	0	1	0	0	0	4.0
		195	77	100	4	5	2	1	2	0	0	0	12.0**
		650	58	100	10	12	0	0	1	0	0	0	18.0**
	Formaldehyde	0	100	100	0	0	0	0	0	0	0	0	0
		33	94	100	5	0	1	0	0	0	0	0	6.0*
		66	94	100	4	0	1	1	1	0	0	0	6.0*
		99	85	100	21	0	55	1	53	0	0	0	71.0**
	Glutaraldehyde	0	100	100	1	0	0	0	0	0	0	0	1.0
		3	94	100	0	0	0	0	0	0	0	0	0
		10	87	100	3	0	0	0	0	0	0	0	3.0
		30	46	100	7	1	1	0	1	0	0	0	7.0
	Guaiacol	0	100	100	0	0	0	0	0	0	0	0	0
		200	90	100	12	3	0	0	0	0	0	0	15.0**
		400	83	100	19	4	5	2	8	0	0	0	34.0**
		800	74	100	30	6	16	1	29	0	0	1	64.0**
		1000	69	100	47	7	21	3	24	0	0	0	63.0**
	Hydrogen peroxide	0	100	100	2	0	0	0	0	0	0	0	2.0
		150	96	100	1	0	0	1	0	0	0	0	2.0
		300	90	100	3	1	8	9	2	0	0	0	16.0**
		450	78	53	3.8	1.9	9.4	0	5.7	0	0	0	15.1**
	Iodoform	0	100	100	0	0	0	0	0	0	0	0	0
		80	105	100	0	1	1	0	0	0	0	0	2.0
		120	94	76	2.6	0	0	0	0	0	0	1.3	4.0
		160	87	12	3.0	0	0	0	0	0	0	0	3.0
		240	8	10	0	0	0	0	0	0	0	0	0
	Thymol	0	100	100	0	0	0	0	0	0	0	0	0
		130	94	100	1	1	1	1	0	0	0	0	4.0
		260	91	100	1	1	1	0	0	0	0	0	3.0
		390	83	100	2	0	3	0	0	0	0	0	5.0
		520	8	100	Few metaphases								
	Zinc oxide	0	100	100	0	0	0	0	0	0	0	0	0
		60	99	100	4	0	0	0	0	0	0	0	4.0
		120	90	100	4	1	6	2	2	0	0	0	8.0*
		180	27	9	0	0	66.7	0	0	0	0	0	66.7**
Oral mucosal antiseptic	Benzalkonium chloride	0	100	100	0	0	0	0	0	0	0	0	0
		0.8	101	100	0	0	0	0	0	0	0	0	0
		3	98	100	0	0	0	0	0	0	0	0	0
		8	105	100	1	0	0	0	0	0	0	0	1.0
		30	ND	100	2	0	0	0	0	0	0	0	2.0
	Benzethonium chloride	0	100	100	0	0	0	0	0	0	0	0	0
		0.7	98	100	2	0	1	0	0	0	0	0	3.0
		2	108	100	1	0	0	0	0	0	0	0	1.0
		7	101	100	2	0	0	1	0	0	0	0	3.0
		20	ND	100	3	0	1	0	0	0	0	0	3.0
	Chlorhexidine	0	100	100	1	0	0	0	0	0	0	0	1.0
		0.3	98	100	2	0	0	0	0	0	0	0	2.0
		1	103	100	0	0	0	0	0	0	0	0	0
		3	104	100	0	0	0	0	0	0	0	0	0
		10	3	16	2	0	0	0	0	0	0	0	2.0
	Iodine	0	100	100	0	0	0	0	0	0	0	0	0
		400	75	100	2	3	1	1	0	0	0	0	5.0
		600	62	100	9	5	1	0	0	0	0	0	13.0**
		800	61	100	10	8	1	2	2	0	0	0	19.0**

CG, chromatid gaps; ICG, isochromatid gaps; CB, chromatid breaks; ICB, isochromatid breaks; E, exchanges; O, ring chromosomes; D, dicentric chromosomes; F, fragmentations. ND, Not done. *Significantly different from control ($P<0.05$, χ^2 test). **Significantly different from control ($P<0.01$, χ^2 test).

Table 2. The frequencies of chromosome aberrations in Syrian hamster embryo cells treated with chemical agents used in dental practice in the presence of rat liver post-mitochondrial supernatant

Chemical agent	Concentration (μM)	Number of metaphases scored	Type of aberrations (%)								Aberrant metaphases (%)
			CG	ICG	CB	ICB	E	O	D	F	
m-Cresol	0	100	0	0	0	0	0	0	0	0	0
	100	100	9	1	46	5	38	0	0	0	52.0**
	300	100	9	1	52	8	48	0	0	0	69.0**
	1000	100	18	0	60	8	62	0	0	0	77.0**
Eugenol	0	100	0	0	0	0	0	0	0	0	0
	6.5	100	1	0	4	0	3	0	0	0	5.0
	20	100	4	1	12	3	10	0	0	0	20.0**
	65	100	5	0	17	4	19	0	0	0	27.0**
Benzalkonium chloride	0	100	2	0	0	0	0	0	0	0	2.0
	0.8	100	0	1	0	0	0	0	0	0	1.0
	3	100	1	0	0	0	0	0	0	0	1.0
	8	100	1	0	0	0	0	0	0	0	1.0
	30	100	1	0	0	1	0	0	0	0	2.0
Benzethonium chloride	0	100	2	0	0	0	0	0	0	0	2.0
	0.7	100	1	0	0	0	0	0	0	0	1.0
	2	100	0	1	0	0	0	0	0	0	1.0
	7	100	1	0	0	0	0	0	0	0	1.0
	20	100	1	1	2	0	1	0	0	0	4.0
Chlorhexidine	0	100	0	0	0	0	0	0	0	0	0
	0.3	100	1	0	0	0	0	0	0	0	1.0
	1	100	0	0	0	0	0	0	0	0	0
	3	100	0	0	2	0	1	0	0	0	3.0
	10	100	1	1	3	1	1	0	0	0	5.0
Glutaraldehyde	0	100	0	0	0	1	0	0	0	0	1.0
	3	100	0	0	0	0	0	0	0	0	0
	10	100	0	0	2	0	0	0	0	0	2.0
	30	100	1	0	0	0	0	0	0	0	1.0
Iodoform	0	100	0	0	0	0	0	0	0	0	0
	8	100	1	0	1	0	0	0	0	0	2.0
	24	100	0	0	1	0	0	0	0	0	1.0
	80	100	0	0	1	1	1	0	0	0	1.0
	240	100	0	0	2	0	1	0	0	0	2.0
	800	100	0	0	2	1	0	0	0	0	2.0
Thymol	0	100	0	0	0	0	0	0	0	0	0
	130	100	2	2	1	0	2	0	0	0	6.0*
	260	100	4	1	10	0	8	0	0	0	19.0**
	390	100	5	0	13	1	8	0	0	0	17.0**

For abbreviations and marks (*,**), see the footnote of Table 1.

and 2). Among the 6 chemical agents examined, only thymol induced statistically significant increases in the levels of chromosome aberrations in this assay (Table 2).

Discussion

The ability of 14 chemical agents used in dental practice to induce chromosome aberrations in SHE cells was examined in the presence or absence of exogenous metabolic activation. Nine of the 14 chemical agents described below induced chromosome aberrations. The other 5 agents failed to induce chromosome aberrations even in the presence of exogenous metabolic activation.

1) CC induces morphological transformation, unscheduled DNA synthesis (UDS), and sister chromatid exchanges (SCEs) in SHE cells (5). As far as we know, there are no reports on the genotoxicity of camphor which is one constituent of CC. Phenol, another constituent of CC, has been reported to be noncarcinogenic (6), yet the incidences of leukemia were elevated in exposure groups of male rats treated with phenol (7). Also phenol has been tested extensively in the two-stage mouse skin model and shows consistent promoting activity (8). Cell transformation, gene mutations, chromosome aberrations, aneuploidy, SCEs, and UDS in SHE cells were induced by phenol (3), suggesting a possible involvement of phenol in the clastogenic activity of CC. Since pretreatment of mice with camphor reduces the γ-irradiation-induced SCE frequency in bone marrow cells (9), the clastogenic activity of CC may vary with the camphor content of CC.

2) *m*-Cresol treatment results in morphological transformation in SHE cells (10). This chemical agent induced chromosome aberrations in SHE cells. The inducibility of chromosome aberrations by *m*-cresol was enhanced in the presence of exogenous metabolic activation. UDS in SHE cells treated with *m*-cresol is not detected in the absence of exogenous metabolic activation, but detected in the presence of exogenous metabolic activation (11). The positive responses in both assays with SHE cells for chromosome aberrations and UDS indicate that a combination of SHE cells and rat liver post-mitochondrial supernatant may be sufficient for converting *m*-cresol to genotoxic metabolite(s), because SHE cells themselves retain endogenous metabolizing enzymes that exhibit oxidative and peroxidative activities (12). *m*-Cresol is negative in *Salmonella* mutagenicity tests with and without exogenous metabolic activation (13). Some additional factors may be necessary for metabolic activation of *m*-cresol in the bacteria system.

3) Eugenol induces chromosome aberrations in Chinese hamster lung (CHL) cells (14). Eugenol exhibits cell-transforming activity in SHE cells (5). Eugenol also induces UDS in SHE cells in the presence of exogenous metabolic activation (5). Although eugenol is negative in the *Salmonella* mutagenicity tests with or without exogenous metabolic activation (13), its metabolite, eugenol-2',3'-oxide is positive in the tests (15). In the present study, eugenol exhibited clastogenic activity in SHE cells that was enhanced in the presence of exogenous metabolic activation. Oral administration of eugenol produces liver tumors in mice (16). However, eugenol fails to give rise to DNA adducts in mouse liver as detected by ^{32}P-postlabeling (17).

4) Formaldehyde is carcinogenic to animals, mutagenic to bacteria, yeast and *Drosophila melanogaster*, and cytotoxic and clastogenic to mammalian cells and plants (18, 19). Morphological transformation and UDS are induced in SHE cells treated with formaldehyde (10, 11).

5) Guaiacol induces morphological transformation in SHE cells (10). Guaiacol also exhibits a positive response in a SHE cell UDS assay with exogenous metabolic activation (11). Guaiacol is a major constituent of creosote that induces mutations in *Salmonella typhimurium* TA 1537, TA 1538, TA 98, and TA 100 with exogenous metabolic activation (20). These findings suggest that the clastogenic activity of guaiacol found in the present study may be correlated to the DNA-damaging or mutagenic activities of creosote.

6) Hydrogen peroxide interacts with the ubiquitous tissue enzyme catalase, which converts hydrogen peroxide to water and gaseous oxygen. When hydrogen peroxide is applied to root canals to cleanse, the release of gaseous oxygen mechanically loosens and moves tissue debris in the canals. Hydrogen peroxide induces DNA damage, SCEs, and chromosome aberrations in mammalian cells (21). Cell transformation and UDS in SHE cells are also induced by hydrogen peroxide (10, 22).

7) Zinc oxide induces cell transformation, UDS, and SCEs in SHE cells (23). DNA-single strand breakage is induced in Chinese hamster V79 cells exposed to an extract of N_2® or Canals®, which are zinc-oxide eugenol-based root canal sealers (24).

8) Iodine induces morphological transformation (10) and SCEs (T. Miyachi, unpublished observations) but not UDS (22) in SHE cells. In the UDS assay, iodine was administered for 1 h. The other halogen compound, sodium fluoride, induces UDS in SHE cells when treated for more than 12 h (25), suggesting that a prolonged treatment period may be necessary for UDS experiments.

9) The clastogenic activity of thymol was observed in SHE cells with exogenous metabolic activation. Thymol is mutagenic in the Ames tests with *Salmonella typhimurium* (26). Morphological transformation, UDS, and SCEs in SHE cells are induced by thymol (5).

There are few reports on the clastogenicity of the

other 5 chemical agents which failed to induce chromosome aberrations in SHE cells. Glutaraldehyde shows a weak mutagenic response in a *Salmonella* mutagenicity test with TA 100 in the presence of metabolic activation (27), but is negative for chromosome aberrations, gene mutation at the *hprt* locus, and SCEs in CHO cells (27). Iodoform is positive for *Salmonella* mutagenicity (13), but no evidence has been reported on the clastogenic activity of iodoform.

The 9 chemical agents that induced chromosome aberrations in the present study were positive in the SHE cell transformation assay (5, 10, 23). In addition, the other 5 chemical agents that failed to induce chromosome aberrations were negative in the cell transformation assay, except for iodoform (5, 10, 23), indicating that the clastogenicity of these chemical agents correlates well with their transforming potential in SHE cells. Since the SHE cell transformation system has significant potential to predict the carcinogenicity of chemicals (28), the tested chemical agents with clastogenic activity are potentially carcinogenic. Other genotoxicity tests remain to be done to clarify the mutagenicity and carcinogenicity of these chemical agents used in dental practice.

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