Assessment of Genotoxicity of Dental Antiseptics: Ability of Phenol, Guaiacol, *p*-Phenolsulfonic Acid, Sodium Hypochlorite, *p*-Chlorophenol, *m*-Cresol or Formaldehyde to Induce Unscheduled DNA Synthesis in Cultured Syrian Hamster Embryo Cells

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ABSTRACT—To assess the genotoxicity of seven dental antiseptics, the ability of these agents to induce unscheduled DNA synthesis (UDS) was examined using Syrian hamster embryo (SHE) cells. Treatment of SHE cells with phenol or formaldehyde induced UDS in a concentration-dependent manner as determined by direct scintillation counting of [³H]thymidine incorporated into DNA during repair synthesis. Guaiacol or *m*-cresol induced UDS only in the presence of exogenous metabolic activation. *p*-Phenolsulfonic acid, sodium hypochlorite and *p*-chlorophenol failed to induce UDS in the presence or absence of exogenous metabolic activation. Our results suggest that phenol, guaiacol, *m*-cresol and formaldehyde are genotoxic to mammalian cells.

Keywords: Dental antiseptic, Genotoxicity, Mammalian cell

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 quantitatively assay the genotoxicity of such agents using cultured mammalian cells. Unscheduled DNA synthesis (UDS) is one of a battery of genotoxicity tests for detecting the ability of a chemical to damage DNA and to induce DNA-repair synthesis. This assay has been used to identify DNA-damaging agents as a possible screen for mutagens/carcinogens (1). There are many UDS assay systems using cultured mammalian cells. It is, however, important to use an assay system that is able to concomitantly study the *in vitro* carcinogenic activity and genotoxicity of a chemical agent to understand the mechanism of carcinogenesis of the agent. In this report, we quantitatively assessed the genotoxicity of 7 dental antiseptics used mainly in endodontics, employing the Syrian hamster embryo (SHE) cell UDS assay system described previously (2). SHE cells in culture have been utilized for a number of years to study chemical carcinogen-induced neoplastic transformation (3, 4). This system is an important assay for carcino-

gen screening and for mechanistic studies of carcinogenesis (4, 5). By comparing the ability of a chemical to induce cellular transformation and UDS or somatic mutations in the same target cells, one can gain insight into the mechanism of action of a chemical agent (2, 4, 5).

SHE cell cultures were established from 13-day-gestation fetuses and grown as described previously (4). All procedures were done in accordance with guidelines outlined by the Institutional Animal Care and Use Committee of the Nippon Dental University School of Dentistry at Tokyo. Phenol (redistilled), guaiacol (≥99% pure), *p*-phenolsulfonic acid, *p*-chlorophenol (≥95% pure), *m*-cresol (≥98% pure) and formaldehyde solution (37% formaldehyde) were obtained from Wako Pure Chemical Industries (Osaka). Sodium hypochlorite solution (12.6% sodium hypochlorite) was purchased from Koso Chemical Co. (Tokyo). Immediately before use, all the agents were diluted with culture medium to the desired concentrations and applied to the cells. Final concentrations of the antiseptics were less than 0.3% (v/v); this concentration did not affect colony-forming efficiencies (CFEs) and UDS of SHE cells. The cytotoxicities of the agents were determined by the CFEs of SHE cells treated with the agents for 48 h (Table 1). Statistically significant decreases in the CFEs were ob-

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Table 1. Relative survival of SHE cells treated with dental antiseptics

Antiseptic	Concentration	Relative cell survival (%) ^a
None	0	100 ^b
Phenol (μ M)	1	95.2 $\pm$ 8.3
	3	90.3 $\pm$ 11.3
	10	83.8 $\pm$ 9.5
	30	78.4 $\pm$ 6.5*
	100	75.2 $\pm$ 4.1**
Guaiacol (μ M)	0.3	111.5 $\pm$ 10.9
	1	98.5 $\pm$ 8.3
	3	93.2 $\pm$ 10.5
<i>p</i> -Phenolsulfonic acid (μ M)	1	95.4 $\pm$ 6.8
	3	101.3 $\pm$ 7.5
	10	100.5 $\pm$ 4.2
Sodium hypochlorite (μ g/ml)	3	105.3 $\pm$ 6.0
	30	96.4 $\pm$ 8.6
	300	1.2 $\pm$ 1.0**
<i>p</i> -Chlorophenol (μ M)	1	95.4 $\pm$ 6.4
	3	97.1 $\pm$ 8.9
	10	101.3 $\pm$ 9.8
<i>m</i> -Cresol (μ M)	1	98.9 $\pm$ 10.7
	3	92.5 $\pm$ 10.1
	10	89.4 $\pm$ 7.7
Formaldehyde (μ g/ml)	0.3	86.6 $\pm$ 7.3
	1	81.5 $\pm$ 8.4*
	3	27.7 $\pm$ 4.1**

Cells (2.5×10^5) were plated into 75-cm² flasks, incubated overnight and treated with the agents for 48 h. Control cultures were incubated with culture medium alone. After harvesting by trypsinization, 2000 cells were replated into 100-mm dishes and incubated for 7 days to form colonies. The colonies were fixed with methanol and stained with 3% aqueous Giemsa solution. The number of colonies was then counted, and the % survival expressed as the number of colonies in the treated cultures divided by the number in the control cultures $\times$ 100. ^aMean $\pm$ S.D. of 4 dishes; ^bActual colony-forming efficiencies of control cultures were 11.3 ± 0.9 (SD)%; * $P < 0.05$, ** $P < 0.01$, as compared with the control cultures (*t*-test).

served in cells treated with phenol, sodium hypochlorite or formaldehyde, but not in cells treated with any of the other 4 antiseptics.

To measure UDS, we used the quantitative assay system as described previously (2). When SHE cells were treated with each of the 7 dental antiseptics in the absence of exogenous metabolic activation, only phenol and formaldehyde induced UDS (Table 2). Among the other 5 antiseptics exhibiting a negative response in the UDS assay, guaiacol and *m*-cresol induced significant levels of UDS in the presence of exogenous metabolic activation (Table 2). The concentrations of formaldehyde at which UDS was induced were not identical to those that induced cytotoxicity, suggesting that the UDS-inducing ability of formaldehyde is independent of the cytotoxic effect of the compound.

Phenol has been reported to be non-carcinogenic, yet the incidences of leukemia were elevated in male rats treated with phenol (7). Also, phenol has been tested extensively in the two-stage mouse skin model and shows consistent pro-

Table 2. The ability of dental antiseptics to induce unscheduled DNA synthesis (UDS) in SHE cells

Antiseptic	Concentration	Induced UDS (cpm) in the absence or presence of exogenous metabolic activation ^a	
		Absence	Presence
Phenol (μ M)	1	321.3 $\pm$ 43.0**	ND ^b
	3	503.6 $\pm$ 111.2*	ND
	10	585.2 $\pm$ 58.5**	ND
	30	678.7 $\pm$ 134.1**	ND
	100	765.0 $\pm$ 71.7**	ND
Guaiacol (μ M)	0.3	0	130.5 $\pm$ 23.4
	1	0	245.3 $\pm$ 13.3*
	3	0	397.9 $\pm$ 12.3*
<i>p</i> -Phenolsulfonic acid (μ M)	1	0	0
	3	0	0
	10	0	0
Sodium hypochlorite (μ g/ml)	3	0	0
	30	0	0
	300	0	0
<i>p</i> -Chlorophenol (μ M)	1	0	0
	3	0	0
	10	0	0
<i>m</i> -Cresol (μ M)	1	0	419.9 $\pm$ 40.4*
	3	0	689.3 $\pm$ 23.6**
	10	0	829.4 $\pm$ 112.2**
Formaldehyde (μ g/ml)	3	249.1 $\pm$ 12.4*	ND
	10	358.6 $\pm$ 18.6**	ND
	30	539.4 $\pm$ 41.7**	ND

The methods for UDS in SHE cells have been described previously (2) and are summarized briefly: cells (10^5) were plated in quadruplicate on 15-mm-diameter glass coverslips in 16-mm tissue culture cluster dishes (Coster, Cambridge, MA, USA) in complete medium. After overnight incubation, complete medium was replaced with medium containing 1% fetal bovine serum (FBS) (1% FBS medium), and then the cultures were incubated for 48 h. The cells were treated with the indicated concentrations of the agents for 1 h. When exogenous metabolic activation was used, the cultures were treated for 1 h in a reaction mixture consisting of 5% rat-postmitochondrial supernatant and other cofactors prepared by the method as described previously (6). To measure UDS, the cells were washed after treatment with 1 ml of phosphate-buffered saline containing Ca²⁺ and Mg²⁺ (pH 7.4) and 1 ml of 1% FBS medium containing 10 mM hydroxyurea (1% FBS-HU medium). Following addition of [³H]thymidine [370 kBq/ml, specific activity 1.89 TBq/mmol, Amersham International plc (Tokyo)] in 1% FBS-HU medium to the cultures, the cells were incubated for 6 h. After washing 3 times with cold trichloroacetic acid (5%), the coverslips were put into scintillation vials to determine the radioactivity in the samples. All experiments were repeated at least twice with similar results. The number of cells on a coverslip was counted using the culture cluster dishes treated with the same conditions used in the experiments for detection on UDS with [³H]thymidine. No significant difference in the number of cells was found between the control and experimental groups. ^aMean $\pm$ S.D. of 4 cultures. The background levels of [³H]thymidine incorporation per culture well in control cultures were < 1000 cpm, and these were subtracted from each determination. ^bNot done; * $P < 0.05$, ** $P < 0.01$, as compared with the control cultures (*t*-test).

moting activity (8). In the SHE cell assay system, phenol induces gene mutations at two loci and sister chromatid ex-

changes (SCEs) (9).

Guaiacol is a major constituent of creosote. To our knowledge, there are few reports on the genotoxicity of guaiacol. As creosote shows mutagenic properties towards *Salmonella typhimurium* TA1537, TA1538, TA98 and TA100 in the presence of rat-liver S9 fractions (10), the DNA-damaging activity of guaiacol as determined by UDS induction in the present study may be attributable to the mutagenic activity of creosote.

UDS was not detected in SHE cells treated with *m*-cresol. However, in the presence of exogenous metabolic activation, detectable levels of UDS were observed in a concentration-dependent manner. *m*-Cresol induces chromosome aberrations and SCEs in SHE cells. The inducibility of chromosome aberrations by *m*-cresol is enhanced in cells treated in the presence of exogenous metabolic activation (T. Tsutsui et al., unpublished data). The positive response in the SHE cell UDS assay indicates that a combination of SHE cells and rat liver postmitochondrial supernatant may be sufficient for converting *m*-cresol to genotoxic metabolite(s), because SHE cells themselves retain a representative spectrum of endogenous metabolizing enzymes that exhibit oxidative and peroxidative activities (11). *m*-Cresol is not mutagenic in *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation (12). Some additional factors may be necessary for metabolic activation of *m*-cresol in the bacterial system. *m*-Cresol fails to induce SCEs in cultured human fibroblasts, nor is any induction seen in bone marrow, alveolar macrophages, or regenerating liver cells in mice (13). The lack of a genotoxic effect of *m*-cresol in these mammalian systems may be attributable to very low levels of cytochrome P450 in human fibroblasts (13) and to rapid conjugation of active genotoxicants of *m*-cresol with glucuronide or organic sulfates in mice (13).

Formaldehyde induces squamous-cell carcinomas of the nasal cavity in animals (14). DNA damage has been observed in bacteria, yeast and mammalian cells exposed to formaldehyde (14). Formaldehyde is mutagenic to bacteria, yeast and *Drosophila melanogaster*; and it is clastogenic to mammalian cells and plants (14). Morphological transformation in mouse 10T1/2 cells is induced when formaldehyde treatment is followed by exposure to 12-*O*-tetradecanoyl phorbol-13-acetate (14). Formaldehyde induced UDS in SHE cells. This antiseptic also induces chromosome aberrations and SCEs in SHE cells (T. Tsutsui et al., unpublished data).

p-Phenolsulfonic acid, sodium hypochlorite and *p*-chlorophenol did not induce UDS in SHE cells, regardless of exogenous metabolic activation. *p*-Phenolsulfonic acid and *p*-chlorophenol are also negative for clastogenic activity in SHE cells (T. Tsutsui et al., unpublished data). Sodium hypochlorite is not carcinogenic to rats and mice (15).

Sodium hypochlorite causes chromosome aberrations in Chinese hamster CHL cells but not in human fibroblasts (15). No induction of micronuclei, aneuploidy or chromosome aberrations was observed in the bone marrow of mice (15). Mutations are induced in *Salmonella typhimurium* but not in *Bacillus subtilis* (15). Sodium hypochlorite induces SCEs and chromosome aberrations in SHE cells (T. Tsutsui et al., unpublished data). In these SHE cell assays, sodium hypochlorite is administered for 24–48 h, suggesting the necessity of performing UDS experiments using prolonged treatment durations. As UDS correlates well with the ability of chemical agents to cause DNA damage in SHE cells (5), dental antiseptics exhibiting positive responses in the present study are potentially genotoxic to mammalian cells. The UDS-inducing antiseptics were positive for the SHE cell transformation (T. Tsutsui et al., unpublished data). In addition, the antiseptics negative in the UDS assay were negative for the cellular transformation, except for sodium hypochlorite (9) (T. Tsutsui et al., unpublished data). These suggest that the ability of the antiseptics to cause DNA damage is a causal mechanism of the in vitro carcinogenicity of the compounds. Other genotoxicity tests remain to be done to clarify the mutagenicity and carcinogenicity of these dental antiseptics.

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