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Shipp	oing Option: E-Mail				
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Notes	ments: s: LD H-Yes, by: DEC 15, 2007				
	Journal Title: Mutation	research			
	Volume: 89 Issue: 2 Month/Year: 1981Pag	ges: 145-9			
	Article Author: Tomita Y;Mihashi S;Nagata K;Ueda S;Fujiki M;Hirano M;Hirohat				
	Article Title: Mutagenicity of smoke condensates induced by CO2-laser irrad				
	Imprint:				
	Item #: Call #:				
			Date Received.: 11/30/2007 08:59:35 AM		
			Status:		
		D Reas	ate Cancelled:son Cancelled:		
			Date Sent:		

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Number of Pages:_

Mutation Research, 89 (1981) 145-149 Elsevier/North-Holland Biomedical Press

MUTAGENICITY OF SMOKE CONDENSATES INDUCED BY CO_2 -LASER IRRADIATION AND ELECTROCAUTERIZATION

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(Received 29 September 1980) (Revision received 22 December 1980) (Accepted 12 January 1980)

Summary

Smoke condensates generated from mucous membrane of the canine tongue irradiated with a CO₂ laser showed mutagenicity on Salmonella typhimurium TA98 under metabolic activation with S9 mix. Strain TA100 was not so sensitive to the condensates with or without S9 mix. Smoke condensates from electrocauterization on the mucosa of the canine tongue also showed mutagenic activity on TA98 and TA100 with S9 mix. The revertant number per mg of the smoke condensates from laser irradiation was one-half that of the smoke condensates from electrocauterization (1623 and 3371) in TA98. The mutagenic potency observed was comparable to that of cigarette smoke. The amount of these smoke condensates from 1 g of tissue was equivalent to those from 3—6 cigarettes as to total mutagenicity.

The carbon dioxide laser has become available for various surgical fields, and its value is recognized. However, laser surgery is accompanied by the generation of a large amount of smoke from irradiated tissues, which interferes with the surgery by masking the operator's view, clouding a focusing lens, and producing an unpleasant odor similar to that produced by electrocautery with an electric knife.

Smoke condensates from broiling fish and meat are reported to show mutagenicity for Salmonella typhimurium TA100 and TA98 [5,6]. Therefore, it may be reasonable to expect smoke condensates from laser-irradiated tissues or electrocauterized tissues to be mutagenic. In this paper, we report the mutagenic activity of such smoke condensates.

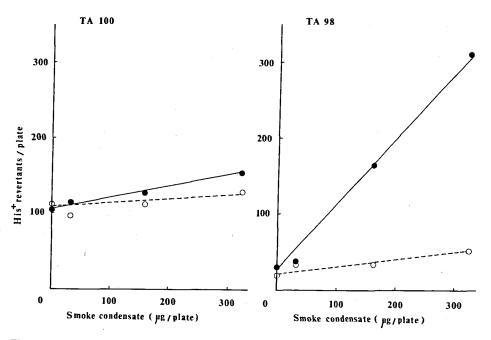


Fig. 1. Mutagenic activity of smoke condensates from CO₂-laser-irradiated canine tongue. Assays were carried out with (•) or without (0) S9 mix. One of 6 similar Expts. is reported.

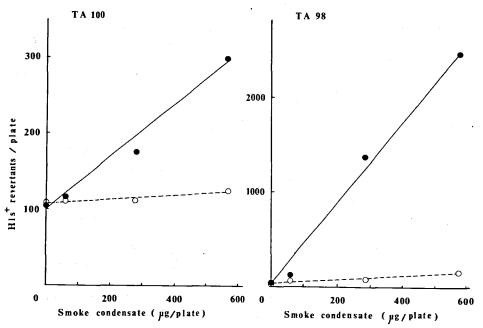


Fig. 2. Mutagenic activity of smoke condensates from electrocauterized canine tongue. Assays were carried out with (•) or without (0) S9 mix. One of 3 similar Expts. is reported.

Materials and methods

Chemicals. NADPH was purchased from Kohjin Co. Ltd., glucose 6-phosphate (G-6-P) from Sigma Chemical Company, and polychlorobiphenyl (Kanechlor KC-500) from Gasukuro Kogyo Co. Ltd. Other chemicals were of special grade and obtained from Wako Pure Chemical Industries Ltd.

Smoke condensates. The excised canine tongue was used as test material. The smoke condensates were generated by a CO₂ surgical laser unit and an electric surgical unit in a closed box (2.7 × 10⁴ cm³). (a) Laser irradiation. The CO₂ surgical laser unit as developed in our department was used [2–4]. The apparatus has a 25-W output and the mode of the beam is single. The irradiation was performed through a 5-cm focusing lens. The incident energy was 15 W for 60 sec, namely 900 J. (b) Electrocauterization. The cauterization was carried out by the Bovie 400-SR made by the Ritter company. The power was 40 degrees and the duration 60 sec. Smokes generated were collected on a glass filter paper (Whatman GF/C, 2.5 cm) by sucking them for 30 min with a vacuum line (400 mm Hg), and were dissolved in 5 ml of DMSO. The solution was sterilized with Fluoropore filter (Sumitomo electric FP-022). Smoke condensates obtained from CO₂-laser-irradiated and electrocauterized canine tongues are referred to as "LIS condensates" and "ECS condensates", respectively. The amount of condensate was calculated by the difference between the weights of a filter before and after collection.

Microbial strains. Salmonella typhimurium TA100 and TA98 were kindly supplied by Dr. Bruce N. Ames (Univ. California) [1]. Bacteria were precultured in nutrient broth for 14 h at 37°C just before the mutation assay.

Mutation assay. The mutation assay was carried out according to the modified method of Yahagi et al. [9]. S9 mix contained 50 μ moles sodium phosphate buffer (pH 7.4), 4 μ moles MgCl₂, 16.5 μ moles KCl, 2.5 μ moles G-6-P, 2 μ moles NADPH, and 150 μ l of S9 fraction (prepared from rat liver pretreated with polychlorobiphenyl) in a total volume of 0.5 ml.

Results and discussion

LIS condensates showed mutagenicity on TA98 in the presence of S9 mix, and His[†] revertants were induced with an increased dose of the condensates (Fig. 1). TA100 was not so sensitive to LIS condensates either in the presence or absence of S9 mix. The ECS condensates exhibited mutagenic activities on both strains in the presence of S9 mix (Fig. 2). No killing effect was observed in either condensate at least within the dose of 1 mg per plate (data not shown). The findings suggest that the primary mutagen(s) in these condensates may be premutagen(s) requiring metabolic activation and may induce frameshift type mutation [1]. In Table 1 are summarized the number of His[†] revertants per mg condensate for LIS and ECS in the presence of S9 mix. The mutagenic ability of LIS condensates was one-half that of ECS condensates for TA98. TA98 was 10-fold more sensitive than TA100 to these condensates. The following discussion is limited to the data from TA98 with S9 mix because the mutagenicity of condensates was clearly observed under this condition as stated above.

TABLE 1
MUTAGENICITY OF SMOKE CONDENSATES

Smoke condensates	His [†] revertants/mg/plate a		
	TA98	TA100	
LIS condensates	1623	194	
ECS condensates	3371	280	

a Averaged data of 6 Expts. for LIS and 3 Expts. for ECS in the presence of S9 mix.

With LIS condensates, CO₂-laser irradiation induced a rapid increase of temperature at local sites [3]. Biomolecules in tissues may be converted to various molecules with higher aromatic ring structure (possibly unsaturated radicals) at high local temperatures. Some of these, such as Trp-P-1 and Trp-P-2, are mutagens [7]. The production of mutagenic substances may depend on the energy of a laser beam and constituents of tissues. ECS condensates showed twice the mutagenicity of LIS condensates. The electrocauterized conditions may be more favorable for the generation of mutagens than laser irradiation. More precise conditions for generation of mutagenic substances are under investigation.

The mutagenic potency was much greater than that of broiled fish or beef (about 300—400 revertants/mg for fish and negligible for meat) [5], and less than obtained by pyrolysis of biomolecules such as lysozyme or histone (8311 and 5012 revertants/mg, respectively) [6]. The mutagenic potency of LIS condensates was comparable to that of cigarette smoke (about 1200—1300 revertants/mg) [8]. About 40 mg of both condensates were collected from 1 g of vaporized or cauterized tissues. This amount of condensate was equivalent to those from 3 (LIS condensates) or 6 (ECS condensates) cigarettes (Sato et al. [8] and our data, not shown) as to the total mutagenicity on TA98 with metabolic activation.

We have no human data to evaluate possible health hazards of LIS or ECS. The mutagenic activities of LIS or ECS are comparable to or less than that of cigarette smoke. As compared with cigarette smoking, the exposure to LIS or ECS is rather limited according to our experience at the Kurume University Hospital. For example, the surgeons are exposed to LIS or ECS 2—3 times a week and for about 0.5 h for each operation. Further, we have not yet identified specific mutagenic substance(s), nor do we know the pharmacokinetics after inhalation. Therefore, more work is needed to evaluate human health hazards of LIS or ECS. However, we believe we should keep in mind the potential hazard of LIS or ECS to the health of surgeons, anesthetists, nurses and patients until evidence from further studies has proved otherwise.

Acknowledgements

This work was partly supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

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