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Induction of Squamous Cell Carcinomas in the Salivary Glands of Rats by Potassium Iodide

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In a 2-year carcinogenicity study of potassium iodide (KI) in F344/DuCrj rats, squamous cell carcinomas (SCCs) were observed in the salivary glands of 4/40 males and 3/40 females receiving 1000 ppm KI in the drinking water. Ductular proliferation with lobular atrophy was observed at high incidence in the submandibular glands of the high-dose animals, and squamous metaplasia was frequently evident within the proliferative ductules and the larger interlobular ducts. A transition from metaplasia to SCC was apparent. The results suggest that squamous metaplasia in proliferative ductules, occurring secondarily to lobular impairment induced by KI, may develop into SCCs via a non-genotoxic, proliferation-dependent mechanism.

Key words: Squamous cell carcinoma — Salivary gland — Potassium iodide — Rat

In a carcinogenicity study of potassium iodide (KI), squamous cell carcinomas (SCCs) derived from the salivary gland were observed in several male and female rats given water containing excess amounts of KI. However, there have been no reports of salivary gland carcinogenesis by chemical substances containing iodine. In order to elucidate the pathogenesis of the SCC induction, further detailed morphological examinations were performed in the present study.

Four-week-old male and female F344/DuCrj rats were purchased from Charles River Japan Inc. (Atsugi), and divided into 4 groups consisting of 40 rats each at 5 weeks of age. They were given potassium iodide in the water at concentrations of 0, 10, 100 and 1000 ppm for 2 years. The highest dose has been reported to result in pronounced physiological effects in experimental animals.[1)] KI intakes of rats during the treatment period were calculated from the water consumption. After the termination of treatment, all animals were killed under ether anesthesia and dissected. Salivary glands and tissue masses observed macroscopically in the subcutis of the neck were fixed in neutral-buffered 10% formalin, routinely processed for production of sections stained with hematoxylin-eosin (HE), and examined histopathologically. In addition, immunohistochemistry to identify cells positive for the proliferating cell nuclear antigen (PCNA) was performed. PCNA labeling indices of epithelial cells in normal ducts and proliferative lesions were determined as percentage of PCNA-positive cells in more than 500 cells counted.

Histopathological examination revealed SCCs in 4/40 male and 3/40 female rats receiving 1000 ppm of KI. The lesions were all considered to be derived from the salivary glands rather than the Zymbal's gland, based on their anatomical locations and histological patterns (Fig. 1, Table I). Ductular proliferation accompanied by lobular atrophy was also frequently observed in submandibular glands of both sexes of the 1000 ppm group, the incidence being 31/40 in males and 34/40 in females (Fig. 2, Table I). These ductular proliferative lesions were well-circumscribed and triangular in shape, with no or only a few acini in association, and appeared to be restricted to single lobules. These lesions were usually multiple in the bilateral submandibular glands, and frequently featured squamous metaplasia within the proliferating ductules (Figs. 3 and 4). In addition, the interlobular ducts draining the affected lobules also generally featured squamous metaplasias (Fig. 3). The lesions were usually associated with various degrees of inflammatory cell infiltration. Similar changes were also observed in the sublingual and parotid glands of the 1000 ppm group, although at much lower incidences and without squamous metaplasia. There was a morphological continuum from squamous metaplasia of proliferating ductules to SCCs. As compared with normal ductules, PCNA labeling indices were elevated in proliferating ductules, squamous metaplasias and SCCs, the values for SCCs being highest (Table II). PCNA labeling indices for interlobular ducts exhibiting squamous metaplasia were also higher than those of normal ducts from control rats. The histological findings and cell kinetics suggest that squamous metaplasia originating from either proliferating ductules in lobular atrophy or interlobular ducts might progress to SCCs.

The incidences of SCC in the 1000 ppm group, 4 of 40 males and 3 of 40 females, were not statistically significant as compared to those in the concurrent control group.

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Table I. Chemical Intake and Incidences of Histopathological Lesions in the Salivary Glands of Rats Given Water Containing Potassium Iodide for 2 Years

	Male				Female			
	0	10	100	1000 (ppm)	0	10	100	1000 (ppm)
Chemical intake (mg/kg/day)	0	0.6	5.3	53.0	0	0.7	6.7	66.6
No. of animals	40	40	40	40	40	40	40	40
Submandibular gland								
Lobular atrophy and ductular proliferation	0	1	0	31	0	0	1	34
Squamous cell carcinoma	0	0	0	4	0	0	0	3
Parotid gland								
Lobular atrophy and ductular proliferation	1	1	1	4	0	0	0	0
Sublingual gland								
Lobular atrophy and ductular proliferation	0	0	0	1	0	0	0	2

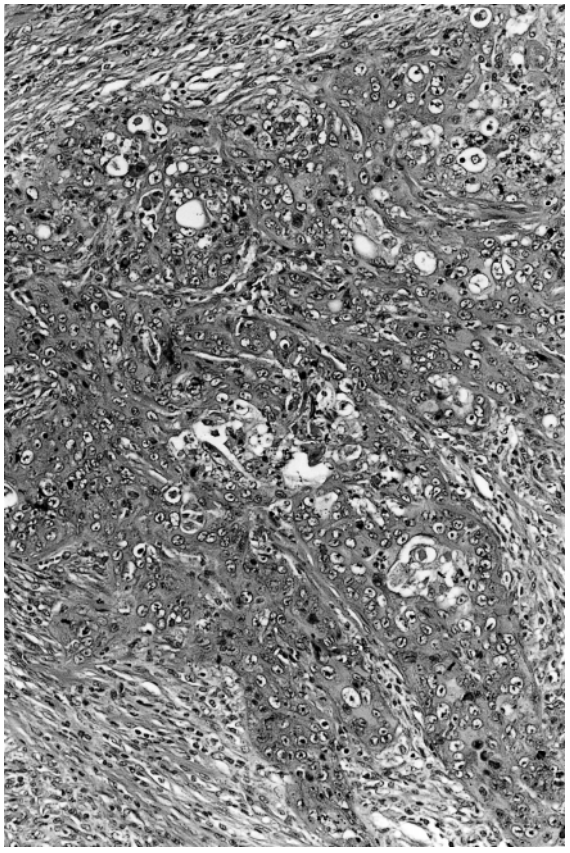

Fig. 1. A squamous cell carcinoma observed in the submandibular gland of a male rat given 1000 ppm KI in the water for 2 years. Tumor cells exhibit nuclear atypia. HE staining, ×130.

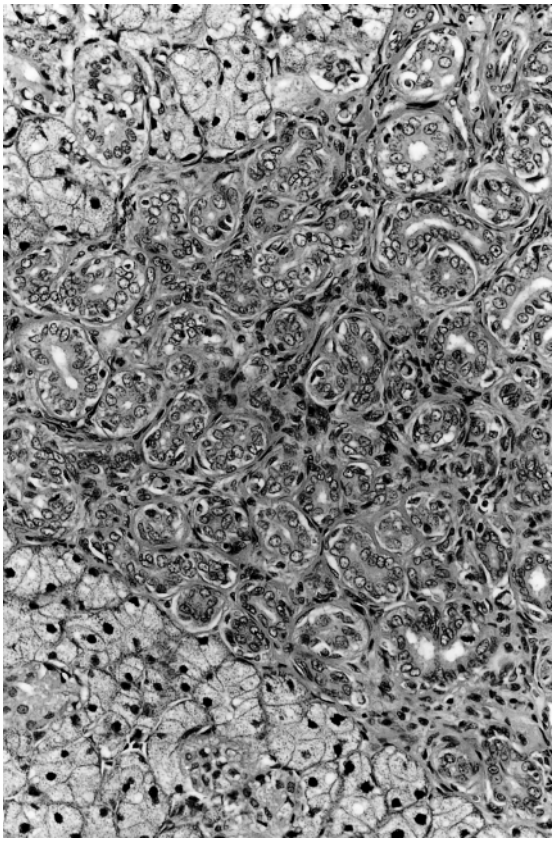

Fig. 2. Ductular proliferation observed in the submandibular gland of a male rat given 1000 ppm KI in the water for 2 years. Only few acini are evident in this lesion. HE staining, ×210.

However, in the historical data for untreated control F344 rats from carcinogenicity studies performed in our laboratory[2–9] and in the literature historical data on control F344 rats[10–12] used for carcinogenicity studies, no spontaneous occurrences of SCCs in the salivary gland are recorded. Therefore, the incidence of SCCs in the 1000 ppm

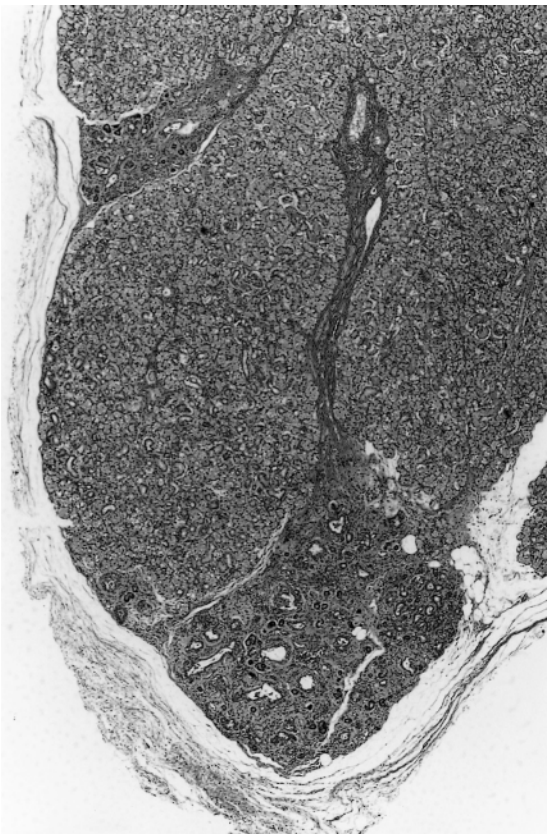

Fig. 3. Lobular change in the submandibular gland of a male rat given 1000 ppm KI in the water for 2 years. Note the ductular proliferation and acinar atrophy, with extensive squamous metaplasia. HE staining, ×32.

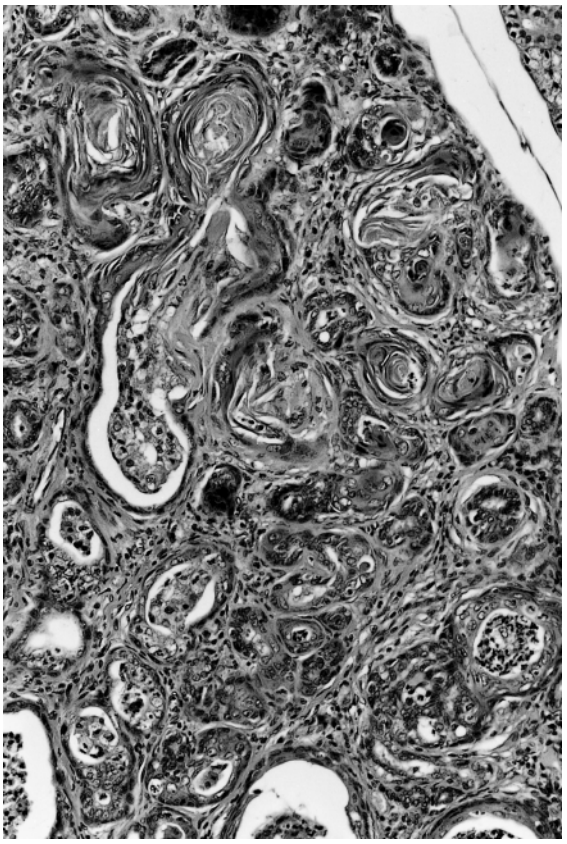

Fig. 4. Squamous metaplasia observed within an area of ductular proliferation in the submandibular gland of a female rat given 1000 ppm KI in the water for 2 years. HE staining, ×160.

Table II. PCNA Labeling Indices of Cell Populations in the Submandibular Glands of Rats Given Water Containing 1000 ppm Potassium Iodide for 2 Years

Cell population	PCNA labeling index (%)	
	Male	Female
Normal ducts	0.04±0.08[a)](5)	0(5)
Ductular proliferation without squamous metaplasia	0.78±0.74(5)	0.39±0.18(3)[c)]
Ductular proliferation with squamous metaplasia	3.50±1.13(5)[c, e)]	6.89±2.85(5)[c, e)]
Squamous carcinoma	11.64±6.06(4)[b, d)]	16.80±14.77(3)[b)]

a) Mean±SD.
b, *c*) Significantly different from the normal duct value at $P<0.05$ and 0.01, respectively.
d, *e*) Significantly different from the value for ductular proliferation without squamous metaplasia at $P<0.05$ and 0.01, respectively.
Figures in parentheses are numbers of animals examined.

group in the present study can not be ignored, and the possibility must be entertained that the SCCs are related to the treatment with large amounts of KI.

Although there have been no previous reports of SCCs of the salivary gland in animals treated with iodine-containing chemicals, there are some references to support the

possibility that SCCs can be induced in the salivary gland. For example, they can be caused in rats by DMBA-treatment,[13, 14] being preceded by the development of duct-like structures and dilated excretory ducts, concomitantly with elevation of cell proliferation and squamous metaplasia.[13] In addition, squamous metaplasia is well known to occur in other non-squamous tissues, such as the mammary gland[15] and bronchi[16] in man. It has also been reported that squamous metaplasia arises in respiratory and olfactory epithelia subjected to prolonged or continuous injury by irritants or infectious inflammation, with occasional cellular atypia suggestive of a preneoplastic nature.[17]

Squamous metaplasia of ductules or ducts is probably a feature of salivary glands suffering repeated injury associated with inflammation. Metaplasias may, for example, occur in sialadenitis caused by conditions blocking secretion, such as sialolithiasis,[18] and in hyperplastic ductal epithelia with inflammation in the cases of sialolithiasis after fine-needle aspiration in humans.[19] In the present study, ductular proliferation in lobules appeared to be a reaction to acinar atrophy. However, it was unclear whether the acinar change was caused by direct injury or was secondary to disturbed excretion due to interlobular duct lesions.

Chemicals containing iodine have in fact been reported to impair salivary gland function in man and rats. In human beings, I-131 used for thyroid tumor therapy has been demonstrated to cause sialadenitis in the submandibular or parotid glands with reduction of serous cell numbers and duct proliferation.[20] Contrast media may also induce so-called iodide mumps, characterized by swelling of submandibular, sublingual or parotid glands and/or sialadenitis.[21, 22] In rats, sodium iodide induces inflammatory lesions and squamous metaplasia of ducts in the submandibular gland[23] that are in line with those seen in the present study. Moreover, treatment with iodinated glycerol is associated with focal atrophy and squamous metaplasia of the salivary glands.[24] Based on these reports, it can be considered that the salivary gland of the human and the rat is one of the major target organs of compounds containing iodine, and acinar atrophy, inflammatory lesions, duct proliferation or squamous metaplasia are induced by these compounds.

In the present study, the observed SCCs clearly originated in the duct system, in association with ductular proliferation and metaplasia. However, since KI has been found to be negative in genotoxicity tests,[25, 26] the underlying mechanisms are presumably epigenetic. The 1000 ppm dose of KI applied in the present study is about 2500 times the maximum tolerable daily intake (1.0 mg iodine/day) settled by FAO/WHO Joint Expert Meeting on Food Additives,[27] with the average human body weight assumed to be 50 kg. The fact that the lower doses employed (10 and 100 ppm) were not associated with tumor development is a clear pointer to a non-genotoxic carcinogenic potential for the salivary glands in rats. The risk to humans can therefore be considered negligible except in cases of excessive exposure to iodine compounds.

A full report on the 2-year carcinogenicity study of this chemical will be presented in the near future.

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