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Regeneration of the Guinea Pig Parotid Gland after 4-hydroxyaminoquinoline-1-oxide-Induced Necrosis (39503)

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4-Hydroxyaminoquinoline-1-oxide (4-HAQO), a presumed proximate carcinogen resulting from the metabolism of 4-nitroquinoline oxide, is carcinogenic in several organs of rodents (1-3). Recently it has been shown that a single iv injection of 4-HAQO induces necrosis of pancreatic acinar cells in rats and guinea pigs (4-7). Furthermore, we have demonstrated that pancreatic acinar necrosis in the guinea pig induced by 4-HAQO is followed by significant regeneration (5, 6). The present study was undertaken to investigate the effect of 4-HAQO on the parotid gland, since the morphology and function of the parotid gland are somewhat similar to those of the exocrine pancreas. We now report that a single iv injection of 4-HAQO in a dose of 22 mg/kg body weight causes profound necrosis of the guinea pig parotid acinar tissue within 48 hr and that the necrosis is followed by active regeneration.

Materials and methods. Randomly bred male guinea pigs weighing between 250-300 g, obtained from Small Stock Industries, Inc., Arkansas, were used in these studies. They were housed in groups of three to four per cage and maintained on guinea pig Purina chow. 4-HAQO (a generous gift from Dr. Elizabeth Weisberger, NIH), dissolved in 0.01 *N* HCl immediately before use, was injected into the antecubital vein at a dose of 22 mg/kg body weight. The control animals were injected with a corresponding volume of 0.01 *N* HCl solution. Three to seven animals in the test group and three animals in the control group were sacrificed at 6-hr intervals beginning from 24 hr after injection up to 108 hr. For light microscopy, all the salivary glands were fixed in 10% neutral buffered formalin and embedded in paraffin, and 5-μm sections were routinely stained with hematoxylin-eosin stain.

To assess DNA synthesis and cell division in the parotid glands, [^{3}H]thymidine (sp act 53.3 Ci/mmole, New England Nuclear Corp., Boston, Massachusetts) uptake was investigated by autoradiography at the light-microscope level. The control and HAQO-treated animals were injected with [^{3}H]thymidine at a dose of 0.4 μCi/g body weight ip 1 hr before sacrifice at 6-hr intervals starting from 48 hr. Tissue was processed for autoradiography as described previously (5). Nuclei overlaid with more than five grains were scored as labeled. Approximately 3000 parotid acinar cells from each animal were counted to obtain the labeling and mitotic indices.

Results. A single iv injection of 4-HAQO has induced severe necrosis of the acinar cells of the parotid gland between 24 and 48 hr. The necrosis was followed by regeneration between 54 and 108 hr. The control animals, injected with HCl alone, revealed no evidence of necrosis in the parotid or other salivary glands at any interval.

Necrotic phase. Grossly, the parotid glands of guinea pigs 24 hr after 4-HAQO injection resembled that of controls. However, after 30 hr they were pale and edematous. Histologically, at 24 hr, the lobular and acinar architecture was well preserved. The cytoplasm of the acinar cells was vacuolated with decrease in granularity. An isolated acinar cell showed nuclear pyknosis or necrosis. The interstitial tissue was edematous and devoid of inflammatory reaction. Between 30 and 48 hr there was progressive increase in cellular injury with distortion of lobular architecture (Figs. 1 and 2). An occasional uninvolved acinar cell was present, scattered among the necrotic acinar cells. The excretory ducts of different sizes showed no evidence of cellular damage. There was no histologic evidence of necrosis

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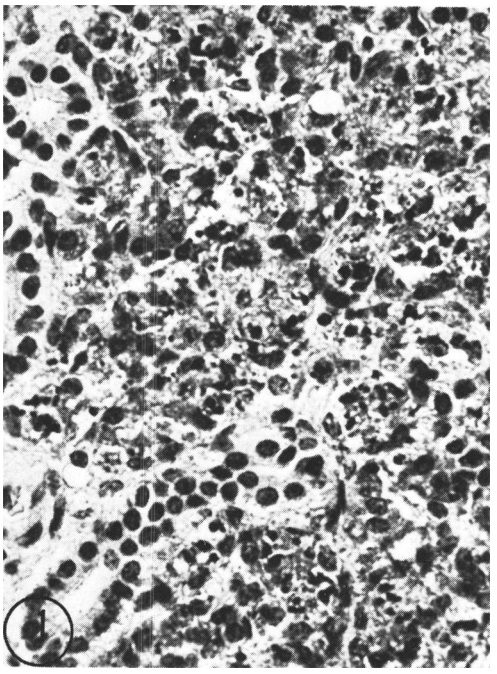

FIG. 1. At 30 hr after 4-HAQO injection, the parotid acinar tissue shows necrosis of acinar cells. H & E, ×450.

in the submandibular or sublingual glands.

Regenerative phase. At 54 hr, there was marked loss of the necrotic tissue. Regenerative activity was prominent at 54 hr and repair of damaged acini, as well as formation of new acini, appeared to proceed rapidly thereafter. Several mitoses were noted in the parotid acinar cells at 54 hr after 4-HAQO treatment. The mitotic count was greatest in animals killed at 72 and 84 hr after 4-HAQO injection (Figs. 3 and 4). The newly formed acinar cells contained a large hyperchromatic nucleus and scant cytoplasm (Figs. 4 and 5). Between 60 and 108 hr, newly formed acinar cells showed a progressive increase in the amount of cytoplasm. No mitotic activity was evident in cells lining the duct system.

The effect of 4-HAQO administration on the incorporation of [^{3}H]thymidine in the parotid gland is shown in Fig. 3. A progressive increase in the percentage of labeled nuclei was noted between 54 and 72 hr; the highest degree of labeling of acinar cells being 15% at 72 hr. Thereafter, a gradual decline in nuclear labeling of parotid acinar cells was noted. No significant nuclear labeling was encountered in the cells lining the ducts.

Comment. The data presented here demonstrate that a single iv injection of 4-HAQO causes necrosis of the parotid gland in the guinea pig between 24 and 48 hr and that the necrosis is followed by marked regeneration and restitution of the acinar tissue. The 4-HAQO-induced necrosis of the salivary tissue in the guinea pig was limited to the parotid gland only; the submandibular and submaxillary salivary glands remained unaffected. The necrotic effect of 4-HAQO in parotid glands appeared similar to that induced by this compound in exocrine pancreas (5, 6). In the pancreas, as well as in the parotid gland, the maximum necrosis occurred by 48 hr after the injection of this compound. The mechanism by which 4-HAQO causes necrosis of the parotid acinar tissue remains to be elucidated. In the pancreas, the necrogenic effect of 4-HAQO has been considered to be caused, possibly, by two mechanisms: (1) the ability of this compound to bind to DNA, leading to interference of DNA-directed RNA synthesis, and (2) enhanced uptake of this carcinogen by the pancreatic exocrine tissue (7,

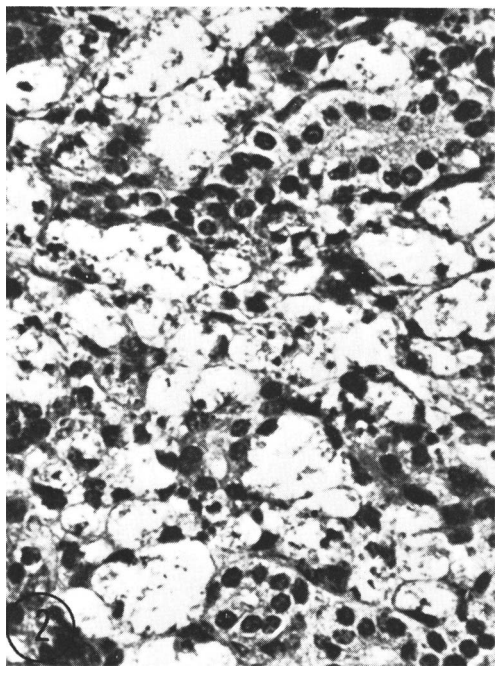

FIG. 2. At 48 hr after 4-HAQO, The necrosis of acinar tissue was marked. H & E, ×450.

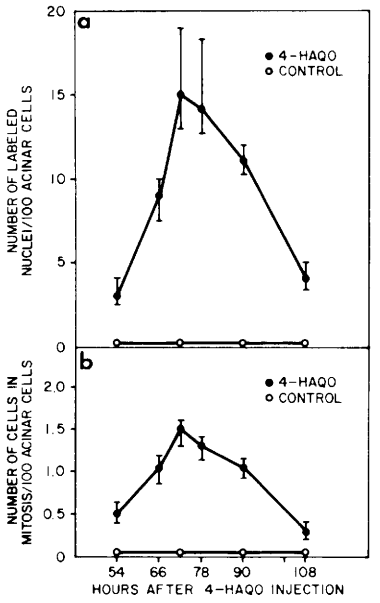

FIG. 3. The effect of 4-HAQO injection on [^{3}H]thymidine acinar cell nuclear labeling between 54 and 108 hr in autoradiographic studies. The effect of 4-HAQO injection (a) on the incorporation of [^{3}H]thymidine into the parotid acinar cell nuclei as determined by nuclear labeling in autoradiographic studies, and (b) on the number of mitoses in acinar cells. The values are mean ± SEM for three animals.

8). Presumably these two mechanisms may be in operation in the 4-HAQO-induced necrosis of the parotid epithelium. The studies of Iqbal *et al.* (9) have shown that 4-HAQO induces DNA damage in the guinea pig pancreatic slices *in vitro* and that repair of the damaged DNA occurs fairly rapidly. Additional studies are needed to ascertain if 4-HAQO induces DNA damage and DNA repair in the parotid gland. The concurrent occurrence of necrosis of the exocrine pancreatic and parotid tissues in the guinea pig following iv injection of 4-HAQO may be due to greater affinity of these exocrine tissues to concentrate this chemical. There is, however, very little information available regarding the uptake and metabolism of carcinogens by the exocrine glands.

The regenerative changes in the parotid gland which followed the 4-HAQO-induced necrotic phase appeared similar to those described in the pancreas after a single iv dose of this carcinogen (5, 6). Regeneration and repair of parotid acinar tissue occurred between 54 and 108 hr after 4-HAQO injec-

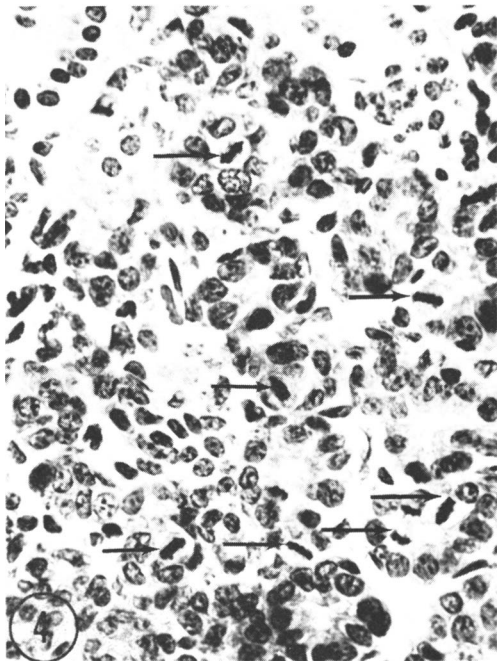

FIG. 4. At 72 hr after 4-HAQO injection, numerous mitoses (arrows) are seen in the parotid acinar cells. H & E, ×560.

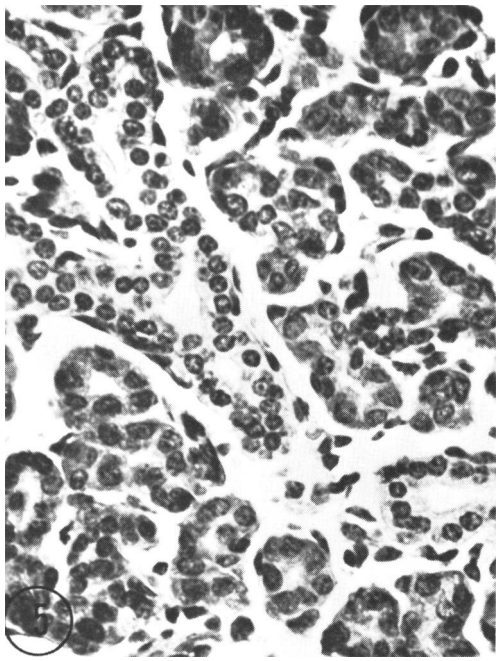

FIG. 5. At 96 hr after 4-HAQO injection, the newly formed acini are lined by low cuboidal cells containing hyperchromatic nuclei and moderate cytoplasm. H & E, ×450.

tion. However, even at 108 hr, the newly formed acini were small and appeared functionally immature in view of the diminished cytoplasmic granularity of the acinar cells. The increased [^{3}H]thymidine labeling of acinar cells, as well as increased mitotic activity in the parotid acinar cells resulting from 4-HAQO treatment, are clear indications that normally "nondividing tissue" (10), in which mitoses are rare, can be stimulated to proliferate actively by experimental manipulation. Although the precise mechanism of 4-HAQO-induced parotid acinar cell regeneration is not clear, it is reasonable to assume that proliferative response is triggered by necrosis and loss of acinar tissue. The regenerative response of the parotid gland observed in the present studies is comparable to that described in the rat parotid gland after ethionine administration (11, 12) or following irradiation (13), both of which are associated with necrosis of acinar tissue. In contrast, however, the studies of Barka (14) have unequivocally demonstrated that the highly differentiated parotid acinar cells can be stimulated to proliferate by the administration of isoproterenol without antecedent necrosis.

Summary. A single iv injection of 4-hydroxyaminoquinoline-1-oxide (4-HAQO) in a dose of 22 mg/kg body weight induced marked necrosis of parotid acinar tissue in guinea pigs within 48 hr. After the necrotic phase, marked regenerative activity of the acinar cells was noted. [^{3}H]Thymidine autoradiographic studies revealed a labeling index of 15% cells in the parotid gland at 72 hr after 4-HAQO injection. A significant increase in the mitotic activity of the parotid acinar cells was also evident at 72 and 78 hr after 4-HAQO administration. The acinar cell repair, as well as formation of the new acini, were complete at 108 hr. It is clear from the studies that 4-HAQO, a pancreaticotoxic agent and a potent carcinogen, also causes necrosis of the parotid acinar tissue, which is followed by regeneration and restitution.

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