Carcinogenic Effect of N-Nitroso(2-hydroxypropyl)(2-oxopropyl)amine, a Postulated Proximate Pancreatic Carcinogen in Syrian Hamsters

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ABSTRACT

N-Nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) proved to be a potent carcinogen in Syrian golden hamsters. The compound is an in vivo metabolite of N-nitrosobis(2-hydroxypropyl)amine, N-nitrosobis(2-oxopropyl)amine (BOP), and N-nitroso-2,6-dimethylmorpholine and a postulated proximate pancreatic carcinogen in hamsters. As with BOP, HPOP induced a higher incidence of pancreatic ductular adenocarcinomas than did N-nitrosobis(2-hydroxypropyl)amine and N-nitroso-2,6-dimethylmorpholine, and these neoplasms showed a great tendency for invasion and metastasis. Also, HPOP induced tumors of the forestomach, liver, gallbladder, kidneys, and vagina (as did BOP). However, HPOP [unlike BOP, but like N-nitrosobis(2-hydroxypropyl)amine and N-nitroso-2,6-dimethylmorpholine] led to tumor development in the nasal cavity, larynx, trachea, intestine, Harderian gland, lips, and flank organ. The possible mechanisms of HPOP carcinogenicity are discussed.

INTRODUCTION

Of the nitrosamines that we have tested in Syrian golden hamsters, 2 postulated ß-metabolites of N-nitrosodi-n-propylamine (5–7), BHP1 and BOP, as well as a synthetic BHP derivative, BAP, and NDMM (Chart 1), were potent pancreatic carcinogens (8, 11, 12, 18). The similar carcinogenic effects of BHP and BAP relative to the tumor spectrum were related to in vivo conversion of BAP to BHP (3). Further biochemical studies showed that these pancreatic carcinogens have HPOP as a common metabolite (Chart 1). This compound was therefore assumed to represent a more proximate pancreatic carcinogen (3), based on evidence that the greater affinity of BOP (compared to BHP) for the pancreas (12, 22) correlated with the quantitative differences in HPOP formation from BHP and BOP. Relatively larger amounts of HPOP were found after BOP administration than after BHP treatment (3). To investigate the significance of HPOP in pancreatic carcinogenesis, we tested its biological effect in the same species.

MATERIALS AND METHODS

We used 8- to 10-week-old Syrian golden hamsters (from the Eppley colony) with an average initial weight of 110 g (females) and 105 g (males). They were kept in plastic cages (Macrolan) on San-i-cel bedding in groups of 5/sex under standard laboratory conditions and given Wayne pelleted diet (Allied Mills, Chicago, Ill.) and water ad libitum. Animals were checked 3 times daily, weighed biweekly, and sacrificed when moribund. HPOP was synthesized by previously described methods (3). The LD50 for HPOP was determined by the method of Weil (25) after an 8-day observation period. In the chronic study, groups of hamsters (15 females and 15 males each) received weekly s.c. injection of HPOP (dissolved in 0.9% NaCl solution) for life in concentrations of 1/10 (Group 1), 1/20 (Group 2), or 1/40 (Group 3) of the LD50. Controls (Group 4) received the solvent only.

After complete autopsies, organs were fixed in 10% buffered formalin, prepared for histology by conventional methods and stained with hematoxylin and eosin. Step sections were prepared from nasal and paranasal cavities, pharynx, esophagus, larynx, trachea, lungs, pancreas with attached extrahepatic bile ducts (including gallbladder), kidneys, urinary bladder, and urethra with attached reproductive sex organs. Tumors were counted in representative histological sections, and tumor size was determined by measuring the diameter in mm. The tumor latency was given in weeks from the beginning of the experiment until first tumor appearance at autopsy. The significance of the differences between tumor incidence rates for each site was ascertained by the χ² test. The occurrence of statistically significant differences in tumor rates is mentioned where appropriate.

RESULTS

The LD50 of HPOP was 406.1 ± 20.1 (S.D.) mg/kg body weight for females and 353.5 ± 18.8 mg/kg body weight for males. Necrosis of pancreatic acinar cells, liver, kidneys, and intestines and hemorrhages of lungs, heart, and abdominal organs were found in animals that died during the first 5 days of the observation period.

Data in the chronic study are summarized in Table 1 and concern average survival, average body weight, and tumor incidence in the main target organs for HPOP. All but one HPOP-treated animal developed tumors of types other than those found in the controls (and those known to occur spontaneously). The non-tumor-bearing hamster died shortly after initial treatment due to an intercurrent disease. There was a dose-response relationship relative to average survival in that hamsters receiving the highest dose (Group 1) died earlier than those treated with the lowest HPOP dose (Group 3) compared to controls (Table 1). However, the difference was not statistically significant. The multiplicity of induced neoplasms seemed to increase at the lower doses because of prolonged survival.
Carcinogenic Effect of HPOP in Syrian Hamsters

Many hamsters simultaneously had multiple tumors in several organs and/or in the same tissue.

Pancreatic Neoplasms. Nearly all HPOP-treated animals had pancreatic neoplasms, and those with none had died in the early stage of the experiment. However, these animals had proliferative and preneoplastic ductular alterations (as early as 13 weeks), indicating that the tumor incidence could have been 100% had the animals survived longer. Tumors of different histological patterns, as described in previous studies (10, 14), appeared in both sexes; however, adenocarcinomas of ductal origin were more common in males. The latency of individual lesions did not differ markedly. Although there were no statistically significant differences in pancreatic tumor incidences among the 3 treated groups, in some instances adenocarcinomas developed earlier and at a higher frequency than did the other tumor types. In addition, the average number of adenocarcinomas was higher compared to that of ductular lesions did not differ markedly. Although there were no statistically significant differences in pancreatic tumor incidences among the 3 treated groups, in some instances adenocarcinomas developed earlier and at a higher frequency than did the other tumor types. In addition, the average number of adenocarcinomas was higher compared to that of ductular lesions. In some instances, adenocarcinomas developed earlier and at a higher frequency than did the other tumor types. In addition, the average number of adenocarcinomas was higher compared to that of ductular lesions. In some instances, adenocarcinomas developed earlier and at a higher frequency than did the other tumor types.

Many hamsters with pancreatic neoplasms also had tumors of other sites (Table 1).

Tumors of the Respiratory Tract. The incidence and multiplicity of nasal cavity neoplasms varied among the groups; it was highest in Group 1 females, whereas males in Group 2 and 3 developed tumors more frequently than those in Group 1 (Table 1). For males, the tumor incidence was significantly higher compared to that of females. Hemorrhagic ascites was found in many hamsters with adenocarcinomas and in all of those in which tumors were invasive or had metastasized. Local fat necrosis was observed in a male hamster. One control female and male each had a small solid ductal adenoma.

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In many hamsters with or without lip tumors, concomitant hyperplasia of pilosebaceous glands, was found. 3830 had adenocarcinomas; 2 of these (microcarcinomas) in males were located in the cecum (one male had, in addition, a rectal polyp), one was located in the rectum (male, 70 weeks), and the remaining 2 (females in Groups 1 and 3) were found in the duodenum, near the opening of the common duct. Based on their morphological patterns (Figs. 1 and 2), both duodenal neoplasms had apparently originated from Brunner's gland.

Liver neoplasms were mostly cholangiocellular adenomas (cholangiomas) which occurred in 20 to 60% of females and in 33% of Group 2 males. Statistically significant differences in tumor incidence (p < 0.025) were found only in males of Group 1, compared to those in either Group 2 or Group 3. Kupffer cell sarcomas were found in 7 to 13% of the females and 0 to 4% males, and hepatocellular adenomas (hepatomas) were found in 7% of Group 1 females and in 13 and 7% in males of Groups 2 and 3, respectively; one hamster exhibited cholangiocellular carcinoma and another had hepatocellular carcinoma. The tumor latency was generally shorter for benign lesions. Proliferation of biliary ducts, often associated with hyperplasia and goblet cell metaplasia of intra- and extrahepatic bile ducts (including the gallbladder and common bile duct), was found in almost all treated hamsters, but none were seen in controls. Four males in Groups 2 and 3 showed a marked proliferation of the common duct epithelium consistent with papillary polyps. Similar lesions were found in the gallbladder of 19 hamsters with the shortest latency in Group 1 male hamsters (26 ± 1 weeks). A Group 1 female (38 weeks) presented a papillary carcinoma that invaded the gallbladder wall and adjacent liver tissue.

**Tumors of Urogenital Tract.** Renal neoplasms appeared only in treated hamsters and most frequently in those of Groups 2 and 3; male predominance was obvious (Table 1). Among males, but not among females, the tumor incidence was significantly lower (p < 0.025) in Group 1 than in either Group 2 or Group 3. Although the first tumor appeared in a Group 1 male at 24 weeks, the neoplasms generally developed after 40 weeks. Tumors were usually solid and rarely multiple (uni- or bilateral). Morphologically, they were tubular adenomas (in 7% of Group 3 females and in 13 and 27% of Group 2 and 3 males, respectively), adenocarcinomas, often of a poorly differentiated type (in 7% of Group 3 females and in 20 and 13% of Group 2 and 3 males, respectively), and mixed mesenchymal epithelial tumors (7% in both Group 3 females and males). Some of the carcinomas reached a considerable size and invaded perirenal tissue. No distant metastases, however, were found. Small transitional cell papillomas of the urinary bladder (all near the external orifice) were observed in 3 treated hamsters, but not in controls (Table 1). A Group 1 female (29 weeks) had a similar lesion in the middle segment of the urethra. Granulosa cell tumors of the ovaries were present in 2 Group 1 hamsters (41 ± 4 weeks) and in one Group 2 animal (41 weeks); none were seen in controls. Squamous cell papillomas of the vagina occurred in 87 to 93% of all treated hamsters (Table 1), as early as 35 ± 6 weeks in Group 1. In most animals, these tumors were multiple (up to 5 tumors) and were predominantly located near the labia. Control hamsters had endometrial polyps (2 hamsters), an endometrial adenocarcinoma (one hamster), and a vaginal papilloma (one hamster).

**Tumors of Other Sites.** Harderian gland adenomas with patterns described earlier (20) were detected in many hamsters, especially in those of Group 3, and occurred predomi-
ductular cells, including the islet cell precursors, which apparently have sugar receptors (24). We do not yet know why these lesions was consistent with trichoepithelioma, and another 3 were squamous cell carcinomas (Figs. 3 and 4). The first tumor appeared at 35 weeks, whereas carcinomas developed up to 20 weeks later. Two of these hamsters simultaneously had tumors of the lip. Hyperplasia of the epidermis and of the adenexes of the flank organ was observed in many treated male hamsters, but not in controls.

Other neoplasms (all benign) were found in the adrenal, thyroid, and parathyroid glands of treated and control hamsters in an incidence within the range of spontaneous diseases in this hamster colony (17, 19-21).

**DISCUSSION**

HPOP, a common in vivo metabolite of BOP, BHP, and NDMM (2, 3), was shown to be a potent pancreatic carcinogen. Its neoplastic effect on the hamster pancreas was similar to that of BOP (12, 22) and thus stronger than those of BHP (18) and NDMM (8). HPOP was formed in vivo more readily after BOP treatment than after BHP and NDMM administration (3) and, accordingly, may represent a proximate pancreatic carcinogen in Syrian hamsters. As with BOP, a great number of HPOP-induced pancreatic tumors were ductular adenocarcinomas, which were invasive and had metastasized. Also indicative of the great affinity of HPOP for the pancreas was the high number and earlier development of ductular adenocarcinomas, compared to that of ductal adenomas and intraductal carcinomas. The lower incidence of intraductal carcinomas in this experiment, as compared to that with BOP and BHP, further confirms the role of ductular, as opposed to ductal, epithelium in pancreatic carcinogenesis (10). Thus, the ductular cells, including the islet cell precursors, apparently represent the main target cells for the carcinogen (10). In this context, the molecular structure of HPOP deserves particular attention. The affinity of the diabetogen and pancreatic carcinogen streptozotocin for islet cells has been attributed to the glucose moiety of streptozotocin, which may act as a carrier for the methylnitroso group (1, 4, 26). We have previously shown (3) that HPOP exists in 2 tautomeric forms. The cyclic tautomer results from an intramolecular hemiacetal formation. The similarity of the cyclic form of HPOP to the pyranose form of hexose sugars and to the glucose moiety of streptozotocin (Chart 2) may also account for the predilection of the compound for pancreatic ductular cells, including the islet cell precursors, which apparently have sugar receptors (24). We do not yet know why streptozotocin affects the mature islet cells and HPOP affects the islet cell precursors.

Despite the similar potent carcinogenic effect of HPOP and BOP on the pancreas, there are some differences in their overall neoplastic action. In contrast to BOP, HPOP induced tumors in the nasal cavity, larynx, trachea, and other organs, with a spectrum similar to that of BHP (18). This may be due to use in this study of doses of HPOP (which were based on the LDo50) that were about 4 times greater than those of the more toxic BOP in the comparable experiment. Since both BOP and HPOP are metabolized to some extent to BHP and more BHP is formed from HPOP than from BOP (2, 3), it can be assumed that much larger amounts of BHP have been formed from HPOP than from BOP, thus accounting for the similar tumor spectra induced by HPOP and BHP. This suggestion is also consistent with the finding that the higher the administered dose of BOP (and consequently the amount of the metabolite BHP formed), the broader is the tumor spectrum (12, 22).

Thus, the suggestion that HPOP may be the proximate pancreatic carcinogen when BOP or BHP is administered to hamsters appears to be supported by this study. However, the fact that HPOP is also formed from BOP and BHP in the rat, which is not susceptible to pancreatic carcinogenesis by these compounds (9, 23), implies either that the metabolic control of the rat and hamster pancreas is different or that HPOP is a precursor to some other proximate carcinogenic metabolites, which may not be formed from HPOP in the rat pancreas. The similarity of BOP metabolites in pancreatic and biliary secretions in the rat and hamster the specificity of BOP and HPOP for the islet cell precursors suggest that neither secretion of nitrosamine metabolites in the pancreatic duct nor reflux of bile containing metabolites into the pancreatic duct (16) are mechanisms for pancreatic carcinogenesis in this animal model. Further studies are required to determine more precisely the mechanism(s) of pancreatic carcinogenesis of HPOP and related nitrosamines for the hamster pancreas.

**REFERENCES**

Fig. 4. Higher magnification of the same lesion in Fig. 3 showing partial keratinization of tumor cells H & E. x 300.

Fig. 2. High-powered view of Fig. 1 showing mucous glands invading the duodenal wall. Note the similarity of tumor cells to those of Brunner glands. H & E, x

Fig. 3. Keratinizing squamous cell carcinoma of the flank origin. Hyperplasia of the adjacent glands (lower left corner). H & E, x 60.

Fig. 1. Duodenal adenocarcinoma composed of branching glands lined by Brunner gland-type mucous cells. H & E, x 80.

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1. P. Pour et al.


22. Weil, C. S. Tables for convenient calculation of median effective dose (LD50 or ED50) and instructions in their use. Biometrics, 8: 249–263, 1952.

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