Augmentation of Carcinogenesis by \textit{N}-Nitrosobis(2-oxopropyl)amine Administered during \textit{S} Phase of the Cell Cycle in Regenerating Hamster Pancreas

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ABSTRACT

Pancreatic acinar cells in various rodent species are capable of undergoing modulation to duct-like structures upon extended exposure to pancreatic carcinogens. Although the majority of malignant pancreatic neoplasms induced in rat and guinea pig are of acinar cell origin, some adenocarcinomas closely resembling those of ductal derivation have been reported. In hamster, on the other hand, carcinoma-induced duct-like modulation of acinar cells is followed exclusively by the development of ductal adenocarcinoma. The present experiments, in which the carcinogen \textit{N}-nitrosobis(2-oxopropyl)amine (BOP) was administered initially to hamsters during ethionine-induced pancreatic regeneration at a time when the maximum number of acinar cells was in \textit{S} phase of the cell cycle, were undertaken to ascertain the extent and nature of acinar cell response to such treatment and the pattern of tumorigenesis. BOP was also administered weekly following the cessation of regeneration for periods ranging from 1.5 to 9 weeks to achieve total doses of 120, 90, and 40 mg of BOP per kg. Controls consisted of hamsters with normal nonregenerating pancreas that were treated with BOP on identical schedules to those of the experimental groups. The largest number of carcinomas (12 in 16 animals or 75%) developed in the highest-dose test group as compared to 10 in 26 animals or 38% in its control group. The difference was statistically significant ($p < 0.05$). Other groups of hamsters with regenerating and nonregenerating pancreas receiving lower doses of carcinogen had tumor incidences which were not statistically different from one another. These experiments yielded ductal adenocarcinomas exclusively, although it is of interest that the two most common benign lesions encountered in these animals were cystadenomata often lined by zymogen-containing cells and duct-like modulation of acinar cells. Finally, in some of the animals with carcinomas, nests of duct-like structures, some of which appeared to arise from acini, were lined by severely atypical epithelium with numerous mitoses. The increased incidence and exclusive development of ductal adenocarcinoma in animals to whom carcinogen was administered during pancreatic regeneration coupled with the changes noted above are interpreted as presumptive evidence that acini may be involved in the pathogenesis of pancreatic ductal adenocarcinoma in the hamster.

INTRODUCTION

Pancreatic carcinomas induced in the Syrian golden hamster by \textit{\beta}-oxidized derivatives of \textit{N},\textit{N}-dipropyl nitrosamine are of considerable interest because they are ductal adenocarcinomas (18, 19) closely resembling those that constitute the most common malignant pancreatic neoplasm in humans (6, 14). It is noteworthy that ductal adenocarcinomas should develop so readily in the pancreas where ductal cells constitute a minor component of the pancreatic cell population, as contrasted to acinar cells which represent 82% of the total volume of the organ (3). The exclusive development of ductal adenocarcinoma in hamster following chronic exposure to carcinogens may be due to, a special metabolic property of duct cells (14), a greater sensitivity of ductal epithelium to carcinogenic metabolites or to a histogenetic pathway for tumorigenesis not limited to duct epithelium. Although the first 2 possibilities remain to be established, the latter has been repeatedly suggested by the results of numerous carcinogenesis experiments involving the pancreas of the guinea pig (21), rat (2, 7), and hamster (28). In each instance, duct-like structures appear to arise from acinar cells following exposure to chemical carcinogens and prior to the development of pancreatic carcinoma.

The present experiments were undertaken to ascertain whether exposure of proliferating acinar cells to carcinogen during pancreatic regeneration has any effect on the nature and yield of pancreatic neoplasms induced. This was accomplished by administering carcinogen to animals during pancreatic regeneration at the time when predominantly acinar cells are in \textit{S} phase of the cell cycle, as compared to only rare islet and duct cells (30). In view of the fact that cells in the process of synthesizing DNA have been shown to be more susceptible to the tumorigenic effects of carcinogens (4, 5, 10), acinar cells at this stage of regeneration should be at highest risk for undergoing the neoplastic transformation.

MATERIALS AND METHODS

Pancreatic Regeneration. Forty-eight postweanling (5 to 6 weeks) male hamster (Charles River Breeding Laboratories, Wilmington, Mass) weighing 35 to 40 g were maintained on a pelleted full amino acid diet (26) for 2 weeks prior to start of the experiment, at which time they were changed to a methionine-deficient diet. Simultaneously, these animals received daily i.p. injections of \textit{\alpha}-ethionine (500 mg/kg body weight) in 0.9% NaCl solution for 8 days. Pancreatic regeneration was initiated on Day 9 by a single i.p. injection of \textit{\lambda}-methionine (800 mg/kg body weight) and returning the animals to the full amino acid diet (30).

Carcinogenesis Experiments. Hamsters with regenerating pancreas were divided into 3 groups and exposed to different doses of carcinogens to determine the sensitivity of regenerating acinar cells to...
various doses and patterns of carcinogen administration. Final size of the groups varied because of death of some animals (27% mortality) during the induction of regeneration. Group 1 received a single s.c. injection of BOP2 (30 mg/kg body weight) 60 hr after regeneration was initiated when the largest number of acinar cells, approximately 22.4%, are in S phase of the cell cycle (30). This was followed by 10 mg BOP per kg body weight each week for 9 weeks for a total dose of 120 mg/kg. Group 2 animals were treated with 2 doses of 5-mg/kg body weight administered at 60 and 66 hr following initiation of regeneration and subsequently received 10 mg BOP per kg body weight administered on Monday and Friday of each week for 4 weeks for a total dose of 90 mg/kg; and Group 3, in which animals were treated identically to those in Group 2 with the exception that postregeneration treatment with BOP was limited to 1.5 weeks instead of 4, received a total dose of 40 mg/kg. Three groups of hamsters maintained on the full amino acid semisynthetic diet, in which pancreatic regeneration was not induced and receiving identical total doses of BOP, served as controls. These included the following: Group 1A consisting of 26 animals received an initial s.c. injection of 30 mg BOP per kg body weight followed by 9 weekly injections of 10 mg BOP per kg body weight; Group 2A consisting of 10 animals was treated with 2 doses of 5 mg BOP per kg body weight administered s.c. 6 hr apart during the first week followed by 10 mg BOP per kg body weight injected on Monday and Friday of each week for 4 weeks; and Group 3A animals were treated identically as those in Group 2A except that following the first week, administration of carcinogen was continued for only 1.5 weeks. Animals were sacrificed when moribund or 26 to 28 weeks from the start of the experiment. The pancreas was removed, fixed in buffered formalin, and embedded. Step sections were cut so that 4 slides were prepared from each organ for light microscopic study. The statistical significance of tumor incidence was tested by the 2-sided $\chi^2$ method with correction for continuity.

RESULTS

Tumor Incidence. In Group 1, ductal carcinomas were induced in 12 of 16 animals (75%) in which the initial dose of BOP was administered at the height of S phase during regeneration. This incidence was statistically significant ($p < 0.05$) when compared to that of its control, Group 1A, in which only 10 carcinomas were induced in 26 animals (38%) in which an identical initial dose of carcinogen was administered to animals with a normal (nonregenerating) pancreas. With lower doses of carcinogen administered during regeneration, there was a marked decrease in tumor incidence, 30 and 11%, respectively, for Groups 2 and 3, which were not significantly different from the incidence in their control, Groups 2A and 3A, suggesting that regeneration did not render acinar cells sufficiently sensitive to lower doses of carcinogen to increase tumor yield. These results are summarized in Table 1.

Type and Frequency of Pancreatic Lesions. The most frequent pancreatic lesion present in all animals receiving carcinogen was the benign neoplastic proliferation, cystadenoma. This consisted of foci of cystic duct-like structures lines by cuboidal to flattened epithelium. In each instance, some of these cells contained zymogen granules and basal nuclei, cytological characteristics of acinar cells (Fig. 1). A second almost equally frequent tissue change involved acinar cells in pancreatic lobules. Acini were dilated, the apical cytoplasm was attenuated, and there was a variable loss of zymogen granules (Fig. 2). Such acini were encountered with greatest frequency in Group 1 animals, where they assumed the profiles of small ducts. These were present as long as 28 weeks from the start of the experiment and involved large areas of the pancreas. Not infrequently, such duct-like structures were the focus of increased mitosis and severe cellular atypia (Fig. 3). Unequivocal evidence of malignant change in such foci was not found. However, their frequent apposition to areas of ductal cancer, coupled with the changes mentioned above, suggests that they may represent precursor lesions. Intraisular ducts were present; however, in no instance were either atypical or preneoplastic changes encountered in them. Florid atypical hyperplasia of small and medium-sized pancreatic ducts consisted of frond-like proliferation of epithelium which extends into the duct lumen (Fig. 4); ducts including large branches of the main pancreatic duct were the focus of in situ adenocarcinoma with its characteristic cribriform pattern of proliferation (Fig. 5). All of the malignant neoplasms induced, including invasive ones, were moderately well-differentiated ductal adenocarcinomas (Fig. 6). These consisted of crowded ducts of varying sizes lined by epithelial cells with bizarre hyperchromatic nuclei and numerous mitoses. In no instance were benign or malignant lesions of acinar, mixed ductal-acinar, or ductal-islet cell type encountered in any of the 82 animals that developed pancreatic pathology as a result of treatment with BOP.

DISCUSSION

Augmentation of pancreatic tumorigenesis when BOP was administered to regenerating pancreas was not unexpected and supports the current view that replicating cells are more susceptible to the deleterious effects of carcinogens. In regenerating pancreas, acinar cells are exquisitely sensitive to even a single dose of BOP as shown by a significant prolongation of mitosis, the induction of numerous mitotic abnormalities, and nucleolar segregation (29). It is noteworthy, however, that ductal adenocarcinomas were induced exclusively even when the cells at highest risk in regenerating pancreas during the initial exposure to BOP were acinar cells with a mitotic index of approximately 16.8 per 1000 acinar cells as compared to only 1.8 and 1.4 per 1000 cells for islet and duct epithelium, respectively, as determined in a previous study (30). These results can be interpreted in one of 2 possible ways, either that acinar cells are unable to metabolize BOP to an active carcinogenic form and are not target cells, or that exposure to carcinogen leads to a phenotypic alteration of acinar cells to duct-like epithelium, which in turn undergoes malignant change. The first possibility can be dismissed in view of studies which demonstrate that hamster pancreas can activate the pancreatic carcinogen BOP to mutagens (31) and, further, that isolated acinar cells show a significantly greater capacity to metabolize such carcinogens to a variety of oxidized metabolites, which are known to be carcinogenic for pancreas (27), than do isolated islet cells. These findings corroborate an earlier report (25) in which the pancreatic carcinogen $[3H]^{-}$NNDM was localized by ultrastructural autoradiography principally to acinar cells of the hamster. However, Reznik-Schüller et al. (25) did not localize labeled carcinogen in duct epithelial cells, suggesting these cells do not metabolize NNDM. This differs from the results of our study in which DNA repair was observed in nuclei of ductal epithelium following incubation in

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2 The abbreviations used are: BOP, N-nitrosobis(2-oxopropyl)amine; NNDM, N-nitroso-2,6-dimethylmorpholine.
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In this paper, we present additional biological and morphological evidence that acinar cells may be involved in the pathogenesis of pancreatic neoplasms in the hamster. However, in contrast to Flaks et al. (8), we believe that pancreatic ducts do participate in the development of pancreatic cancer in the hamster. This conclusion is based on the presence of numerous neoplastic lesions as well as noninvasive and invasive adenocarcinoma in both small and large pancreatic ducts. Accordingly, we postulate that a dual pathway may exist for the development of chemically induced pancreatic cancer in the hamster involving both acini and ducts. Since metabolites of BOP are present in pancreatic juice following its administration to hamsters (11), it seems reasonable to assume that these are released by acinar cells into the ducts and therefore are also capable of affecting duct epithelium. Whether such is also true for pancreatic carcinoma in humans where the majority of neoplasms appear to be of ductal and duct derivation remains to be established.

ACKNOWLEDGMENTS

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<table>
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<th>Group</th>
<th>Effective animals (mg)</th>
<th>Total dose of carcinogen (mg)</th>
<th>Gross tumors (total)</th>
<th>Cancer Invasive (total)</th>
<th>In situ (total)</th>
<th>Carcinomas (total %)</th>
<th>Cystadenoma (total)</th>
<th>Appearance of first tumor (wk)</th>
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<td>7 (27)</td>
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<tr>
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<td>10 (91)</td>
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a Numbers in parentheses, percentage.

vitro with NNDM, suggesting that it is metabolized by duct cells (27). Phenotypic alteration of acinar cells was described some years ago. In these studies, duct-like structures developed in pancreatic acini following protracted exposure to diverse carcinogens such as 7,12-dimethylbenz(a)anthracene (2, 7), azaserine (13), N-methyl-N-nitrosourea (21, 23), BOP (28), and N-nitrosobis(2-hydroxypropyl)amine (12). Although such modulation of pancreatic acini has been described in 3 rodent species, pancreatic ductal adenocarcinomas have been induced reproducibly only in the hamster. Pancreatic carcinogenesis in rat and guinea pig appears to involve the acinar cell (13, 20, 21, 22) except, in the case of 7,12-dimethylbenz(a)anthracene in the rat, where the carcinomas induced more closely resembled poorly differentiated ductal adenocarcinomas than acinar cell carcinomas (2, 7). Conversion of acinar cells to duct-like epithelium is also encountered in sublethal traumatic injury to pancreas 4 and may simply represent a general reaction of such cells to injury. The view that acinar cells can, under the appropriate stimulus, be caused to undergo the transition to duct-like epithelium conflicts with the observation that during pancreatic development, acinar cells are derived from duct epithelium (15). The involvement of acinar cells in the genesis of ductal adenocarcinoma is also supported by their greater sensitivity than either islet or duct epithelium to carcinogens such as 7,12-dimethylbenz(a)anthracene, azaserine, and N-methylnitrosourea, which is a pancreatic carcinogen in that species. Our investigations. Am. J. Pathol., 83: 517–522, 1976. 2. Bockman, D. E., Black, O., Jr., Mills, L. R., and Webster, P. D. Origin of tubular complexes developing during induction of pancreatic adenocarcinoma by 7,12-dimethylbenz(a)anthracene. Am. J. Pathol., 90: 645–656, 1978. 3. Bolender, R. P. Stereological analysis of the guinea pig pancreas. I. Analytical model and quantitative description of nonstimulated pancreatic exocrine cells. J. Cell Biol., 61: 269–287, 1974. 4. Craddock, V. M. Induction of liver tumours in rats by a single treatment with NNDM, suggesting that it is metabolized by duct cells (27). Phenotypic alteration of acinar cells was described some years ago. In these studies, duct-like structures developed in pancreatic acini following protracted exposure to diverse carcinogens such as 7,12-dimethylbenz(a)anthracene (2, 7), azaserine (13), N-methyl-N-nitrosourea (21, 23), BOP (28), and N-nitrosobis(2-hydroxypropyl)amine (12). Although such modulation of pancreatic acini has been described in 3 rodent species, pancreatic ductal adenocarcinomas have been induced reproducibly only in the hamster. Pancreatic carcinogenesis in rat and guinea pig appears to involve the acinar cell (13, 20, 21, 22) except, in the case of 7,12-dimethylbenz(a)anthracene in the rat, where the carcinomas induced more closely resembled poorly differentiated ductal adenocarcinomas than acinar cell carcinomas (2, 7). Conversion of acinar cells to duct-like epithelium is also encountered in sublethal traumatic injury to pancreas 4 and may simply represent a general reaction of such cells to injury. The view that acinar cells can, under the appropriate stimulus, be caused to undergo the transition to duct-like epithelium conflicts with the observation that during pancreatic development, acinar cells are derived from duct epithelium (15). The involvement of acinar cells in the genesis of ductal adenocarcinoma is also supported by their greater sensitivity than either islet or duct epithelium to the acute and chronic effects of pancreatic carcinogens that induce ductal carcinoma (12, 28). Focal necrosis and subsequent regeneration soon after administration of carcinogen are sustained with continued treatment and are almost totally limited to acinar cells. This regeneration renders them more sensitive to carcinogen-induced alterations of DNA and could lead to their neoplastic transformation. In an earlier report, it was shown that regenerating pancreas covalently binds [3H]-NNDM 20-fold over that bound by normal nonregenerating pancreas (30). Mitosis of duct and ductular epithelium appears only after protracted exposure to carcinogen and is much less intense than in acinar cells.

Morphological details of the manner by which acinar cells may be involved in the pathogenesis of pancreatic ductal adenocarcinoma are not yet entirely clear. In all the reports including our own, their implication is indirect and presumptive. Flaks et al. (8, 9) have established that the cells which comprise the multifocal cystadenomatous lesions in the hamster, which they consider to be putative precursors of pancreatic ductal carcinoma, possess the cytological and ultrastructural characteristics of acinar cells. A similar conclusion was reached by Rao and Reddy (20, 21) from their analysis of acinar cell alterations they term pseudoductular changes encountered in guinea pig pancreas chronically exposed to N-methyl-N-nitrosourea, which is a pancreatic carcinogen in that species; however, they were not able to unequivocally establish that these evolved into ductal carcinomas. Pour et al. (16–18) and others (1, 24, 32), on the other hand, maintain that chemically induced pancreatic adenocarcinoma in the hamster arises solely from preexisting ducts and proliferating intralobular, perisinus, and intrainsular ductules, the latter often accompanied by proliferation of islets. Their reports make no mention of either the sustained mitogenic effects of pancreatic carcinogens on acinar cells or their early and persistent modulation to duct-like structures.
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...nuclei and apical cytoplasmic granules identical to zymogen granules in pancreatic acinar cells. Toluidine blue, x 900.

The origin of such "ducts" from acini is suggested by the presence of residual zymogen granules figures in the epithelial cells of some of the "ducts" indicate an increased proliferative capacity. H & E, x 390.


...Early changes in regenerating hamster pancreas following a single dose of N-nitrosobis(2-oxopropyl)amine (NBOP) administered at the peak of DNA synthesis. Cancer (Phila.), 47: 1552–1561, 1981.


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Fig. 5. A high magnification of intraductal \textit{in situ} adenocarcinoma showing the characteristic cribriform pattern of epithelial proliferation and several foci of cell necrosis. BOP was administered during the peak of pancreatic regeneration. H & E, x 500.

Fig. 6. Invasive moderately well-differentiated ductal adenocarcinoma from an animal treated with BOP during the height of pancreatic regeneration. H & E, x 320.
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