Orotic Acid Enhancement of Preneoplastic and Neoplastic Lesions Induced in the Pancreas and Liver of Hamsters by N-Nitroso(2-hydroxypropyl) (2-oxopropyl)amine

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ABSTRACT

The effect of dietary orotic acid (OA) in liver-pancreas carcinogenesis induced in female Syrian hamsters by N-Nitrosobis(2-hydroxypropyl)amine (HPOP) was evaluated. All animals infused with the carcinogen received the same doses. Results of the control group which received no OA or carcinogen were compared with the results of: (a) hamsters treated with HPOP and fed a regular 20% protein synthetic diet (group 1); (b) hamsters fed the OA diet for a brief time period during initiation with the carcinogen (group 2); and (c) hamsters in which OA was administered after carcinogen infusion for life (group 3).

All animals of the control group were normal at autopsy, while those in group 1 (HPOP alone) revealed the spectrum of lesions accepted as classical in the multitissue hyperplasia-dysplasia-carcinoma in situ (CIS) sequence of carcinogenesis. Results of group 2, in light of group 1, revealed an increased incidence of the following lesions in the common pancreatic duct: dilatation, 2.5 times; flat and papillary hyperplasia, 2 times; and dysplasia (atypical hyperplasia), 12 times. No significant increase of CIS and invasive cancer in the body and tail of the pancreas was observed; in addition, the incidence, nature, and location of pancreatic adenocarcinomas were not affected. Yet, the effect of OA administered after carcinogen infusion (group 3) when compared to group 1 seemed to enhance a further increase in the incidence of practically all lesions throughout the pancreas. An obvious overall step-up incidence along the multitissue hyperplasia-dysplasia-CIS-invasive cancer process in the pancreas was observed. The increase in incidence of flat, papillary, and atypical epithelium of the common pancreatic duct in group 3 was mild compared to that found in the same duct of group 2, but the increase in incidence of these same three lesions when found in the main ducts was marked: flat hyperplasia, 3-fold; papillary hyperplasia, 2.5-fold; atypical hyperplasia, 3-fold. The increase in incidence of CIS in this group was 5-fold and papillary adenocarcinomas, 3-fold, when compared to 5% found in groups 1 and 2. Hepatic malignancies (cholangiocarcinomas) occurred in 6% of the cases in group 3 compared to none in group 2; the incidence of malignancy in the gallbladder was the same in groups 2 and 3 but three times greater than that in group 1. Our results suggest that not all ductal cells of the pancreas respond to OA promotion, which implies that various pancreatic tumors in the hamster, and by extrapolation, in humans, may have different etiologies.

INTRODUCTION

BOP and its reduced analogues HPOP and N-nitrosobis(2-hydroxypropyl)amine (4), induce tumors of the pancreas in the Syrian golden hamster, which are adenocarcinomas of the ductal phenotype and are mostly localized in the body of the gastric lobe. In many respects, these tumors are similar to those found in the human pancreas.

The hamster model has been studied extensively using BOP as the carcinogen of choice because of its greater carcinogenic effectiveness compared to other pancreas-specific nitrosamines. Although BOP is known to induce a high incidence of pancreatic tumors, it has a modest effect on main duct abnormalities such as hyperplasia, papillary hyperplasia, dilatation, and obstruction (2, 5), unless tumors invade this duct or its branches. BOP-induced pancreatic tumors in the hamster model are believed to originate from ductal ductules in the body and tail of the gastric lobe. Ductal adenocarcinomas are often associated with intraluminal proliferation of atypical epithelium in papillary or microglandular patterns. Such an association is indicative of tumor origin. Neoplasms and preneoplastic lesions in the head of the pancreas are a secondary feature of the BOP-hamster model. Cystadenomas or adenocarcinomas in the head of the hamster pancreas, originating either from large ducts or from ductular epithelium, comprise 1 to 2% of all induced tumors (2). The incidence of the above lesions can be increased by prolonged treatment with BOP, but even then the incidence is not high enough for the study of such lesions experimentally.

In contrast to the BOP-hamster model, about one-half of pancreas exocrine tumors in humans are localized in the head and peripancreatic region (6-8), whereas the other one-half is distributed in the body and tail of the pancreas. With the advent of diagnostic procedures such as retrograde pancreatography, papillary lesions occurring in large ducts in the cephalic portion of the pancreas have recently attracted attention. Papillary projections lined with tall columnar epithelium and mucus-secreting cells in the main pancreatic duct of human pancreas are often associated with intraductal mucinous papillary carcinomas in a dysplasia-CIS-cancer sequence of events (9, 10). These projections are further associated with diffuse or segmental dilation of pancreatic ducts (11, 12) and possibly with the formation of cysts. The extreme low incidence of premalignant lesions which are believed to lead to mucus-hypersecreting tumors of the main pancreas, in the hamster model are believed to originate from dysplastic ductules in this duct or its branches.4 BOP-induced pancreatic tumors in the hamster model are believed to originate from ductal ductules in the body and tail of the gastric lobe. Ductal adenocarcinomas are often associated with intraluminal proliferation of atypical epithelium in papillary or microglandular patterns. Such an association is indicative of tumor origin. Neoplasms and preneoplastic lesions in the head of the pancreas are a secondary feature of the BOP-hamster model. Cystadenomas or adenocarcinomas in the head of the hamster pancreas, originating either from large ducts or from ductular epithelium, comprise 1 to 2% of all induced tumors (2). The incidence of the above lesions can be increased by prolonged treatment with BOP, but even then the incidence is not high enough for the study of such lesions experimentally.

In contrast to the BOP-hamster model, about one-half of pancreas exocrine tumors in humans are localized in the head and peripancreatic region (6-8), whereas the other one-half is distributed in the body and tail of the pancreas. With the advent of diagnostic procedures such as retrograde pancreatography, papillary lesions occurring in large ducts in the cephalic portion of the pancreas have recently attracted attention. Papillary projections lined with tall columnar epithelium and mucus-secreting cells in the main pancreatic duct of human pancreas are often associated with intraductal mucinous papillary carcinomas in a dysplasia-CIS-cancer sequence of events (9, 10). These projections are further associated with diffuse or segmental dilation of pancreatic ducts (11, 12) and possibly with the formation of cysts. The extreme low incidence of premalignant lesions which are believed to lead to mucus-hypersecreting tumors of the main pancreatic duct impedes a better study of these tumors using the current BOP-hamster model. Further development of the hamster model is needed to selectively increase the incidence of intraductal tumors and their preceding associated lesions. In pursuing a higher incidence of premalignant lesions and a wider spectrum of pancreatic tumors, this laboratory has introduced promoting dietary regimens following a defined initiating period with HPOP (13, 14) a carcinogen which is more versatile and less toxic than BOP (3). Based on the assumption that pancreatic cancers may initially originate from more than one cell type (15-18) and as such may respond differently to various stimuli, promoting regimens are expected to modify not only the incidence of tumors but also their spectrum. Here we report the effect of orotic acid, a promoter which has been shown to alter the rate of DNA synthesis in target organs in this and other experimental models of gastrointestinal carcinogenesis (19). Since rates of DNA synthesis and cell replication have been widely recognized as important components in cancer initiation, modulation of such rates is expected to further promote the understanding of the mechanism of induction and origin of pancreatic tumors.

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3 The abbreviations used in this paper are: BOP, N-nitrosobis(2-oxopropyl)amine; HPOP, N-nitrosobis(2-hydroxypropyl)amine; CIS, carcinoma in situ; H&E, hematoxylin and eosin; OA, orotic acid.

4 D. M. Kokkinakis, unpublished observations.
MATERIALS AND METHODS

Chemicals. HPOP was synthesized according to methods published previously (20). HPOP was administered continuously at a total dose of 170 mg/kg for 9 days via 2001 Alzet (Alza Co., Palo Alto, CA) osmotic pumps, which were implanted s.c. as previously described (13).

Diets. The following diets were purchased from Dyets, Inc. (Bethlehem, PA) in the form of pellets: (a) regular medium protein (20% casein) synthetic diet having a composition identical to that described in a previous study (Ref. 14; herein referred to as regular diet); and (b) medium protein diet similar to 1 (regular diet), but supplemented with 1% OA at the expense of cornstarch (herein referred to as OA diet).

Animals. Two hundred and twenty 4-week-old female golden Syrian hamsters (Charles River, Wilmington, DE) were placed on regular diet immediately upon arrival. Animals were kept on this diet for approximately 15 days until they reached 70 g. At that time, they were randomly distributed into three groups of 40 animals per group, respectively. All hamsters in the first group (−OA) remained on the regular diet; then, when they reached 85 g, they were treated with HPOP as described previously (13). These animals were fed the same diet until sacrificed at 30 weeks after carcinogen treatment. Animals in the second group (+OA,I) were switched to the OA diet until they reached 85 g (approximately 12 days); then they were treated with HPOP as described above and returned to the regular diet 2 weeks after completion of carcinogen treatment. A third group, (+OA,P), received the same diet and HPOP treatment as group 1; however, they were switched to the OA diet 2 weeks following completion of carcinogen infusion and were kept on that diet until sacrificed. Two additional groups consisting of 12 hamsters each were placed on regular and OA diets, respectively, 2 weeks after implantation of osmotic pumps containing saline. All animals were killed and autopsied 28 weeks after the implantation of pumps. Pancreases, livers, lungs, and kidneys were removed, fixed in 10% buffered formalin, embedded in paraffin, sectioned at 20-μm intervals (five sections), stained with H&E, and examined histologically.

Statistical Analysis. Two-tailed χ² analysis was used for pairwise comparisons of the incidence of various lesions among the three groups.

RESULTS

HPOP induced a modest incidence of pancreatic and hepatic lesions in female Syrian hamsters when infused s.c. at a rate of 18.9 mg/kg/day for 9 days. In both pancreas and liver, the carcinogen affected the ductal mucosa, giving rise to tumors of ductal phenotype. In the pancreas, hyperplastic changes were determined in the common and main pancreatic ducts from at least four serial sections of paraffin-embedded specimens. Hyperplastic changes were classified, depending on their location (main or common pancreatic ducts), and on microscopic appearance (flat or papillary). Lining of ducts by tall columnar epithelial cells arranged in a unilayer fashion, with or without the presence of goblet cells, was regarded as flat hyperplasia. Fibrovascular projections of the mucosa lined by columnar cells, with or without the presence of goblet cells, was regarded as papillary hyperplasia. Following this original classification, ducts with flat or papillary hyperplasia were further screened for the presence of cytological atypia. When segments of the common or the main pancreatic ducts appeared pseudostratified and showed dysplastic changes characterized by columnar and goblet cells having enlarged, often elongated hyperchromatic nuclei and with small nucleoli, the term atypical hyperplasia was applied. Atypical hyperplasia was not subclassified into papillary or flat for statistical considerations. Enlargement of the diameter of hyperplastic ducts by 3-fold or more as compared to normal was regarded as dilation. Ducts or clusters of papillary projections lined by atypical epithelial cells showing nuclear pleomorphism, loss of polarity, and mitotic figures were regarded as CIS. Tumors were classified into papillary and nonpapillary, according to the criteria first described by Pour (2) for the hamster model.

Group 1: Carcinogen without OA Added to Regular Diet (−OA)

Pancreas. Treatment with HPOP alone resulted in the induction of ductal pancreatic hyperplasia, dysplasia, and neoplasia. Two striking observations emerged: (a) the cases of hyperplasia and dysplasia were found only in the head and body, with none of the malignancies occurring in the head and all CIS and invasive carcinomas confined to the body and tail; and (b) most changes occurred either in the common duct or in the major ducts of the head, except for the cases of hyperplasia with goblet cell metaplasia, which were more common in ductules of the body. Flat hyperplasia occurred in 35% of the cases in both common and main ducts of pancreatic head. Papillary hyperplasia occurred in 30 and 10%, respectively, in common and main ducts of pancreatic head (Table 1). Flat hyperplasia was often accompanied by focal papillary proliferation of tall columnar cells. Ductal dilatation and concomitant destruction (atrophy) of adjacent lobules was observed in 20% of the animals. Such changes were confined to the pancreatic common duct and were not induced in the hepatic section of the bile duct or in the gallbladder of the affected hamsters. Atypical hyperplastic changes or goblet cell metaplasia in the common pancreatic duct were uncommon, found only in 2 of 40 animals. The incidence of pancreatic cancer in animals treated with HPOP alone (−OA) was moderate (25%), due to the low dose of carcinogen, compared to that used in previous studies (14). In situ carcinomas were present in four animals, and invasive cancers were present in another six animals. The latter varied between 0.4 and 0.6 cm in diameter and were either poorly differentiated (2 of 40) or well-differentiated mixed-tubulo-papillary adenocarcinomas (4 of 40). All six tumors were surrounded and infiltrated by lymphocytes. All in situ and invasive carcinomas were restricted to the body and tail of the pancreas.

Liver/Gallbladder. Liver tumors were exclusively cholangiocarcinomas, occurring in 20% of the cases (8 of 40) and ranging from 0.5 to 2 cm in diameter.

Table 1. Effect of OA administration on HPOP carcinogenesis in the hamster

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective no. of hamsters</th>
<th>Pancreas</th>
<th>Liver/Gallbladder</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>40</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Group 2</td>
<td>38</td>
<td>55</td>
<td>5</td>
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<tr>
<td>Group 3</td>
<td>32</td>
<td>0</td>
<td>20</td>
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</table>

+ OA alone (−OA). Regular diet throughout.

+ OA plus OA at initiation (+OA,OA). OA diet 2 weeks before and after HPOP treatment. Returned to regular diet until sacrificed.

+ OA postinitiation (+OA,P). Regular diet before, during, and after 2 weeks after HPOP treatment and then switched to the OA diet until sacrificed.

P values for group 2 as compared to group 1.

P values for group 3 as compared to group 1.

NS, not significant.

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to 1.5 cm in diameter. Gallbladder adenocarcinomas were of the papillary type and occurred in 5% (2 of 40) of the animals. There was no association between tumors of the gallbladder and pre-malignant or malignant lesions of the pancreas. In four cases, tumors of the liver and pancreas coexisted. In another four, cholangiocarcinomas were present in animals with various degrees of hyperplasia in either the main or common pancreatic duct. Finally, in four cases, the liver was the only organ affected.

Group 2: OA Treatment Prior to and during Initiation with Carcinogen (+OA,I)

Pancreas. Administration of OA prior to and during treatment with OA did not result in significant modification in the incidence of cancer or preneoplastic changes in the body or tail of the pancreas. The treatment, however, doubled the incidence of hyperplastic changes in the common pancreatic duct as compared to treatment with HPOP alone. Thus, flat hyperplasia of the duct rose from 35 to 68% and papillary hyperplasia increased from 30 to 63%. At the same time, OA increased the incidence of atypical hyperplasia of the common duct 12 times and tripled the incidence of dilation of that duct as compared to treatment with HPOP alone. The effect of OA coadministration on HPOP-induced lesions of the common pancreatic duct is shown in Fig. 1A (papillary hyperplasia and dilation) and in Fig. 1B (moderate to severe atypia). Differences in pancreatic malignancies, hyperplastic changes, atypia, or dilation of the main pancreatic duct were not apparent between the (−OA) and (+OA,I) regimens.

In spite of the impressive effect of OA coadministration on papillary hyperplastic changes in the head of the pancreas, the number and frequency of lesions in the rest of the tissue was not affected by (+OA,I) treatment. The number and frequency (multiplicity) of pancreatic adenocarcinomas was also no different from that induced by HPOP alone. Overall, 6 invasive and 4 in situ carcinomas were found in 10 animals. Two-thirds of the invasive tumors were classified as mixed papillary tubular adenocarcinomas, while the rest were poorly differentiated. Therefore, the administration of OA prior to and during initiation with HPOP did not affect the incidence, nature, or location of pancreatic adenocarcinomas.

Liver/Gallbladder. No cholangiocarcinomas developed in this group compared to 20% (8 of 40) of cases in group 1 (−OA). However, coadministration of OA resulted in: (a) increased incidence of papillary hyperplasia of the gallbladder from 5% in group 1 to 26% in group 2; (b) increased incidence of malignancy of the gallbladder (CIS-invasive carcinoma) from 5% in group 1 to 16% in group 2 (+OA,I), although due to low tumor incidence, such increase does not have statistical significance; and (c) increased incidence in the concurrence of gallbladder hyperplasia-CIS-invasive carcinoma and severe pancreatic ductal hyperplasia.

In spite of such concurrence, the lesions in the common ducts were benign and not the result of extension of the gallbladder tumors, nor were any associated malignant pancreatic lesions present in these cases. In three of these animals, the pancreatic common duct hyperplasia extended into the main ducts. These three cases also revealed severe ductule proliferation and hyperplasia, which occurred at the junctions of the common and main ducts, all of which were lined with tall columnar epithelium (Fig. 2).

Group 3: Postinitiation Treatment with OA (+OA,P)

Pancreas. The incidence of all pancreatic lesions increased significantly when HPOP-treated animals were fed a diet containing 1% OA 2 weeks after termination of carcinogen administration for 28 weeks (+OA,P). Invasive primary and in situ carcinomas were found in 44% of the animals in this group versus 15% found in (−OA) or (+OA,I) regimens. Furthermore, the incidence of atypical hyperplasia in the main pancreatic ducts (Fig. 3) was at least three times greater than that observed in the previous groups (P < 0.001). Ductal adenocarcinomas induced by HPOP and a postinitiation treatment of OA were in general well-differentiated tubular ductular or mucinous ductular adenocarcinomas (Fig. 4). An association of these tumors with the main duct was suggestive, due to the presence of hyperplastic changes and premalignant or malignant lesions within segments of the main duct surrounded by the tumor (Fig. 4). In addition to papillary or mixed papillary tumors, two animals developed ductular-acinar cell carcinomas that appeared to originate from zymogen-containing cells.

The incidence and nature of hyperplastic changes (mucinous cell metaplasia, dilation, and atypia) in the common pancreatic duct in this group were similar to those observed in the previous group treated with OA prior to and during initiation. Papillary hyperplasia associated with goblet cell metaplasia and atypia were present in 69% of the animals. All affected ducts were also dilated. Flat hyperplasia and/or
papillary hyperplasia with no evidence of atypia were observed in the remainder of the animals. Hamsters treated with HPOP and promoted with OA had extensive alterations in their main pancreatic ducts, particularly in those of the gastric and duodenal lobes. Hyperplastic changes were observed in 75% of the animals in this group. Focal atypical hyperplasia and papillary changes were present in 63 and 38% of these animals, respectively. Extreme dilation of the main duct, indicating obstruction, was present in six cases and was always associated with extensive papillary proliferation of the epithelium to form cystic papillary tumors (Figs. 5 and 6). Dilation of the main duct was always associated with the presence of papillary hyperplasia in the same duct. In the majority of the animals with cystic papillary carcinomas, dilation of the main duct was present at several places upstream from such tumors.

Liver/Gallbladder. Surprisingly, the incidence of hepatic cholangiocarcinomas in this group was lower than that observed in group 1 when OA was omitted (Table 1). Animals in this group developed papillary adenocarcinomas of the gallbladder (Fig. 7). In one case, the tumor extended into the liver. The incidence of gallbladder papillary hyperplasia was significantly greater than that observed in animals treated with HPOP alone (26% versus 5%). However, the incidence of gallbladder lesions was the same as that observed in animals of the second group given OA prior to and during initiation. An association between gallbladder tumors and abnormalities in the common pancreatic duct in the head of the pancreas may be proposed in this group since all animals having papillary lesions in their gallbladder also showed extensive and severe atypical hyperplastic changes in the common pancreatic duct.
DISCUSSION

The spectrum of pancreatic tumors induced by continuous infusion of HPOP in female hamsters does not appear to differ substantially from that reported in the literature using multiple bolus injections of BOP (1, 2, 5). A notable difference between the two models is a higher incidence of hyperplastic changes in the common duct in the head of the pancreas of HPOP-treated animals. Such changes include flat and papillary hyperplasia with no or modest atypia and severe dilation of the duct. Orotic acid appears to enhance both the incidence of the above lesions and also the extent of atypia in the common duct and to further result in promoting such changes into the main pancreatic ducts. Significant enhancement of the incidence of the above lesions by OA in the hamster-nitrosamine model offers an advantage in the study of main duct hyperplasia and its relation to the development of intraductal carcinomas. In addition to its effect on the pancreas, OA appears to modify liver and gallbladder carcinogenesis in this model. Thus, OA inhibits the induction of liver cholangiocarcinomas initiated by HPOP but enhances development of biliary hyperplastic changes and most probably the development of gallbladder papillary adenocarcinomas. In this regard, the HPOP-OA hamster model is useful for the study of early events leading to pancreatic intraductal tumors and other peribiliary cancers.

The role of OA in carcinogenesis is not well understood. Although it appears to enhance carcinogenesis in several tissues (19, 21), its mechanism of action as a tumor promoter has been studied almost exclusively in the rat liver. In this organ, OA is a mitotic inhibitor for
normal hepatocytes, but it has a less inhibitory effect on the proliferation of initiated hepatocytes, thus offering a selective advantage for the growth of malignant cells (22–26). In this study, the effect of OA was determined: (a) during initiation, when OA may influence rates of DNA synthesis and, therefore, the frequency of induction of permanent somatic mutations resulting from the replication of damaged DNA in select cell populations of target organs; and (b) at postinitiation, when OA is expected to offer a selective advantage for growth of “initiated” cells. In group 2 (+OA, I), OA exposure occurred predominantly prior and during the burst of mitogenic activity induced by the carcinogen in the pancreas (27). In contrast in group 3 (+OA, P), exposure to OA took place after that mitogenic activity had subsided and DNA synthesis had returned to normal. Since carcinogen-induced mitogenic activity is believed to be strongly linked with initiation, the two regimens do not only differ in terms of the duration to OA exposure (3 versus 28 weeks) but also in terms of timing of such exposure to target initiation and postinitiation periods.

The most striking effect of OA is on the common pancreatic duct of HPOP-treated animals. Regardless of the time of exposure to OA, common ducts of such animals become hyperplastic, frequently with extensive focal papillary projections. Such projections are lined with tall columnar cells. Mild atypia and mucus cell metaplasia are frequent in such lesions. The induction of hyperplastic changes in the common duct of HPOP-treated female hamsters is first observed shortly (2 to 3 weeks) after placing the animals on the OA diet. These lesions do not recede when OA is discontinued (data not shown) but

Fig. 5. Intraductal papillary tumor in the main pancreatic duct (tail of gastric lobe) of a female hamster initiated with HPOP and promoted with OA (group 3). Papillary fronds are lined with tightly packed columnar cells with interspread goblet cells. H&E, × 50. Inset: papillary structure lined with cells showing mild atypia. H&E, × 250.

Fig. 6. Papillary hyperplasia in the tail of the main pancreatic duct causing obstruction and dilation of the duct in a hamster treated with HPOP and OA (group 3). Ductule proliferation with concomitant destruction of acinar tissue is observed upstream. The ectatic duct is lined with tall columnar epithelium and interspread goblet cells. H&E, × 40.
Fig. 7. A. papillary adenocarcinoma projects and fills the lumen of the gallbladder in a female hamster treated with HPOP and OA (group 3). H&E, × 50. B, densely packed papillary epithelium of the same tumor shows tall columnar cells morphologically similar to those seen in the pancreas. No goblet cells are present. H&E, × 200.

Persist for the entire life span of the animal and are therefore believed to be true initiated lesions. In this regard, the mechanism of HPOP-OA-induced common duct hyperplasia differs from that proposed for liver parenchymal hyperplasia caused by (28) and cyproterone acetate (29), or the mechanism of renal hyperplasia caused by lead nitrate (30), or that for the onset of biliary duct hyperplasia produced by α-naphthyl-isothiocyanate (31) and that of pancreatic hyperplasia caused by diets containing trypsin inhibitor (32). The above hyperplasias recede when the stimulus is withdrawn. It is speculated that OA may be trophic to initiated ductal cells in common duct epithelium, which rapidly expand to form papillary structures. However, these cells seem not to be fully dependent on OA and do not undergo apoptosis when the stimulus is withdrawn. The presence of the K-ras mutation in codon 12 in more than 80% of the papillary structures in the common duct epithelium of HPOP-OA-treated animals suggests that cells may have a growth advantage compared to noninitiated cells and may, at some point, become independent of the OA stimulus.

Orotic acid shows a modest promotional effect in the main pancreatic duct of hamsters when it is administered 2 weeks after termination of the carcinogen treatment. The incidence of in situ and frank carcinomas also increases significantly, only when OA is used at

postinitiation. The promotional effect of OA is manifested only on papillary tumors, which most probably originate from papillary hyperplastic lesions in the main pancreatic duct and its branches rather than from ductules. The incidence of nonpapillary tumors in the body of the pancreas, in which an association with main duct neoplastic lesions could not be demonstrated, is not affected by OA. The latter are believed to originate from dysplastic ductules since they are highly differentiated tubular tumors or mixed tumors containing tubular structures (2, 5). Promotion of tall columnar cell proliferation in the main pancreatic duct in conjunction with a higher incidence of papillary tumors, which appear to be associated with this duct, is supportive for the sequence of hyperplasia-dysplasia-CIS in the development of intraductal tumors. However, the location of large tumor masses in the tail and in the body of the pancreas, in spite of the more conspicuous presence of hyperplastic and dysplastic lesions in the head of the organ, points out that additional steps not necessarily represented by morphologically defined states may be involved in pancreas carcinogenesis.

The inhibition and enhancement of liver cholangiocarcinomas and gallbladder tumors, respectively, in animals treated simultaneously with HPOP and OA (+OA,I) suggest that the OA may also have a direct effect on cancer initiation in liver. HPOP induces a rapid proliferation of bile duct cells in hamster liver, which reaches a maximum approximately 1 week after treatment and continues to persist several days thereafter (27). Because of the slow repair of promutagenic DNA damage, bile duct cells entering S-phase during carcinogen-induced mitogenesis have the potential to mutate and become initiated. Orotic acid, when administered along with the carcinogen, cancels its mitogenic effect on biliary cells. Administration of OA at a later time, when the mitogenic effect of HPOP has subsided, may also result in some suppression of cholangiocarcinomas, but the effect is less pronounced (if not insignificant) as compared to that observed on coadministration of OA and HPOP. In addition to a direct effect of OA on the yield of cholangiocarcinomas, it is possible that this compound also acts indirectly. HPOP, when administered alone, results in partial obstruction of the common duct and subsequent accumulation of the bile in the liver, which becomes “cystic” (13, 14). Hepatic cystic lesions almost invariably accompany cholangiomas and cholangiocarcinomas in HPOP treated animals, which suggests an association between bile accumulation and promotion of this particular tumor. Partial obstruction of bile flow in the hamster has also been reported to significantly increase the yield of diisopropynitrosamine-induced cholangiocarcinomas (33), because of the accumulation of bile salts which appear to promote this tumor (34). Obstruction of the bile flow may occur at the level of the common duct due to debris discharged from the liver during the initial cytotoxic assault by the carcinogen. However, in animals treated with OA, such obstruction is possibly relieved following the extreme dilation of this duct. It is further speculated that the mechanism of induction of cholangiocarcinomas differ from that of gallbladder tumors. The striking difference in the effect of OA on the incidence of these two tumors cannot be explained without further information regarding the response of the epithelium of the gallbladder to HPOP and OA exposure.

Like in hamsters, human pancreatic adenocarcinomas arise from the main pancreatic duct and its branches (intraductal) or from ductules. Intraductal neoplasms exhibit papillary features with varying degrees of atypia (35, 36). The histomorphology of these tumors bears close similarity to the intraductal papillary neoplasms of the hamster pancreas described here. The presence of cells with mild and severe atypia in the same type of tumor in the hamster model as well as in humans suggests a sequential or multistep evolution from certain hyperplastic and dysplastic lesions to invasive pancreatic cancer (37). Because of the variation in cytological atypia, ductal dilation, and mucin production, intraductal carcinomas in humans have been known by several names in the literature including papillomatosis, papillomas, mucinous cystadenomas, duct-ectatic mucinous cyst-adenocarcinomas, mucinous hypersecreting neoplasms, etc. (10—12, 38—40). However, the variability in nomenclature does not necessarily mean that they are distinct entities different from each other. Based on the hamster model, we postulate that various degrees of cytological atypia represent part of the morphological spectrum of the same entity, whether such entity originates from the main duct or its branches. Indeed, there are conspicuous similarities among HPOP-OA-induced papillary lesions, whether these were found in the head or tail in the pancreas, suggesting that the process of tumor development may be similar in spite of differences in the environment.

The selectivity of OA in enhancing ductal rather than ductular neoplastic processes poses the question whether tumors originating in the main duct and its branches have different etiologies than those rising from the ductules. Since exposure to promoters may be as important as the exposure to carcinogens for certain pancreatic cancers, it is possible that pancreas carcinogenesis is subject to more than one, possibly diverse, causative agent. The observation that intraductal carcinomas are predominant in elderly persons further supports a multiple etiological cause for this type of cancer. Many epidemiological studies which currently focus on a simple causating agent, with nondefinitive results, could have been more definitive if they had taken into account the type of pancreatic cancer and associated spectrum of lesions and also the habitual exposure of their subjects to more than one suspect agent.

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9 D. M. Kokkinakis, unpublished observations.


Orotic Acid Enhancement of Preneoplastic and Neoplastic Lesions Induced in the Pancreas and Liver of Hamsters by \( N \)-Nitroso(2-hydroxypropyl) (2-oxopropyl)amine

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