Differences in Experimental Pancreatic Carcinogenesis Induced by Oral or Subcutaneous Administration of 2,2‘-Dihydroxydi-n-propylnitrosamine in Duct-ligated Hamsters

K. Rückert, B. Pracht, and G. Klöppel

Department of Surgery, University of Mainz, Langenbeckstrasse 1, D-6500 Mainz [K. R., B. P.], Institute of Pathology, University of Hamburg, Martinistrasse 52, D-2000 Hamburg 20 [G. K.], Germany

ABSTRACT

After ligation of the main pancreatic duct in the splenic lobe, Syrian golden hamsters were treated, either p.o. or s.c., for 15 weeks with dihydroxydi-n-propylnitrosamine for induction of pancreatic duct carcinomas. The incidence, location, and type of proliferative lesions distal and proximal to the ligation were recorded and compared to those of dihydroxydi-n-propylnitrosamine-treated hamsters without duct ligation. Proliferative duct lesions, including carcinomas, developed on either side of the ligation. While after s.c. administration the incidence was similar to that in nonligated animals, a markedly decreased incidence was found in p.o.-treated animals with duct ligation. The data suggest that the blood stream might be one major access route of the carcinogen to the pancreatic duct cell. Furthermore, it is assumed that an unimpaired release of pancreatic juice in the duodenum is necessary for the enteral resorption of dihydroxydi-n-propylnitrosamine.

INTRODUCTION

Environmental carcinogens seem to be one of the most likely causes of pancreatic duct carcinomas (1, 3, 8). This is evident from epidemiological (16) as well as experimental studies (8, 9). The way the carcinogens act on the pancreas can be discussed either as a blood-mediated or a bile-mediated process. The study, therefore, deals with the question whether it is possible to induce pancreatic carcinomas in animals by preventing possible bile reflux mechanisms. With DHPN,1 we induced pancreatic carcinomas in hamsters following ligation of the pancreatic duct in the splenic lobe and in nonoperated animals.

MATERIALS AND METHODS

Animals. One hundred outbred 10- to 12-week-old female Syrian golden hamsters (Tierzuchtanstalt Kastengrund; Hoechst Company), with an initial weight of 140 to 160 g, were used. They were housed under standardized conditions in plastic cages in groups of 5 and fed Altromin pellets and water ad libitum.

Surgical Technique. Median laparotomy was performed under ether anesthesia to expose the splenic lobe of the trilobular pancreas of the hamster. This lobe is located laterally to the descending duodenum, the pancreas, and the spleen were completely removed and embedded en bloc. The pancreas was examined in 6 or more step sections by routine histology (hematoxylin and eosin, periodic acid-Schiff, and aldehyde fuchsin).  

Histological Recording. The proliferative lesions of the pancreatic duct cells were recorded with regard to localization and type of the lesions. The histological characterization followed a previously described classification (5), which distinguishes between precancerous and those of a definitely cancerous nature. The precancerous lesions showed the following sequelae leading to definitive carcinoma: focal hypertrophy and hyperplasia of duct epithelium with occasional cell metaplasia and mild atypia; papillary hyperplasia with involution and stratification of epithelium showing focal moderate atypia; cribriform intraductal proliferation of epithelium with marked atypia of the cells (intraductal carcinoma); and unequivocal carcinoma with proliferation of neoplastic ducts invading the pancreatic parenchyma. Statistical analysis was by $\chi^2$ test.

RESULTS

Precancerous and cancerous lesions developed with varying rates of incidence in all treatment groups except Group 4, which showed only few precancerous proliferations of the duct epithelium, and Group 5 (controls), which had none. All hamsters with carcinomas also had precancerous lesions. The incidence, site, and multiple occurrence of the neoplastic proliferations are recorded in Table 1 and Fig. 1. For the vast majority, the proliferations appeared to originate from the larger ducts in the gland. Whereas the precancerous proliferations were equally distributed in Groups 1 and 2, the carcinomas in Group 1 were located in the body and tail region (Fig. 2), while in Group 2, they were mainly in the pancreatic head (Fig. 3).

The effective number of animals, in which the ligation of the splenic lobe caused complete atrophy of the acinar tissue was 15 of 25 in Group 3, 16 of 25 in Group 4, and 7 of 10 in Group 5. In the atrophied pancreases, the acinar tissue was totally replaced by fatty and slightly fibrotic tissue, in which only the islets and major ducts were still preserved (Fig. 4). Focally periductal infiltrates of lymphocytes and macrophages were

1 The abbreviation used is: DHPN, 2,2‘-dihydroxydi-n-propylnitrosamine.
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found. Around the sutures, a granulomatous tissue had developed.

In Group 3, the number and type of induced carcinomas and precancerous proliferations were similar to those observed in Group 1. Concerning their location it is noteworthy that precancerous proliferations and carcinomas occurred not only proximally to the ligation in the preserved pancreas but also distally to the ligation in the completely atrophied pancreatic lobe (Figs. 5 and 6). The proliferative lesions originated from the remaining large ducts. In Group 4, the incidence of the induced neoplasms was markedly reduced. There were only limited precancerous proliferations of the ductal epithelium in the intact and in the atrophied pancreas, while carcinomas were completely lacking on either side of the ligation. Group 5 showed no proliferative alterations of the pancreatic ducts.

Statistical analysis of the incidence of the proliferative lesions (χ² test) showed a significant difference between Groups 2 and 4 (p < 1%) but none between Groups 1 and 3.

DISCUSSION

Pathological examinations have shown that pancreatic duct carcinomas primarily develop in the head of the pancreas (2, 3, 6). On the basis of the assumption that environmental carcinogens and their metabolites play an important role in pancreatic carcinogenesis (1, 3, 8, 15), there are currently 2 hypotheses which attempt to explain the preferred cancer site (12–15): (a) that carcinogens transported with the bile reach the ducts of the pancreatic head by retrograde flow from the biliary tract or (b) that carcinogens conveyed with the blood are excreted into the pancreatic juice and attain their highest concentration and greatest effect in the collecting ducts of the pancreatic head.

The hamster model for induction of pancreatic duct carcinomas, as introduced by Pour et al. (11), appears to be suitable, in examining this question, because the pancreas of this species has an anatomical structure that is quite similar to the human biliary-pancreatic system (15). After s.c. administration of DHPN for 15 weeks and an additional observation period of 4 weeks, we found precancerous and cancerous ductal lesions randomly distributed in the pancreas. This pattern of distribution may be consistent with the view that the agent reaches the pancreas via blood circulation. However, since there were a higher rate and a preferred localization of carcinomas in the pancreatic head after p.o. administration of DHPN, a reflux mechanism of biliary carcinogens could also be operative. The last assumption is, however, most unlikely, since although the carcinomas were concentrated in the head area the accompanying precancerous lesions, the definite precursors of carcinomas (5, 9), were randomly located in the gland in a way similar to that seen after s.c. administration. The increased incidence of carcinomas after p.o. treatment is therefore probably due to a more effective resorption of DHPN from the small intestine rather than from s.c. resources and cannot be attributed to a bile reflux mechanism.

After s.c. administration of DHPN and proximal ligation of the splenic lobe, precancerous and cancerous duct proliferations occurred not only proximally but also distally to the ligation in

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective no. of hamsters</th>
<th>No. of hamsters with precancerous ductal proliferations and ductal carcinomas</th>
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<tr>
<td></td>
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<td>No. of hamsters with precancerous and cancerous lesions</td>
</tr>
<tr>
<td>1</td>
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<td>12 (71)</td>
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<tr>
<td>2</td>
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<td>3</td>
<td>15</td>
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<td>4</td>
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<td>5</td>
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* Numbers in parentheses, percentage.

Gr. 1: (n = 17)                      Gr. 3: (n = 15)

Gr. 2: (n = 16)                      Gr. 4: (n = 16)

Fig. 1. Distribution of precancerous ductal lesions (○) and ductal carcinomas (□) in DHPN-treated Hamsters without ligation (Groups 1 and 2) and after ligation of the pancreatic duct in the splenic lobe (Groups 3 and 4). Groups 1 and 3 received DHPN s.c., Groups 2 and 4 received DHPN p.o. (n = effective number of hamsters).
the atrophied splenic lobe. These data suggest that the blood stream might be one major access route of DHPN to the pancreatic duct cells. Furthermore, it is of interest that the incidence of the proliferative lesions in these partially ligated pancreases was very similar to that in nonoperated animals, whereas, after p.o. treatment, the frequency of neoplastic lesions in the operated animals notably decreased, with only few precancerous lesions detectable proximal to the ligation in the nonatrophied parts of the pancreases. The most plausible explanation of this finding is that the pancreatic juice, which is reduced in its production due to the ligation of approximately 40 to 50% of the pancreatic parenchyma, may be an important factor for the enteral resorption of DHPN suspended in olive oil. The s.c. absorption, on the other hand, is not impaired by the ligation procedure and thus results in an equal rate of precancerous lesions and carcinomas as in nonligated animals.

It is suggested that nitrosamines, like DHPN, are thought to require metabolic activation to become carcinogenic in the organism. Recent studies with electron microscopic autoradiography showed that in the hamster pancreas a DHPN-related compound, N-nitroso-2,6-dimethylmorpholine, labels acinar and duct cells, the labeling being lower in duct cells than in acinar cells (12). This implies that the acinar cell may be the principal site of the metabolic activation of N-nitroso-2,6-dimethylmorpholine and probably also of DHPN. Acinar cells, however, are lacking in long-term duct-ligated pancreases so that under these circumstances DHPN can affect only either duct or islet cells. Since only ductal neoplasms but no endocrine tumors developed in the duct-ligated pancreases, we assume that DHPN or its metabolite(s) (4) are mainly incorporated in the duct cells, thus leading to their neoplastic transformation.

In conclusion, our studies suggest that the carcinogen DHPN, which induces neoplastic proliferations of the pancreatic duct epithelium, reaches the duct cell mainly via blood circulation and less so by bile regeneration.

REFERENCES


Fig. 2. Pancreatic duct carcinoma in the body of a hamster pancreas after s.c. administration of DHPN for 15 weeks. a, low-power micrograph showing a carcinoma with formation of densely packed atypical tubular structures. H & E, × 40. b, high-power view of pancreatic ducts (D) with atypical epithelium. In direct contacts with the ducts are neoplastic tubular structures (arrows) embedded in an inflammatory infiltrate. H & E, × 250.

Fig. 3. Pancreatic duct carcinomas (Ca) in the head of a hamster pancreas after p.o. treatment with DHPN for 15 weeks. a, 2 tumors with a partly cystic pattern. Arrows, some goblet cell metaplasia. Periodic acid-Schiff, × 40. b, atypical ductules encompassing an islet (arrows). H & E, × 250.

Fig. 4. Duct-ligated hamster pancreas. Total replacement of acinar parenchyma by fatty tissue. A remaining duct (D) is surrounded by islets (arrows). Periodic acid-Schiff, × 115.

Fig. 5. Precancerous lesions in a duct-ligated hamster pancreas (splenic lobe) after s.c. administration of DHPN. The ducts are lined either by flattened epithelium (small circles), hypertrophied cells with moderate atypia (medium circles), or intraluminal papillary proliferations of markedly atypical cells (large circles).

Fig. 6. Pancreatic duct carcinomas in duct-ligated hamster pancreases after s.c. administration of DHPN. a, cystic and papillary adenocarcinomas (Ca) in the tail and b, in the midportion of the splenic lobe. The fatty tissue adjacent to the carcinomas contains only islets and dilated ducts (arrows). Periodic acid-Schiff, × 40.
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