Pancreatic Adenocarcinoma in Inbred Guinea Pigs Induced by N-Methyl-N-nitrosourea

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Summary

Long-term studies of the carcinogenicity of N-methyl-N-nitrosourea (MNU) in inbred guinea pigs were undertaken to develop an animal model of pancreatic adenocarcinoma. MNU, freshly dissolved in 0.015 M sodium citrate buffer, pH 6.0, was administered by gavage once weekly at a dose of 10 mg/kg body weight to inbred strain 13 guinea pigs. By 27 weeks, 54% of guinea pigs given MNU weekly died, mostly due to severe atrophy and fatty metamorphosis of the exocrine acinar cells of the pancreas. Of 34 guinea pigs that survived more than 27 weeks of MNU administration, 10 (29%) developed pancreatic adenocarcinoma between 28 and 44 weeks. Other tumors included adenocarcinoma of the stomach (two animals) and of colon (one animal), lymphoma of mesenteric nodes (three animals), and a hepatoma (one animal).

Atypical changes involving acinar cells were observed in certain pancreatic lobules and included ductular or pseudo-ductular transformation, acinar ectasia, increased mitotic activity, and periacinar fibrosis. These changes are suggestive of acinar cell dedifferentiation, and their role, if any, in the histogenesis of pancreatic adenocarcinoma remains to be elucidated.

These studies suggest the feasibility of using inbred guinea pigs for developing a satisfactory model of pancreatic adenocarcinoma. Additional studies are necessary to minimize the high mortality rate during the first 6 months and to enhance the incidence of pancreatic adenocarcinoma.

Introduction

The incidence of pancreatic adenocarcinoma in the United States and elsewhere in highly developed countries has shown a significant increase in recent years (1, 5, 20, 21). Cancer of the pancreas ranks as the 4th leading cause of death from cancer in the United States (1) and, despite several recent epidemiological studies (9, 11, 14, 20, 21), the factors involved in the causation of pancreatic cancer are not clearly delineated. However, until recently, very little attention has been devoted by the experimental oncologist to develop a suitable animal model of pancreatic adenocarcinoma, which could be of considerable value in the understanding of pathogenetic processes of this tumor. In addition, an animal model may serve as an effective system for various experimental manipulations aimed at preventing or significantly altering the natural progression of the disease. The lack of experimental work on pancreatic carcinogenesis may be attributed, in part, to inherent difficulties in working with pancreas of most laboratory animals.

Druckrey et al. (6) in 1968 reported that prolonged administration of MNU or N-methyl-N-nitrosourea in drinking water to outbred guinea pigs produced adenocarcinoma of the pancreas and stomach. The average induction time of these tumors with MNU was approximately 740 days, and with N-methyl-N-nitrosourea it was about 800 days. In addition to long latency, the overall incidence of pancreatic adenocarcinoma in these studies was observed to be about 25% (2). The unusually long induction period and low yield of pancreatic tumors were considered as serious limitations to the usefulness of this model. The main objectives of the work currently in progress in our laboratory are: (a) to confirm the development of pancreatic carcinoma in guinea pigs treated with MNU and to study the histogenesis of these tumors; (b) to reduce the induction period of >800 days; and (c) to increase the incidence of pancreatic tumors. As indicated elsewhere (19), a short latency period and a high yield of pancreatic adenocarcinomas, without concomitant development of tumors of other organs, are 2 major criteria for a satisfactory animal model of this tumor. In the present communication, we report the development within 44 weeks of pancreatic adenocarcinomas in inbred guinea pigs given MNU once weekly by gavage.

Materials and Methods

Inbred strain 13 guinea pigs, weighing between 250 and 300 g, were obtained from Frederick Cancer Research Center, Frederick, Md. These animals were housed 2 to 3/cage and were maintained on Purina guinea pig chow (Ralston Purina Company, St. Louis, Mo.). After 1 week of acclimation to housing conditions, 62 female and 12 male guinea pigs were started on once-weekly i.g. administration of MNU (Ash Stevens Inc., Detroit, Mich.), 10 mg/kg. Since MNU is rapidly inactivated, it was usually dissolved in 0.015 M sodium citrate buffer, pH 6.0, as 1% solution.
solution 5 to 10 min prior to dosing. The half-life of MNU at pH 6.0 in 0.015 M sodium citrate buffer, as determined in preliminary studies, was approximately 20 hr. The animals were starved for 22 hr before i.g. administration of MNU, in order to ensure an empty stomach. The control group consisted of 12 male and 6 female guinea pigs given identical volumes of citrate buffer by gavage once a week. The guinea pigs were allowed access to food 1 hr after MNU treatment. Autopsies were done on animals that died overnight or were found moribund. For light microscopy, tissues were fixed in formalin and embedded in paraffin. Sections of tumors, pancreas, stomach, and liver were routinely stained with hematoxylin and eosin, but when indicated periodic acid-Schiff and mucicarmine stains were also done.

Results

General. The survival of guinea pigs treated with MNU and tumor incidence are summarized in Table 1. By 27 weeks, 54% of guinea pigs receiving MNU died. During the initial stages of the experiment (1st 4 weeks), deaths were due to pulmonary hemorrhage or pneumonia. Some animals also died of hemorrhagic necrosis of the gastric mucosa, occasionally complicated by perforation and peritonitis. In guinea pigs that died subsequently, there was marked atrophy of exocrine pancreas (Figs. 1 and 2). In these animals, pancreatic lobules were greatly reduced in size and the interlobular connective tissue was infiltrated with fat. The exocrine acinar elements were small and crowded together. The acinar cell cytoplasm was scanty and displayed a prominent solitary fatty vacuole (Fig. 2), which usually displaced the nucleus to 1 side. The thin rim of acidophilic cytoplasm surrounding the fatty vacuole contained few zymogen granules. Necrosis of the acinar cells was not observed at any time. There was no significant mitotic activity in acinar cells that showed fatty vacuolation during the lst 6 months of the experiment. Likewise, mitotic activity in the lining epithelium of the pancreatic ducts was minimal. An occasional duct showed a mild degree of hyperplasia of submucosal or basal glands. The islets of Langerhans appeared very prominent (Fig. 1) but showed no atypical changes. In essence, the most significant alteration, in pancreas of guinea pigs that died within 27 weeks of the experiment, is atrophy and fatty degeneration of the exocrine acinar cells. These animals also had a moderate to severe degree of fatty metamorphosis of the liver parenchyma, possibly resulting from functional insufficiency of pancreatic enzymes.

Atypical Acinar Proliferation. In 4 guinea pigs that died between 24 and 32 weeks, certain atypical changes in some pancreatic lobules were noted. In Fig. 3, a small focus of pseudoductular proliferation is shown at the periphery of an atrophic pancreatic lobule. These glandular structures are lined by cuboidal epithelium which each contain a prominent vesicular nucleus. In a guinea pig that died at 30 weeks, 2 adjacent pancreatic lobules showed atypical changes (Fig. 4). These lobules appeared distinct when compared to other lobules that were atrophic with fatty vacuolation. In these lobules, the acinar component was irregular and surrounded by periacinar fibrosis. The islets of Langerhans were small in these lobules. In another guinea pig (Fig. 5), the pancreatic lobule showed marked desmoplastic reaction and highly atypical acinar component. These acini appeared dilated and tortuous and were irregular in size, closely resembled proliferating ducts. Destruction of islets of Langerhans in these lobules was also evident. At 32 weeks, the pancreas of 1 guinea pig demonstrated severe ectatic changes of exocrine acini in many lobules (Fig. 6). These acini appeared markedly dilated and lined by cuboidal or flat epithelium. Mitotic activity was frequent in the lining epithelium (Fig. 7). In spite of these severe atypical alterations, the lobular pattern of the pancreas was maintained (Fig. 6). These changes suggest that the exocrine acinar cells are capable of dedifferentiation into ductular or pseudoductular structures.

Pathology of Tumors. Of 34 guinea pigs that survived more than 27 weeks of MNU administration, 12 females (19 of 28) and 3 males (5 of 6) developed tumors between 28 and 44 weeks after the beginning of the experiment (Table 1). Pancreatic tumors were found in 7 female (7 of 28) and 3 male (3 of 6) guinea pigs; this accounted for an incidence of

<table>
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<th>Group</th>
<th>Sex</th>
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<td></td>
<td></td>
<td>Initial</td>
<td>Effective*</td>
<td>Pancreas</td>
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<td>Control</td>
<td>Female</td>
<td>6</td>
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<td>Male</td>
<td>12</td>
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<tr>
<td>MNU</td>
<td>Female</td>
<td>62</td>
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<td></td>
<td>Male</td>
<td>12</td>
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<td>3</td>
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* Number of guinea pigs that survived more than 27 weeks.
* Adenocarcinoma of exocrine pancreas.
* Adenocarcinoma.
* Lymphoma of mesenteric nodes.
* Hepatocellular carcinoma of the liver.
Pancreatic Adenocarcinoma in Guinea Pig

29% in animals surviving more than 27 weeks. Among the other tumors were adenocarcinoma of the stomach (2 guinea pigs) and colon (1 guinea pig); lymphoma involving mesenteric lymph nodes (3 guinea pigs); and a hepatocellular carcinoma (1 guinea pig).

Grossly, the pancreatic tumors appeared as either irregular or well-circumscribed gray nodules of varying size, measuring 5 to 32 mm in diameter (Figs. 8 and 9). In 9 animals, the pancreatic tumors were solitary and in 1 the pancreas contained 2 small tumors. These tumors were located either in the duodenal segment ("head") or in the gastric segment ("body") of the pancreas. No tumors were seen in the splenic segment ("tail") of the pancreas. Histologically, the pancreatic tumors were adenocarcinomas showing varying degrees of differentiation (Figs. 10 to 12). The glandular differentiation and marked desmoplastic reaction of the stroma resembled adenocarcinomas of ductal origin, similar to those occurring in the human pancreas (4, 15). Invasion of the duodenum, regional lymph nodes (Fig. 13), and perineural spaces was occasionally encountered. Peritoneal dissemination of pancreatic tumors was not noted.

The adenocarcinomas of the stomach and carcinoma of colon were well differentiated. Lymphomas involved the mesenteric nodes diffusely and were predominantly lymphocytic in nature. The liver tumor was a highly vascular hepatocellular carcinoma.

Discussion

The studies presented here confirm the earlier report of Druckrey et al. (6) that chronic administration of MNU to guinea pigs results in the development of pancreatic adenocarcinomas. However, the promising aspect of this study is that the pancreatic tumors developed in less than 1 year, when compared to long latency period of \( \approx 740 \) days described by Druckrey et al. (6). There are 2 possible explanations for the reduction in induction period of pancreatic tumors. First, outbred guinea pigs were used by Druckrey et al. (6) whereas inbred strain 13 guinea pigs were used in the present investigation, and this may imply relative susceptibility of this inbred strain to the rapid induction of pancreatic adenocarcinoma (18). Second, in the present studies, the freshly mixed carcinogen was administered by gavage once a week in a dose of 10 mg/kg body weight to animals starved for approximately 22 hr prior to dosing to minimize degradation of MNU and to ensure rapid absorption from the gut. Druckrey et al. (6) added the carcinogen to drinking water, 5 days a week, in a daily dose of 2.5 mg/kg body weight. These differences in the delivery of carcinogen may also have accounted for increased mortality (about 54%) of guinea pigs within the 1st 27 weeks of our experiment. For prevention of this high mortality rate, it appears necessary to reduce the dose of MNU in future experiments.

Whatever might be the reason for the reduction in tumor induction time, this inbred strain of guinea pigs may prove to be of considerable value in ultimately developing a satisfactory animal model of pancreatic carcinoma, because of the histological resemblance of these tumors to human pancreatic adenocarcinoma (4, 15). Furthermore, these guinea pigs with pancreatic carcinoma did not develop simultaneous tumors of other organs, which is an important consideration in evaluating the suitability of an animal model of a specific disease entity. Despite this initial optimism, there are, however, several deficiencies that must be rectified before this can be successfully used as an animal model. One of these is high mortality rate among the animals given MNU and the severe atrophy of exocrine pancreas. It is anticipated that a substantial reduction in the amount of carcinogen administered weekly, parenteral administration of selected fat-soluble vitamins, and/or supplementing the diet with lipotrope factors and pancreatic enzymes may prevent excessive mortality during the 1st 6 months of the experiment. Another drawback appears to be the low incidence of pancreatic tumors (about 29%) in guinea pigs that survived more than 27 weeks of MNU treatment. It may be possible to enhance the incidence of pancreatic adenocarcinoma in inbred guinea pigs, if MNU is given to these animals: (a) during experimentally induced pancreatic cell division (3, 7, 12, 17, 19); (b) in association with high-fat or low-fat diets; and (c) in combination with a known carcinogen for pancreas, such as azaserine (13), 2,2'-dihydroxydi-N-propylnitrosamine (10, 16), or 4-hydroxyminoquine 1-oxide (8). These possibilities are currently under investigation in this laboratory in our attempt to develop an animal model that can simulate the pancreatic cancer in man.

Although the histological appearance of pancreatic tumors in guinea pigs, which was characterized by glandular differentiation and desmoplastic stroma, was highly suggestive of ductal origin, it may be premature to speculate on the histogenesis of these tumors. The atypical changes noted in the exocrine acinar element of pancreas (Figs. 5 to 7) raise the possibility that well-differentiated acinar cells are capable of dedifferentiation into ductal structures. Several studies have shown that differentiated acinar cells of pancreas divide following appropriate stimulation (3, 4, 12, 17, 19), and it is plausible to assume that these cells can dedifferentiate into ductal epithelium. Additional sequential morphological studies may shed some light on the role of acinar and ductal cells in the histogenesis of pancreatic adenocarcinoma.

Recent attempts to induce carcinoma of the pancreas in experimental animals have met with considerable success (10, 13, 16, 18). Longnecker and Curphey (13) have observed predominantly acinar-cell carcinomas of the exocrine pancreas in rats treated with azaserine. The studies of Krüger et al. (10) and Pour et al. (16) have demonstrated that 2,2'-dihydroxydi-N-propylnitrosamine produced mostly ductal adenocarcinomas of pancreas in the Syrian golden hamster. This hamster model of pancreatic adenocarcinoma appears to be highly promising because of the high tumor incidence and short induction period. However, 1 obvious disadvantage is that nearly 72 to 100% of hamsters with pancreatic tumors also had tumors of other sites (16). Hayashi and Hasegawa (8) reported the appearance of exocrine adenomas in pancreas of rats treated with
4-hydroxyaminoquinoline 1-oxide. However, this compound failed to induce tumors in guinea pig pancreas (M. S. Rao, unpublished data), suggesting a need to investigate further into the species and strain differences in the induction of pancreatic tumors with these various carcinogens. In conclusion, although some progress has been made in experimental pancreatic oncogenesis in the last 2 to 3 years, a great deal of experimental work has to be undertaken to refine these animal models of pancreatic carcinoma.

References

Pancreatic Adenocarcinoma in Guinea Pig
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Informal Discussion following the Paper by Reddy and Rao

**Dr. Fitzgerald:** I think we have 2 models now that simulate the pancreas cancers seen in the human. I would have a small caveat with Dr. Reddy because, where he shows acinar cells in the dilated ductule areas, he implies that duct cells arise from acinar cells. I don’t believe this, because the identical picture is seen if you tied off splenic pancreas segment duct as we did some years before. Did you do electron microscopy in these suspected areas?

**Dr. Reddy:** No, we have not done ultrastructural studies of these areas.

**Dr. Fitzgerald:** I think one of the big problems in pathology arises when you begin to “see” transition cell types. Propinquity is unreliable in this area. Admittedly, it would be scientific cuckoldry to ignore the juxtaposition of cell types, but it is extremely difficult to interpret these anatomical relationships in precursor or transitional terms. I doubt that acinar cells transform into duct cells. I believe that acinar and islet cells are pretty much the end of the trail in terms of differentiation. So far, “dedifferentiation,” if it exists, is a rare event in animals (if dedifferentiation means that the cells are starting out at the end of differentiation and going back to the primitive cell of origin). I would suspect that the duct cells might be able to differentiate into islet and acinar cells but the reverse, acinar cells to duct cells, I rather doubt. I’ve never seen an example of it in experimental pancreas studies. Otherwise, I think this a very fine model and one which will have considerable value.

**Dr. Reddy:** Thank you for your comments. I do realize that the question of dedifferentiation, particularly in pancreas, is a very difficult problem to prove. However, the illustrations shown here (see Figs. 5 to 7 of the preceding paper) strongly suggest that acini are capable of transforming into, at least, pseudoductules.

**Dr. Preussmann:** I would like to comment on the high mortality in your experiments. You also indicated that a possible explanation for low tumor incidence in our experiments in Germany was that the compound was administered in the drinking water and that decomposition of the carcinogen could have occurred. I think that this is not true. Our animals were housed overnight without water, and the...
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