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FROM THE DEPARTMENTS OF SURGERY AND PATHOLOGY, ULLEVÅL UNIVERSITY HOSPITAL, AND THE DEPARTMENT OF SURGERY, INSTITUTE OF PATHOLOGY AND EXPERIMENTAL ANIMAL DEPARTMENT, THE NATIONAL HOSPITAL, OSLO, NORWAY.

INFLUENCE OF PANCREATIC SECRETION ON LATE RADIATION ENTEROPATHY IN THE RAT

M. HAUER JENSEN, T. SAUER, T. BERSTAD and K. NYGAARD

Abstract

In female Wistar rats a 10 cm long exteriorized mid small intestinal segment was roentgen irradiated 3 weeks after a pancreatic duct-occluding operation/sham operation. Roentgen doses were 19 and 21 Gy as single exposures. Radiation injury was assessed 2, 8 and 26 weeks after irradiation using one macroscopic and 7 histopathologic parameters. The parameters were graded according to severity, and a radiation injury score was calculated by adding the scores for the individual parameters. Two and 8 weeks following irradiation there was no difference between pancreatic duct-occluded and sham operated animals. Twenty-six weeks after irradiation all parameters of radiation injury except the extent of vascular sclerosis were less marked in pancreatic duct-occluded rats than in controls. It is concluded that exocrine pancreatic secretions may influence the development of late changes following irradiation, and that these changes seem to be mainly independent of the degree of vascular sclerosis.

The composition of the contents of the small intestine has been shown to influence the development of the acute intestinal radiation syndrome (6, 16). There is much evidence to support the theory that the most important factor in this respect is pancreatic secretions (11), and that the effect is due mainly to proteolytic enzymes aggravating the mucosal injury (17).

Although the pathogenesis of late radiation enteropathy differs from that of the acute changes, certain observations from previous investigations (5) have been suggestive of an association between the degree of mucosal changes and the extent of late intestinal wall fibrosis.

The present study was designed in order to investigate the effect of a temporary exocrine pancreatic insufficiency at the time of irradiation on the development of late radiation enteropathy in rat small intestine.

Material and Methods

Pancreatic duct occlusion. Seventy-two female Wistar rats weighing 180 to 200 g were used for the experiments. The animals were kept 5 to 6 in each cage with free access to water and pellets. In half of the animals a pancreatic duct occluding operation with metal clips was performed in accordance with the technique described elsewhere (4). With this operation a temporary reduction of pancreatic exocrine secretion of approximately 80 per cent is achieved. In the other half of the animals a sham operation consisting of exposure and manipulation of the pancreatic gland was performed.

Roentgen irradiation. Three weeks following pancreatic duct occlusion/sham operation a 10 cm long exteriorized mid small intestinal segment was roentgen irradiated. The procedure has been described in detail previously (5).

Roentgen irradiation was performed with a Philips Müller MG 300 apparatus, the characteristics being as follows: Focus–intestine distance 40 cm, 250 kV, 10 mA, filter 0.5 mm Cu, HVL 1.5 mm Cu, dose rate 0.92 Gy per minute, temperature 18 to 20°C.

The radiation doses to the exteriorized intestinal segments were 19 and 21 Gy, each dose being ad-

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ministered to 18 pancreatic duct-occluded (PDO) rats and 18 sham operated controls as single exposures.

Evaluation of radiation injury. Animals were killed in groups of six 2, 8 and 26 weeks after irradiation. Specimens were prepared for histopathologic examination, and evaluated as described previously (5).

Radiation injury was assessed using the parameters listed in the Table. These changes were graded according to severity, and a radiation injury score (RIS) was calculated by adding the scores for the individual parameters. In addition to the RIS, scores for 'early' alterations (macroscopic alterations, thickening of serosa, mucosal ulcerations, epithelial atypia) and 'late' alterations (vascular sclerosis, fibrosis of the intestinal wall, lymph congestion, ileitis cystica profunda) were calculated separately.

For evaluation of differences between PDO groups the two-tailed Mann-Whitney test was used.

Table

Parameters of radiation injury

Macroscopic changes	
Visible alterations of irradiated intestine	
+	Pale-gray serosa
++	Palpable thickening of intestinal wall
+++	Stenosis with proximal dilatation
Histopathologic changes	
Thickening of serosa	
+	Slight thickening of serosa; hyperplasia of peritoneal mesothelium
++	Marked thickening of serosa
+++	Extreme thickening and fibrosis of serosa
Mucosal ulcerations	
+	Small, superficial ulcerations
++	Ulcerations involving more than half of the intestinal circumference
Epithelial atypia	
+	Abnormally oriented crypts
++	Irregular crypt regeneration with atypical epithelial cells
+++	Adenocarcinoma
Vascular sclerosis	
+	Slight thickening and hyalinization of vessel wall
++	Vessel wall double normal thickness; hyalinization and stenosis
+++	Extreme sclerosis with marked stenosis or complete occlusion; fibrinoid necrosis
Fibrosis of intestinal wall	
+	Submucosa double normal thickness; broadened and hyalinized collagen fibres
++	Submucosa three to four times normal thickness; abnormal collagen fibres
+++	Massive fibrosis including muscularis
Lymph congestion	
+	Dilated lymph vessels or cystic collections of lymph
Ileitis cystica profunda	
+	Submucosal glandular inclusions
++	Submucosal cysts with polypoid elevation of the mucosa
+++	Large cysts extending into the muscularis

Results

Four control animals died from intestinal obstruction 5 to 11 weeks after exposure with 21 Gy. There was no mortality in the PDO group following this radiation dose, or in either control or PDO animals following 19 Gy.

Two and 8 weeks following radiation exposure there was no significant difference in RIS between PDO animals and controls. However, 26 weeks after irradiation the PDO rats had significantly lower median RIS than the controls (Fig. 1). The difference was more marked following 21 Gy ($p<0.01$) than following 19 Gy ($p<0.05$). Also when scores were calculated separately for 'early' and 'late' alterations, a significant difference between PDO animals and controls was found for both subgroups of changes, following both 19 Gy (Fig. 2a) and 21 Gy (Fig. 2b) ($p<0.05$ and $p<0.01$, respectively).

Twenty-six weeks following irradiation all changes except the extent of vascular sclerosis were consistently less marked in the PDO group than in the control animals. Mucosal ulcerations, epithelial atypia and lymph congestion were found in most specimens from control animals following irradiation with 21 Gy, whereas these changes were virtually absent in specimens from PDO animals which had been exposed to this radiation dose. However, the greater part of the difference in RIS was due to a discrepancy in the extent of macroscopic alterations, serosal thickening, ileitis cystica profunda and intestinal wall fibrosis. These changes were much more prominent in the control animals than in the PDO rats. Consequently since the degree of vascular sclerosis was almost the same in PDO animals and controls, these changes accounted for approximately 50 per cent of the RIS in the PDO group, compared with approximately 20 per cent in the control group.

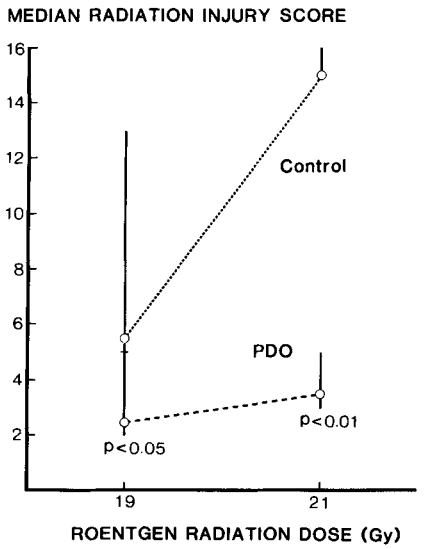

Fig. 1. Median radiation injury score 26 weeks following irradiation (6 animals per group). PDO = Pancreatic duct-occluded. Vertical lines indicate range of the four central values (0.17–0.83 quantiles).

Discussion

The selection of a suitable endpoint is a common problem in studies of the late effects of irradiation. In a previous series, we studied the development of radiation injury 2 to 50 weeks after single dose irradiation of exteriorized rat small intestine (5). The results indicated that the RIS, calculated as in the present investigation, is useful for semiquantitative assessment of late radiation enteropathy. The score correlated nicely both with the radiation dose and with the cumulative mortality observed in the different exposure groups. Therefore, the RIS was used as an estimate of radiation injury in the present study.

The 8 different parameters of radiation injury used for calculation of the RIS appeared to belong to 2 subgroups showing different patterns of development (5). The 'early' alterations were relatively marked already 2 weeks after irradiation, whereas the 'late' alterations were almost absent at this time, and developed during a period of 8 weeks following radiation exposure. Since this pattern of development of the various parameters of radiation injury was found also in the present study, the values for the two categories were calculated separately.

In previous experiments with the same model as that used in the present series, we found that the dose–response curves, both with regard to RIS and mortality, were rather steep in the range of 19 to 21 Gy. However, the mortality with these doses was acceptable from an ethical point of view, as the LD50 was approximately 22 Gy. Therefore radiation doses of 19 and 21 Gy were chosen for the present study.

In the model used, the observations at 2, 8 and 26 weeks following irradiation represent the early, intermediate and late phase in the development of chronic radiation enteropathy (5).

The contents normally present in the small intestine seem to aggravate the acute epithelial changes following irradiation or exposure to radiomimetic drugs (6). In addition to the loss of fluids and electrolytes, intestinal bacteria, bile and proteolytic enzymes have been proposed as factors of pathogenetic importance in development of the acute intestinal radiation syndrome (9, 16).

The significance of intestinal bacteria and bile is probably minor.

OSBORNE et coll. (13) found no prolongation of survival after irradiation of mice given penicillin or streptomycin. On the other hand, a protective effect of metronidazole on the intestinal epithelium following abdominal irradiation of rats was observed by FRIBERG (3). WILSON et coll. (21) reported prolonged survival of germfree mice compared with conventional mice following whole-body roentgen irradiation. However, this seemed to be due to a lower mucosal cell mitotic rate in the germfree mice.

JACKSON & ENTENMAN (9) found that bile duct ligation before irradiation leads to an increase in survival time, but in a study by MORGENSTERN & HIATT (11) the presence of bile in the intestinal lumen did not appear to affect the histopathologic changes following irradiation.

The exocrine pancreatic secretions probably excert a more significant influence on development of the acute intestinal radiation syndrome. Reduction of the amount of pancreatic enzymes in the intestine by pancreatectomy (18), pancreatic duct ligation (12), bypass operation (11) or chemical inhibition of proteases (17) seems to prolong survival in dogs following a lethal radiation dose to the abdomen. Furthermore, absence of pancreatic proteases in the intestine has been shown to attenuate acute radiation-induced histopathologic changes of the mucosa (12).

Although the pathogenesis of late changes following irradiation is different from that of acute injury, evidence both from clinical (1) and experimental (5, 14) studies suggests a relationship between the degree of mucosal changes and late radiation entero-

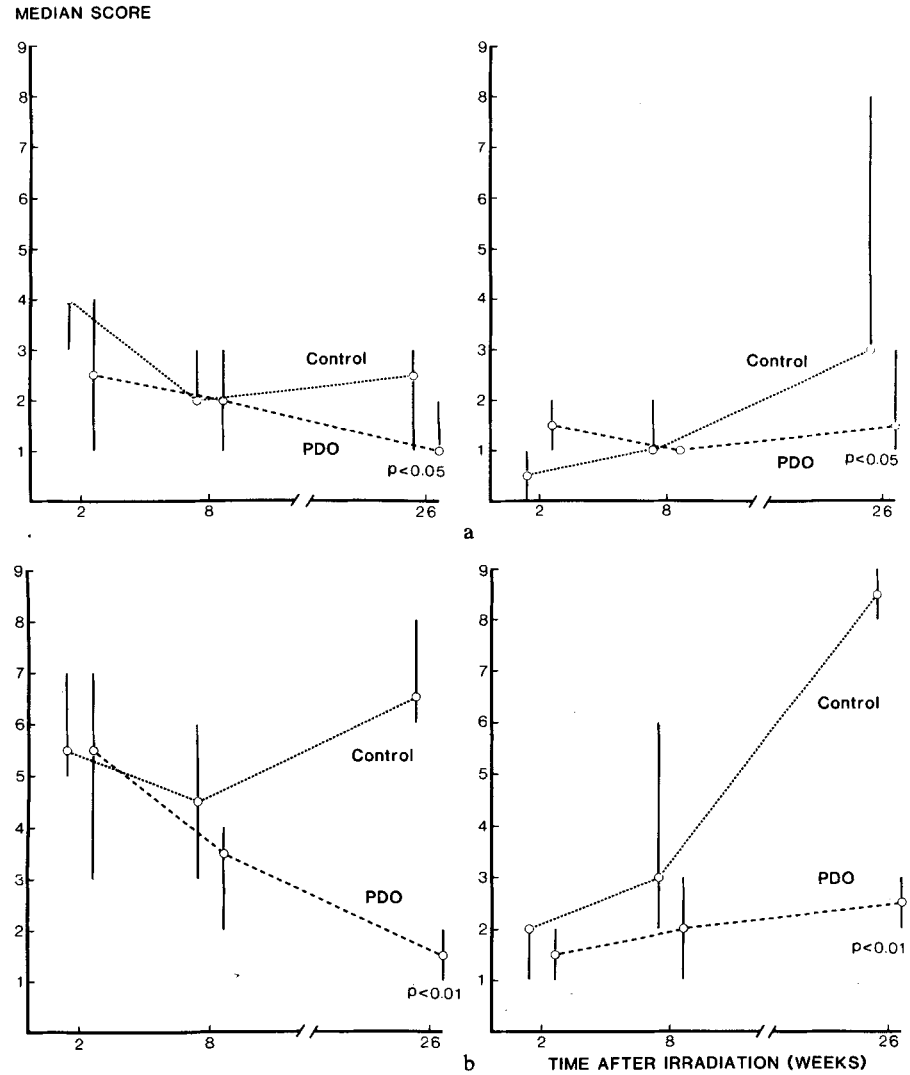

Fig. 2. Development of early (left) and late (right) alterations following a) 19 Gy and b) 21 Gy (6 animals per group). Early alterations: Macroscopic changes, thickening of serosa, mucosal ulcerations, epithelial atypia. Late alterations: Vascular sclerosis, intestinal wall fibrosis, lymph congestion, ileitis cystica profunda. PDO = Pancreatic duct-occluded. Vertical lines indicate range of the four central values (0.17–0.83 quantiles).

pathy. Thus, the present study was performed in order to assess the influence of temporary exocrine pancreatic insufficiency on the development of late radiation injury.

With the technique of pancreatic duct occlusion used, the secretion is reduced by approximately 80 per cent at the time of irradiation, 3 weeks after the duct-occluding operation (4), but recanalization probably occurs within the next 4 weeks (19). Therefore, significant pancreatic insufficiency is probably present only during the first part of the observation period.

No difference between PDO animals and controls was found 2 and 8 weeks following irradiation. However, 26 weeks after radiation exposure the PDO animals had significantly less prominent 'early' and 'late' alterations and lower RIS than the sham operated controls. The difference was greater following 21 Gy than following 19 Gy. This is consistent with the shape of the dose–response curve at this dose interval (5). The apparently beneficial effect of pancreatic duct occlusion seems to be in accordance with the theory that suggests a relationship between mucosal injury and the development of late radiation enteropathy.

Not all parameters of radiation injury seemed to be modified to the same degree by pancreatic duct occlusion. The greatest difference between PDO

rats and controls was found with regard to fibrosis of the intestinal wall, whereas there was little difference in the extent of vascular sclerosis.

The mechanism by which the pancreatic secretions may influence the development of the late radiation-induced changes of the intstine is unclear. Following irradiation destruction of the normal epithelial cell barrier occurs, thereby possibly permitting proteases to penetrate into the intestinal wall and cause tissue damage. The presence of proteases in the intestinal lumen may also lead to a more rapid and complete epithelial cell desquamation and/or delayed reepithelialization.

Whereas many authors regard differences in proliferation rate or proliferative organisation as the main reason for the difference between early and late reacting tissues (7, 20, 22), others maintain that the late changes following irradiation are secondary to vascular damage (8, 10). Increased endothelial permeability following irradiation is considered the primary lesion leading to leakage of fluids and proteins, and subsequent late fibrosis of the vessel wall and perivascular tissue (10). It has also been shown (15) that trypsin inhibitors prevent the increase in vascular permeability induced by intestinal ischemia, possibly by reducing free radical formation. However, a similar mechanism would not explain the less marked intestinal wall fibrosis in animals with pancreatic duct occlusion, as the degree of vascular changes seemed to be relatively little influenced by the procedure. The reason why the vascular changes were less affected by pancreatic duct occlusion than the other alterations remains to be elucidated.

In conclusion, the results of the present study indicate that reduction of the pancreatic exocrine secretions may significantly reduce the degree of some of the late changes following irradiation. Furthermore, since the blood vessel changes seemed to be less affected by pancreatic duct occlusion than the other alterations, our results do not support the theory of vascular injury as the primary cause of late radiation lesions of the small intestine.

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Request for reprints: Dr Martin Hauer Jensen, University of Iowa, Radiation Research Laboratory, Iowa City, Iowa 52242, USA.

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