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RADIOPROTECTIVE EFFECT OF LOCAL ADMINISTRATION OF LYSINE-VASOPRESSIN AND TRIGLYCYL-LYSINE-VASOPRESSIN ON THE RECTAL MUCOSA IN RATS

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Reactions from the rectal mucosa often give rise to troublesome side-effects during and after radiotherapy in the pelvic region. Local vasoconstriction in the rectal mucosa will cause an ischemia which will decrease the sensitivity of the mucosal cells to radiation and thereby these side-effects can be reduced. Triglycyl-lysine-vasopressin applied rectally in 1% Blanose solution gave in the present study significant radioprotection of the rectal mucosa in the doses of 0.8, 1.6, and 3.2 mg. These doses are, however, very high. Triglycyl-lysine-vasopressin in 1.2% Natrosol solution in a dose of 128 µg did not show any certain protective effects. However lysine-vasopressin in 1.2% Natrosol solution in a dose of 16 µg gave significant radioprotection of the rectal mucosa. This dose level has in a previous study not given any significant effects on the systemic circulation. Lysine-vasopressin in Natrosol solution seems to be a suitable combination for further studies.

Radiotherapy in the pelvic region often gives rise to reactions in colorectal mucosa, causing diarrhoea and abdominal discomfort, which can be very disturbing for the patient. In some cases chronic reactions will lead to a persistent damage of the mucosa and fibrosis in the intestinal wall. The risk of complications after abdominal surgery is also increased after abdominal radiotherapy (1–4).

There is a great deal of interest in methods of reducing radiation-induced side-effects in the intestinal mucosa. They range from suitable positioning, which removes the small intestine from the treatment volume (5), to methods of reducing the arterial circulation in the intestinal mucosa. These latter methods are based on the fact that hypoxic cells can tolerate radiation doses 2–4 times as high as normally oxygenated cells (6). To make the intestinal mucosa hypoxic during radiotherapy, intra-arterial injections of starch particles can be used to temporarily block the blood-vessels (7–9). Another way is to use vasoconstrictive agents. These can be given intra-arterially with good protective effect (10–12). Local application of suitable vasoconstrictive agents on the surface of the intestinal mucosa is a further possibility and studies on local application of norepinephrine and sodium sulphite have shown promising results (13, 14).

In a previous study we have shown that lysine-vasopressin and triglycyl-lysine-vasopressin, dissolved in a gel-solution of cellulose-polymer, can be given rectally without any operative measures and at dose-levels below the threshold for systemic effects (15). The present study was done to evaluate the radioprotective properties of the different vasopressin substances given rectally in rats. Initially a study was done to select a suitable radiation dose and both single-dose and fractionated irradiation was used for this study. Thereafter a study was performed to evaluate the radioprotective properties using the chosen radiation dose.

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Material and Methods

Selection of radiation dose

Fifty-six Wistar rats (SPF, Møllegaard), weighing about 200 g were used. The rats were anaesthetised with chloralhydrate intraperitoneally (36 mg/100 g body weight). The animals were then placed in a plastic (Cabulite) shell, formed to fit a rat of this size and thereby ensuring adequate immobilisation during the irradiation. The plastic shell was connected to a lead shield placed 2 cm from the plastic surface. There was a lead shield on both the ventral and on the dorsal side and in each shield an opening (40 × 30 mm) was cut out over the pelvic and lower abdominal region, corresponding to the location of the rectum and the terminal colon. This was checked with x-ray films. The shields were connected to each other, and in this way a cassette was formed which could be turned over, prone versus supine, without changing the position of the rat or the organ to be treated, according to control films.

Irradiation was given with 200 kV x-rays at a focus–skin distance of 400 mm, HVL 1.25 mm Cu, dose-rate 0.82 Gy/min. By the use of two opposing fields, a homogeneous dose could be given to the pelvic region and lower part of the abdomen.

Single dose irradiation was given ranging from 5–15 Gy. The interval between irradiation and autopsy ranged from 2 to 21 days. At each dose-level and interval 2 rats were irradiated.

A two-fraction irradiation was given, ranging from 6 Gy × 2 to 10 Gy × 2. Irradiation was given on days 0 and 4. There was an interval between irradiation and autopsy of 14 or 21 days. At each dose-level and interval 2 rats were irradiated.

A three-fraction regimen was used with doses ranging from 4 Gy × 3 to 8 Gy × 3 and irradiation on days 0, 2 and 4. The interval between irradiation and autopsy was 14 days or 21 days. At each dose-level and interval 2 rats were irradiated.

After irradiation the rats were given water (drinking-bottle) and food (pellets) ad libitum for 2–21 days. They were then sacrificed by an overdose of ether, and the rectum and terminal colon (fixed in 10% buffered formalin) were removed for histologic examination. The histopathologic changes were estimated according to a scoring system where three different parameters were evaluated:

A Inflammation and fibrosis in the mucosa and submucosa.
B Changes in glandular structure in the intestinal mucosa.
C Occurrence of crypt abscesses and/or ulceration in the intestinal mucosa.

Each parameter was graded from 0 to 6 where 0 denoted normal and 6 marked changes in the structure. The pathologist had no information of the type of treatment given to the animal.

Single-dose irradiation led to mortality due to complications from small intestine before suitable reactions in the rectum were achieved. With fractionated irradiation this was avoided, and the dose levels 10 Gy × 2 and 8 Gy × 3 gave suitable reactions. For practical reasons 10 Gy × 2 was chosen for further studies. The reactions in the rectum did not change between 14 and 21 days and an interval of 14 days was thus chosen for the further studies.

Evaluation of radioprotective properties

A total of 102 Wistar rats (SPF, Møllegard, weight 187–394 g) were used as experimental animals. They were treated in pairs at the same time. One rat in each pair was given vasopressin treatment rectally. The vasopressin was dissolved in a cellulose solution to achieve suitable viscosity. The other rat in each pair was used as control and was given inert solution of the respective cellulose substance rectally. The animals were divided into 7 groups. Dose-levels of triglycyl-lysine-vasopressin (GVP) and lysine-vasopressin (LVP) were chosen according to a previous study (15). The cellulose substates used were Blanose (sodium-carboxymethyl-cellulose) and Natrosol (hydroxy-ethyl-cellulose).

Group A (5 pairs) received GVP 0.8 mg in Blanose solution 15 min prior to irradiation. Group B (10 pairs) was given GVP 1.6 mg in Blanose solution 15 min prior to irradiation. Group C (10 pairs) was treated with GVP 1.6 mg in Blanose solution 30 min prior to irradiation. Group D (5 pairs) received GVP 3.2 mg in Blanose solution 15 min before irradiation. Group E (5 pairs) was given GVP 3.2 mg in Blanose solution 30 min before irradiation. Group F (8 pairs) was treated with GVP 128 μg in Natrosol solution 15 min prior to irradiation. Group G (8 pairs) received treatment with LVP 16 μg in Natrosol solution 15 min prior to irradiation.

The rats were pretreated with 20 ml bisacodyl (Toilax) solution (10 mg/300 ml water) in the rectum during a short aether anaesthesia in order to empty the rectum prior to vasopressin treatment. The rats were then anaesthetised with pentobarbital (Mebumal vet) intraperitoneally (6 mg/100 g body weight). The vasopressin, dissolved in 1/2 ml gel-solution, was given via a pediatric Nelaton-Foley catheter introduced into anus and advanced until the tip was 8 cm proximal to the anus. The balloon was then inflated with 1 ml saline to make the catheter stay in the same position and to prevent the vasopressin from spreading to the proximal parts of the gut. To make the solution fill the distal 5 cm of the rectum and terminal colon, the tip of the catheter had been obstructed and two new openings had been made just below the balloon. The animal was then placed in the plastic shell described previously.

Table 1

Histopathologic scores in group A–D. The average scores and the range are given for each group. GVP = triglycyl-lysine-vasopressin

	Vasopressin		Control	
	Average	Range	Average	Range
Group A				
GVP 0.8 mg				
Blanose solution				
15 min interval				
Fibrosis	2.0	1–3	3.4	2–5
Gland. chang.	1.4	1–2	3.4	2–5
Absc./ulcer.	1.4	0–3	3.2	2–5
Group B				
GVP 1.6 mg				
Blanose solution				
15 min interval				
Fibrosis	2.3	1–4	4.3	2–6
Gland. chang.	1.4	0–2	3.9	2–6
Absc./ulcer.	0.4	0–2	3.6	2–5
Group C				
GVP 1.6 mg				
Blanose solution				
30 min interval				
Fibrosis	3.2	2–4	5.2	4–6
Gland. chang.	2.2	2–4	5.6	4–6
Absc./ulcer.	1.2	0–4	5.2	2–6
Group D				
GVP 3.2 mg				
Blanose solution				
15 min interval				
Fibrosis	2.4	2–4	4.4	4–6
Gland. chang.	1.6	0–4	6.0	6–6
Absc./ulcer.	1.2	0–4	4.4	4–6

Table 2

Histopathologic scores in group E–G. The average scores and the range are given for each group. GVP = triglycyl-lysine-vasopressin. LVP = lysine-vasopressin

	Vasopressin		Control	
	Average	Range	Average	Range
Group E				
GVP 3.2 mg				
Blanose solution				
30 min interval				
Fibrosis	4.0	4–4	6.0	6–6
Gland. chang.	1.6	0–4	5.6	4–6
Absc./ulcer.	1.6	0–4	5.2	4–6
Group F				
GVP 128 μg				
Natrosol solution				
15 min interval				
Fibrosis	5.0	4–6	5.0	4–6
Gland. chang.	4.8	2–6	4.0	2–6
Absc./ulcer.	3.8	2–6	4.0	2–6
Group G				
LVP 16 μg				
Natrosol solution				
15 min interval				
Fibrosis	3.8	2–6	5.5	4–6
Gland. chang.	3.0	0–6	5.5	4–6
Absc./ulcer.	3.3	0–6	5.0	4–6

Irradiation was given with 240 kV x-rays at a focus–skin distance of 420 mm, HVL 1.1 mm Cu, dose rate 1.135 Gy/min. The absorbed dose-rate was determined by using an ion chamber placed in a rat phantom made of wax and connected to a Farmer electrometer. A dose of 10 Gy × 2 was given with treatment on days 0 and 4. After irradiation the animals were given water (drinking bottle) and food (pellets) ad libitum. They were sacrificed 14 days after termination of the irradiation, and the rectum and terminal colon, fixed in 10% buffered formalin, were taken for histologic examination. The histopathologic changes were estimated according to the scoring system described previously. At the microscopical examination and grading the most damaged part was regarded as representative for the maximal reaction. The histologic grading was done by the same pathologist during the study, without information on the type of treatment given.

Weight was recorded before start of irradiation and when the rats were sacrificed. Statistical evaluation of the weight development was made by Student's t-test. The evaluation of the intestinal reactions was made by comparing the histologic scores of each vasopressin-treated animal with the scores of its control. By studying which animal in each pair that had the highest scores and by comparing this relation to a binominal distribution, the significance level could be calculated.

Results

Evaluation of radioprotective properties

The average and range of the histopathologic scores are shown in Tables 1 and 2.

In group A all the vasopressin-treated rats had less pronounced reactions than their controls, $p = 0.031$. In group B all the vasopressin-treated rats showed less pronounced reactions than their controls ($p = 0.001$) (Fig. 2). Also in group C all the vasopressin-treated rats had lower histologic scores than their controls ($p = 0.001$) (Fig. 3). The same was true for group D ($p = 0.031$) and group E ($p = 0.031$). In group F no certain advantage of the vasopressin treatment was noted and only 3 of the 8 pairs showed lower reactions in the vasopressin-treated rats. In group G the vasopressin-treated rats had lower histologic scores than their controls in 7 of the 8 treated pairs (0.035).

Most of the rats increased in weight during the study. There were no significant differences in weight development, except in group B where the vasopressin-treated

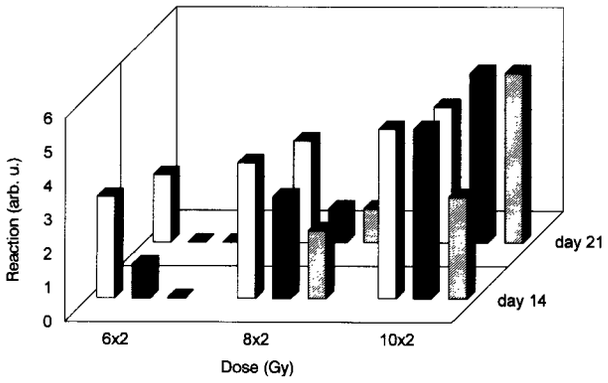

Fig. 1. Reactions in rectum after two-fraction irradiation. Each column represents mean values of 2 rats. White columns denote inflammatory reaction and fibrosis. Dark grey columns denote changes in the grandular structure. Light grey columns denote crypt abscesses and/or ulcerations.

animals had a significantly better gain in weight than their controls ($p < 0.01$).

Discussion

Intra-arterial treatment with vasoconstrictive agents in order to protect the intestinal mucosa is difficult to use during fractionated radiotherapy. Previous studies with local application of vasoconstrictive agents on the mucosal surface have also used operative measures and have not been applied in clinical practice (13, 14). Local application of vasopressin dissolved in a gel-solution can be done without any operative measures. This type of treatment should thus be possible to use in clinical practice.

During the now reported experimental study, the set-up has been easy to use and allowed irradiation with constant positioning of the animal. Single dose treatment gave insufficient reactions for analysis and led to lethal complications from the small intestine. For the study of reactions in the rectal mucosa a higher radiation dose is necessary than reported by Friberg (16) as suitable for the study of reactions in the small intestine. This is probably due to the slower cell renewal in the rectal mucosa compared with the small intestine (17) and to the good regenerative capacity (18). This observation is also in accordance with the findings of Hubmann (19), Sténson (20) and Northway et al. (21). With fractionated irradiation doses of 10 Gy × 2 and 8 Gy × 3 gave reactions in the rectal mucosa, suitable for studies of radioprotective effects. The vasopressin-treatment was well tolerated by the rats and no adverse reactions to the vasopressin were noted.

The radioprotective properties of the vasopressin preparations were impressive in all experiments with one exception. Triglycyl-lysine-vasopressin in the dose of 128 μg in Natrosol solution did not show any radioprotective effects and this dose may have been too low. Triglycyl-lysine-vasopressin in Blanose solution gave significant radioprotection of the rectal mucosa in the doses of 0.8 mg, 1.6 mg or 3.2 mg. The time-interval between vasopressin adminis-

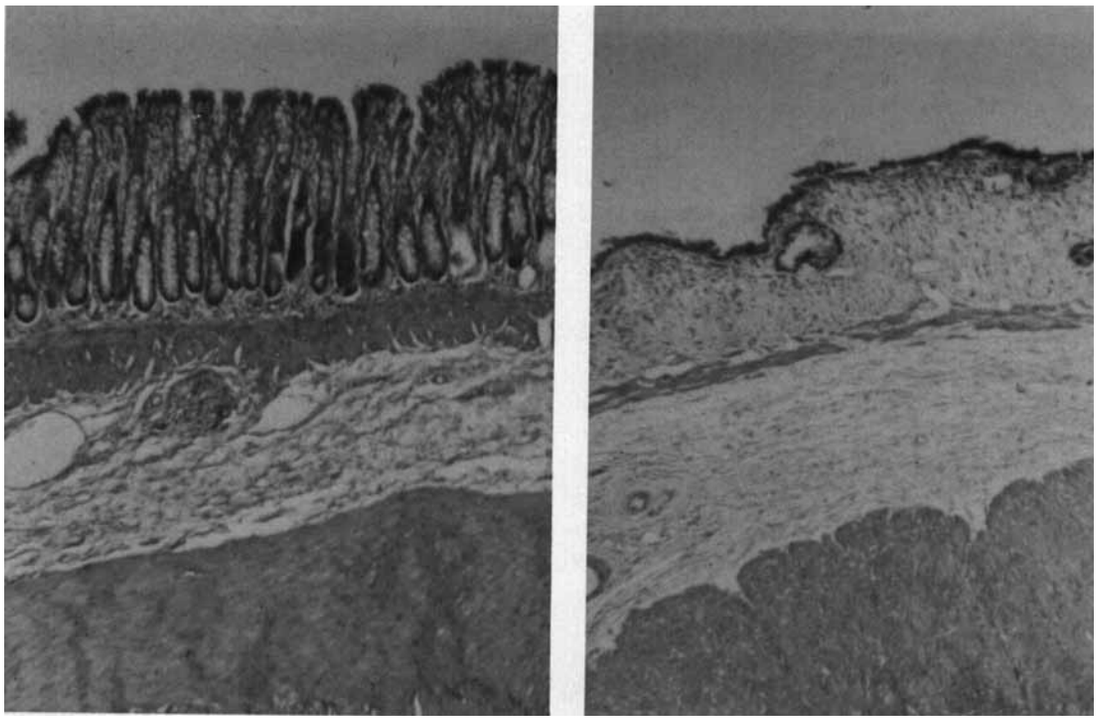

Fig. 2. Postradiological rectal mucosa. To the left almost normal mucosa from a rat treated with 1.6 mg GVP 15 min prior to irradiation. To the right severely damaged mucosa from the control animal.

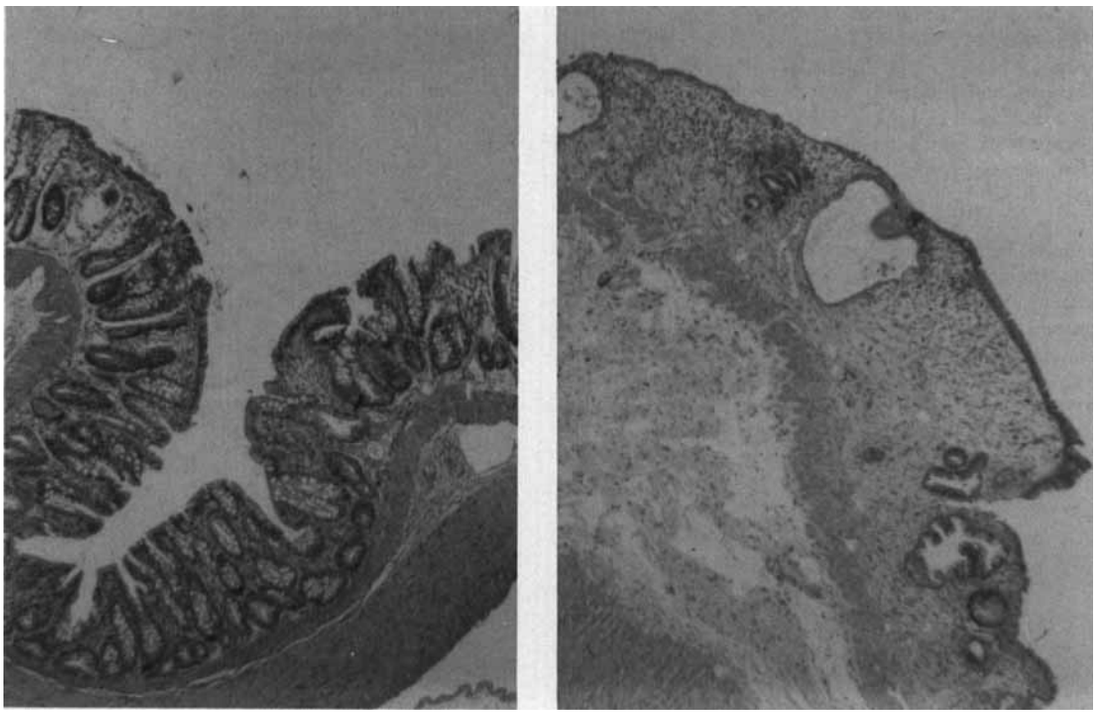

Fig. 3. Postradiological rectal mucosa. Slightly damaged mucosa from a rat treated with 1.6 mg GVP 30 min before irradiation (left). This is in contrast to the pronounced reactions in the control animal (right).

tration and radiotherapy did not change the results and this was expected since the gel-solution acts as a depot for the vasopressin and since this preparation has a longer duration (22). Lysine-vasopressin in the dose of 16 μg in Natrosol solution gave also significant radioprotection. Probably the more long-lasting triglycyl-lysine-vasopressin has no advantage in this setting, since the gel-solution acts as a depot which allows the vasopressin to be gradually absorbed by the mucosa.

The doses of triglycyl-lysine-vasopressin in Blanose solution had to be very high, probably because Blanose is an anionic compound that is likely to bind to the peptid molecule. Doses of this size have been shown to give a tendency for bradycardia which is not desirable (15). However, 16 μg lysine-vasopressin in Natrosol solution has not shown any significant systemic effects (15) and seems to be the most promising combination. Further studies to explore the effects in a clinical setting should be of great interest.

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