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Acute and Chronic Effects of Rat Colon after Photodynamic Therapy and Radiotherapy - A Comparative Study.

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ABSTRACT

After clinical photodynamic therapy (PDT) and radiotherapy (RT) of the colon carcinoma acute and late damages on adjacent normal tissue were seen. Therefore it was the aim of this experimental study to investigate these damages on normal colon tissue of rats after PDT in comparison with RT. For PDT the rats got Photofrin® (5 mg/kg bw, i.v.) 24 h prior to the laser light illumination (630 nm, 100 mW/cm², 20-100 J/cm²) at 20-50 mm proximal from the anus. In the RT-group a localized 300 kV x-irradiation with 23 Gy of a 24 mm long segment of the rat colon, 26-50 mm proximal from the anus, was performed.

Within the first hours after PDT the endoscopic examination showed a severe acute damage. The histopathological examination showed that the acute ulceration depends on the energy density applied within the first three days. Animals receiving a synthetic diet before and during the PDT treatment survived the acute phase and showed no late effects. After x-irradiation the acute damage of the mucosa occurred in the second week post irradiation and could be observed by endoscopy. After 4 to 10 weeks late damage started with ulceration and colon obstruction. All animals receiving the synthetic diet after x-irradiation survived as long as the diet was maintained. When the diet was changed to the standard pellets more than 90% of the rats developed a progressive large bowel obstruction.

This study indicates different progresses of acute effects after PDT and RT, respectively. Late damages were observed only by RT in contrast to PDT. Synthetic diet prevents acute damages after PDT. However, the synthetic diet after RT can prevent the late damage for the duration of the diet administration.

2. INTRODUCTION

The potential of clinical radiotherapy (RT) is limited by side effects on normal tissue which is always present in the irradiation field. One of such an organ at risk in clinical radiotherapy is the colon. Clinically, late damage (6 months - 5 years) on adjacent normal colon tissue has often proved to be more severe than acute injuries ^{1, 2}.

A new promising treatment modality of gastrointestinal cancer ³ is photodynamic therapy (PDT) based on the ability of a photosensitizer to accumulate in tumors to a higher degree than in surrounding normal tissue. Interaction of light with this photosensitizing drug produces cellular tumor damage ^{4, 5}. Acute and late damage were observed post PDT ³. Only few studies are reported on the acute and late damage of the normal colon tissue post PDT ⁶.

The aim of this study is to examine the pathogenesis of acute and late damages of RT and PDT and consequently influence and treat these damages. Therefore we have developed a clinically reliable model for localized photodynamic therapy and x-irradiation of the rat colon and produced a similar clinical damage in the large bowel of these rats.

3. MATERIALS AND METHODS

3.1 Animals

Experiments were performed on female wistar rats (200-250 g). All procedures were achieved under general anaesthesia of a semiclosed inhalation system (enflurane 1.5 % in pure oxygen). For irradiation the rats were

positioned head down. This procedure allows the small intestines to be removed from the irradiation field⁸. During the observation period of 200 days we assessed the rats by daily visual inspection, routine abdominal palpation and weekly endoscopic examination⁹. The endpoint for chronic damage due to PDT and x-irradiation was the rectal obstruction.

3.2 Photodynamic therapy

Rats were treated with Photofrin® intravenously injected at dose of 5 mg/kg bw. 24 h post injection a 3 cm segment of the colon (2-5 cm proximal from the anus) were illuminated with a cylindrical light applicator with diameter of 0.8 cm. Light of an Argon-ion laser pumped dye laser tuned to $\lambda = 630$ nm, energy density was 20-40 J/cm² by a power density of 100 mW/cm². For the study of acute effects, one group of animals were sacrificed and the bowel removed 1-48 hr post illumination, because animals developed a rectal obstruction 48 hr post irradiation.

The other group of animals received a astronaut diet for 4 weeks and then the normal rodent diet. The observation period of chronic effects was 200 days post irradiation.

3.3 X-irradiation

Two groups of animals were irradiated with a localized 300 kV x-irradiation with 23 Gy (ED 90 = 23 Gy) of a 24 mm long segment of the rat colon, 26-50 mm proximal from the anus.

One group of animals received only a normal rodent diet and the other group received a astronaut diet for 4 weeks.

4. RESULTS AND DISCUSSION

The endoscopic image of an untreated control animal is shown in fig. 1 a. On visual inspection the mucosal surface is rose coloured, moist and smooth. Arteries and veins surround the whole circumference of the colon. Between these vessel there is an arborization of smaller arterioles and venules.



(a)



(b)

Figure 1. Endoscopic image of (a) normal rat colon, (b) rat large bowel 2 weeks after 23 Gy x-irradiation showing a reddened hyperaemic erosive zone in the irradiation area ↑. Similar effects were seen 48 h post PDT.

4.1 PDT:

Within the first hours after PDT the endoscopic examination showed a dose dependent severe acute damage (fig. 1 b). The histopathological examination reveals acute ulceration depending on the energy density applied within the first three days. The histological picture (fig 3 a) of the acute effects of the rat colon 24 hours illuminated with a laser light of 40 J/cm² 24 hours post i.v. injection of 5 mg/ kg bw. Photofrin in comparison with unirradiated mucosa (fig. 2). Massive bleeding was observed in the mucosa, capillaries are filled with erythrocytes. The submucosa was judged as edematous and there were no intact epithel cells found in the section. Rats, which were fed with a normal diet developed in the acute phase within the first 4 days a rectal obstruction.

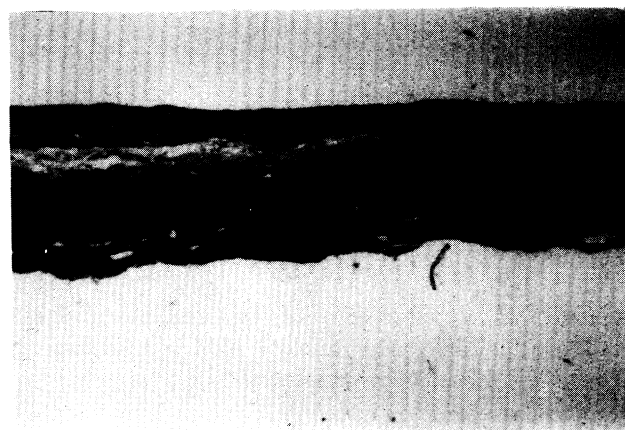
Animals receiving a synthetic diet before and after the treatment (40 J/cm²) for 4 weeks survived the acute phase. After change to the normal rodent pellets does not cause any clinical symptoms of chronic damage and survived all the observation period of 200 days. The crypts of the illuminated part (fig. 3 b) of the rat colon 200 days post PDT were shortened and less densely packed than in the colon of control rats.



Figure 2. Histological section showing an untreated rat colon. M = mucosa, MM = muscularis mucosa, SM = submucosa, MP = muscularis proper (H.E.)



(a)



(b)

Figure 3. Longitudinal section through the illuminated part of the rat colon (a) Acute effects 48 h post PDT (40 J/cm²). (b) 200 days post PDT (40 J/cm²) and synthetic diet for 4 weeks.

4.2 X-irradiation:

The acute radiation damage of the mucosa occurred in the second week post x-irradiation and could be observed by endoscopy (fig. 4 a). It showed the typical signs of a locally limited proctitis. A reddened hyperaemic erosive zone was covered with fibrinous exudate and blood clots in the irradiation area. In fig. 4 b an erosion subsequent to the complete denudation of the epithelial layer and loss of crypts is shown. Submucosa oedema and the erosive zone were covered with fibrinous exudate. All rats survived this period of acute radiation proctitis. After a time period of several weeks without any clinical signs late damage started with the clinical symptoms of colon obstruction about 28 days post 23 Gy. Fig. 7 shows the histological section through the proximal end of a radiation ulcer of the rat colon that caused colon obstruction. In the irradiation zone strong edema of the submucosa is observed. Ulcer is penetrating through the muscularis proper into the serosa.

All animals receiving the synthetic diet after x-irradiation survived as long as the diet was maintained. When the diet was changed to the standard pellets more than 90% of the rats developed a progressive large bowel obstruction.

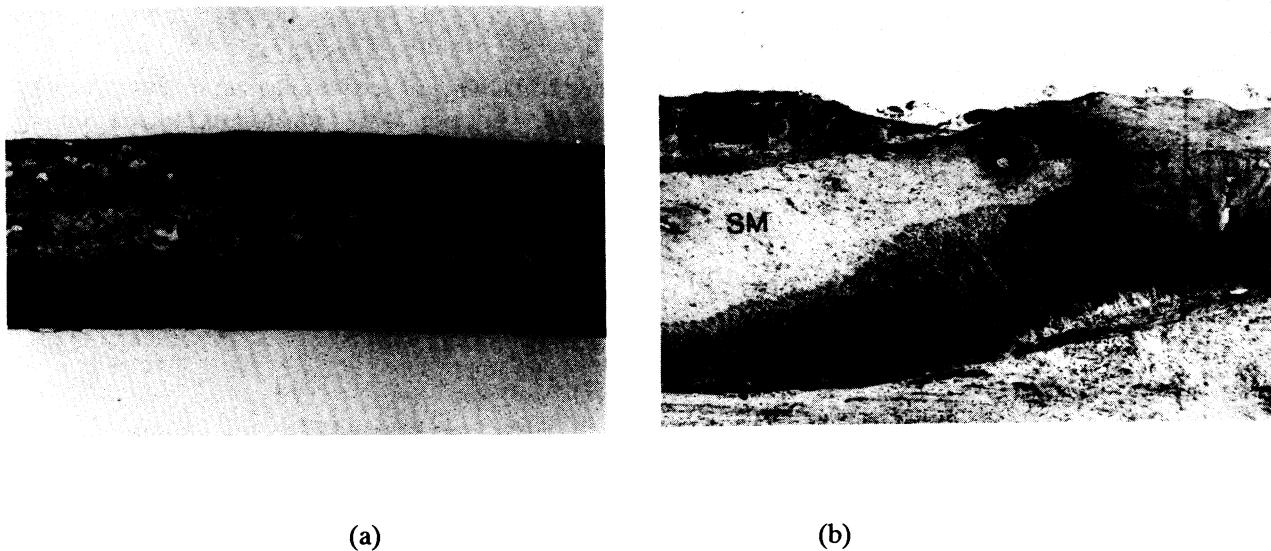


Figure 4. (a) Acute effects 2 weeks post 23 Gy x-irradiation. (b) Radiation ulcer of rat colon inducing colon obstruction 4-6 weeks after 23 Gy. M = mucosa, MM = muscularis mucosa, SM = submucosa, MP = muscularis proper, N = necrosis, GW = wall of granulocytes (H.E.)

5. CONCLUSION

This study indicates different progresses of acute effects after PDT and RT of the normal rat colon, respectively. Late damages were observed only by RT in contrast to PDT. Synthetic diet prevents acute tissue damages after PDT. However, the synthetic diet after RT can prevent the late damage only for the duration of the diet administration.

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