Tetrachlorodecaoxygen anion complex (TCDO) treatment after localized X-irradiation of the rat colon

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Summary

Local X-irradiation of the rat colon with 23 Gy produced ulcer in 90% of the rats resulting in fatal large bowel obstruction about 4 weeks after irradiation. Topical and systemic application of saline had no influence on this effect. However, the systemic applications of the tetrachlorodecaoxygen anion complex (TCDO), given at different doses and at different times after irradiation markedly prolonged the latency time and increased the survival rate of the rats.

Introduction

Late radiation effects in the rat colon are assumed to be due primarily to mucosal atrophy caused by progressive damage to the vascular connective tissues of the mucosa and submucosa. The atrophic mucosa is particularly susceptible to infection, inflammation and mechanical friction exerted by the feces (Trott et al. 1986). Experiments to control these secondary damaging factors by application of cortisone, antibiotics and laxatives (Trott et al. 1986) resulted only in a short prolongation of the median survival time. More protection was obtained after reducing the volume of the faeces dramatically by feeding a residue-free synthetic diet, which was fully absorbable in the jejunum. However, this treatment had to be given continuously after irradiation.

Substances which stimulate tissue regeneration might be an attractive alternative to prevent progression of subclinical radiation injury to gross tissue failure. Since TCDO has been described to have such properties (Hinz et al. 1984 and 1986) we have investigated the effect of TCDO on the progression of chronic radiation injury after local irradiation of rat colon in a series of pilot experiments.

Methods

Localized 300 kV X-irradiation of a defined part of the rat colon from 26 to 50 mm proximal to the anus was carried out with a method described earlier (Kiszel et al. 1984). In all experiments a dose of 23 Gy was given which has been proven to produce fatal ulceration in the colon in over 90% of the animals. The observation period was 100 days after irradiation. After single dose irradiation most ulcers occur in this period.

TCDO was given to the animals after irradiation in various doses, schedules and application techniques as shown in table I. Topical treatment (s c h e d u l e 1) was started on day 2 after irradiation. Two times per week 0.5 ml of a TCDO solution* was injected with a bulb headed canula directly into the irradiated part of the colon. Two different concentrations of TCDO were applied: 10.3 ug/ml in the group of animals receiving undiluted TCDO and 2.6 ug/ml in the group receiving diluted TCDO (TCDO : saline = 1:3). In s c h e d u l e 2, systemic treatment was given by i.v. injection of a more concentrated solution** at weekly intervals starting on day 4 after irradiation. 0.4 ml of WF 10 per kg body weight were injected each week. In a third experiment (s c h e d u l e 3) the number of intravenous applications was increased and the dose per injection was decreased. TCDO was given daily from day 4 to 8 after irradiation and then once weekly with a dose of 0.2 ml per kg body weight. In the last experiment (s c h e d u l e 4) TCDO was applied intraperitoneally twice per week starting at the second day after irradiation. Since the availability after intraperitoneal application is not exactly known, the dose was increased to 0.8 ml WF 10 per kg body weight for each treatment. Control animals in all experiments were given physiological saline in parallel.

* Oxoferin^{R}, OXO Chemie GmbH, Heidelberg, FRG

** WF 10, OXO Chemie GmbH, Heidelberg, FRG

Results

The application of saline (control animals) was without any therapeutic effect (table I). About 3-4 weeks after irradiation control rats in all schedules developed an endoscopically detectable radiation ulcer which extended round the whole circumference of the colon. The consequence of this damage was fatal rectal obstruction. Histologically, (fig.1) mucosa, submucosa and part of the muscularis propria were necrotic and interspersed and covered with bacteria. In the periphery, the lesions were demarcated by a wall of granulocytes which invaded the adjacent circular musculature. Submucosal and subserosal tissues including the larger vessels, were fibrotic and degenerate. The larger vessels were often occluded.

The results after TCDO treatment are shown in the table I. Schedule 3 appeared to be especially promising and data are given in figure 2. TCDO treatment increased the median survival time and also increased the survival rate (table I, fig.2). This effect on the survival rate was less after local application (0-16%) than after systemic application (25% -40%). The results in the systemic application schedules 2-4 did not differ significantly.

From endoscopy all TCDO treated animals developed ulcers and some animals died from these ulcers, as is shown in table I and in fig.2. However, these ulcers occurred later and they were smaller than in control rats. Histologically (fig.3) these ulcers appeared to be more superficial: Necrotic tissue interspersed and covered with bacteria was found only in the mucosa propria. The muscularis mucosae was not continually interrupted: Often we found only erosions covered with bacteria. Granulocytes were rather diffusely distributed and did not demarcate any grossly necrotic tissue. There was pronounced submucosal edema and

inflammation and a distinct subserosal fibrotic reaction. Vessel damage was much less expressed and we never saw occluded vessels.

In animals surviving the observation period after TCDO treatment we even observed endoscopically healing of radiation ulcers. Histologically (fig.4), we found a hyperplastic mucosa with hypertrophic crypts, irregular and often cystic which invaded deep into the muscularis propria. The muscularis mucosae and submucosa and muscularis propria were interrupted or absent. The hyperregenerated mucosa was abutting on the muscularis propria. The serosa showed reactive fibrosis.

Discussion

From these experiments we conclude that TCDO may be able to influence the time of onset, the severity and the healing of radiation ulcers in the rat colon. Endoscopical and histological observations showed that the ulcers were smaller and more superficial than usual and that these ulcers did not inevitably cause functional rectal obstruction but could even heal in a considerable proportion of the treated animals.

Before more extensive studies have been performed on this animal model of late complications of radiotherapy one can only speculate on the possible underlying mechanisms. Contrary to initial expectations, we observed that systemic intravenous or intraperitoneal application was superior to local application. If confirmed in further experiments this type of observations may help to suggest mechanisms of action and future directions of work. Further experiments are planned with different doses and different schedules of drug and of radiation to quantitate

properly the sparing effect observed in the pilot experiments and establish DMF factors and to improve further the already promising initial results.

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Table I: Observed incidences and latency times for radiation damage in the colon of treated rats.

schedule	n. of animals	survival rate (%)	median survival time (days)
1 (topical)			
control	6	0	23
TCDO con.	7	14	31
TCDO dil.	7	0	30
2 (i.v.)			
control	8	0	26
TCDO	4	25	67
3 (i.v.)			
control	8	0	26
TCDO	8	38	34
4 (i.p.)			
control	8	0	28
TCDO	5	40	41

Legends to figures and tables:

Table: Observed incidences and latency times for radiation damage in the colon of treated rats.

Fig.1: Radiation ulcer in the colon of the rat, 4 weeks after single dose irradiation with 23 Gy and treatment with physiological saline (control). M = mucosa; MM = muscularis mucosae; SM = submucosa; MP = muscularis proper; S = serosa; N = necrosis; GW = wall of granulocytes; A = submucosal artery with media necrosis; B = islands of bacteria; SF = subserosal fibrosis.

Fig.2: Survival curves of rats locally irradiated with 23 Gy and systemically treated with TCDO (schedule 3).

Fig.3: Radiation ulcer in the colon of the rat, 6 weeks after single dose irradiation with 23 Gy and treatment with TCDO. N = necrosis; MM = muscularis mucosae.

Fig.4: Healed radiation ulcer after TCDO treatment. 100 days after 23 Gy. MP = muscularis proper; SF = subserosal fibrosis.