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Protective Effects of Trithioformaldehyde against Radiation Damage of Mouse Jejunal Crypt Cells

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ABSTRACT

At present, the treatment of the radiation-induced diseases are only performing by the palliative treatment technique. Moreover, radioprotective drugs are a little toxic for human being and this seriously limits their application with various complication in clinical uses. Accordingly, new radioprotectors need developing.

In our Lab., we synthesized trithioformaldehyde (TTFA), containing three sulfur atom, and examined the effect on mouse jejunal crypt cells after irradiation. Mice treated with TTFA (2.0 g/kg) showed 78% survival ratio at 30 day after 800 rad irradiation. 1.0 g/kg and 2.0 g/kg of TTFA increased resistance of jejunal crypt cells to single doses of radiation by protection factors of 1.17 and 1.23, respectively.

Key Words: Radioprotective drug, Trithioformaldehyde, Jejunal crypt

INTRODUCTION

In determining the doses of radiation in radiotherapy of tumor, one must consider the radioresi stance of normal tissues because normal tissues suffer a severe adverse effect by irradiation. But that dose which not induces a toxic effect is inadequate in the elimination of tumor. Thus new methods of radiotherapy are developed; one is the use of radiosensitizing drugs, such as Ro-07-0582 which enhances the susceptibility of tumor tissues to irradiation (Yuhas *et al.*, 1977; Fowler, 1985), and another is the use of radioprotective drugs, such as WR-2721, which selectively protect normal tissues and thereby allow the use of larger radiation doses to the tumor (Haris *et al.*, 1971; Travis *et al.*, 1986).

Dale *et al.* (1949) reported that in vitro experiment with vertebrate animal cell preinjection of thiourea and thiosulfate protected moderately the damage induced by irradiation. Later, Patt *et al.* (1949) and Bacq *et al.* (1951) verified that cysteamine, glutathione and 5-hydroxytryptamine etc. enhanced the radioresistance of vertebrate organ in vivo and compounds-containing sulfhydryl group inhibited the inactivation of several enzymes by irradiation. Further, many studies were carried out on the radioprotective drugs after or before irradiation. But the application of radioprotectors in clinical radiotherapy is limitted by their toxic effect (large drug doses required to obtain significant therapeutic gains).

In this paper, we synthesized TTFA which is a cyclic aliphatic compound containing three sulfur atom, and examined the protective effect of the mouse jejunal crypt cells after irradiation.

METHODS AND MATERIALS

Mice

The mice used throughout these studies were 8 ~12 week-old ICR mice. Mice were housed 9 per cage with food and water ad lib.

Survival test

Irradiations of 800 rad were delivered to whole bodies of groups of nine ICR mice, using a Linac

Table 1. Radioprotective effect of trithioformadehyde on the irradiated mice

Treatment	Drug dose (g/kg)	Number of mice	Numbers of survived mice days after being irradiated				
			3	10	15	20	30
Control		9	9	5	2	1	1
TTFA	0.5	9	9	7	4	4	4
	1.0	9	9	7	5	4	4
	2.0	9	9	8	7	7	7

Radiation dose is 800 rad of 137 Cs. Dose is 200 rad/min.
Drug was administered 15 min prior to the irradiation.

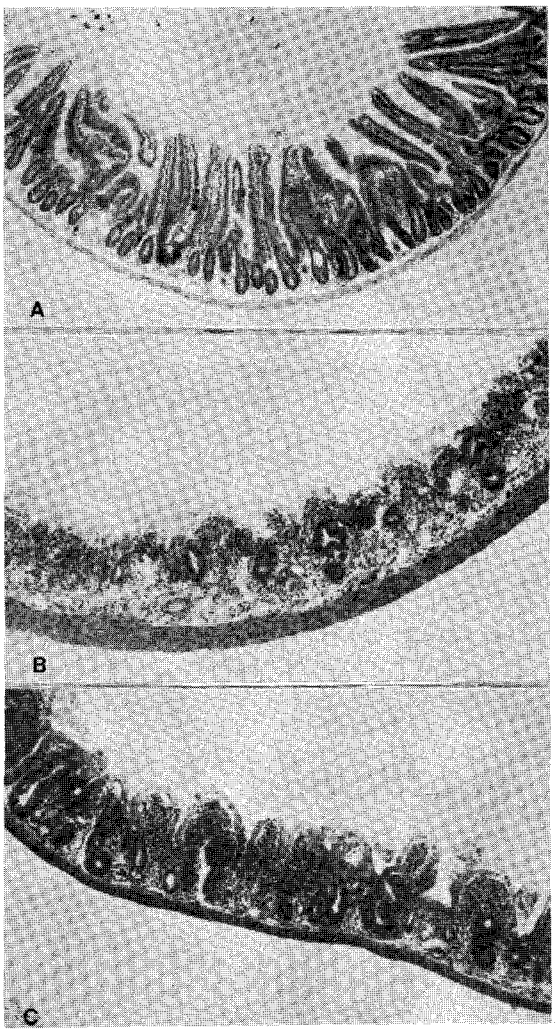

Fig. 1. Photomicrographs of jejunal cross-sections. A, normal jejunum (contains about 140 crypts); B, jejunum irradiated with 1500 rad; C, jejunum irradiated with 1500 rad 15 min after trithioformaldehyde (2.0 g/kg). Hematoxylin and Eosin x 100

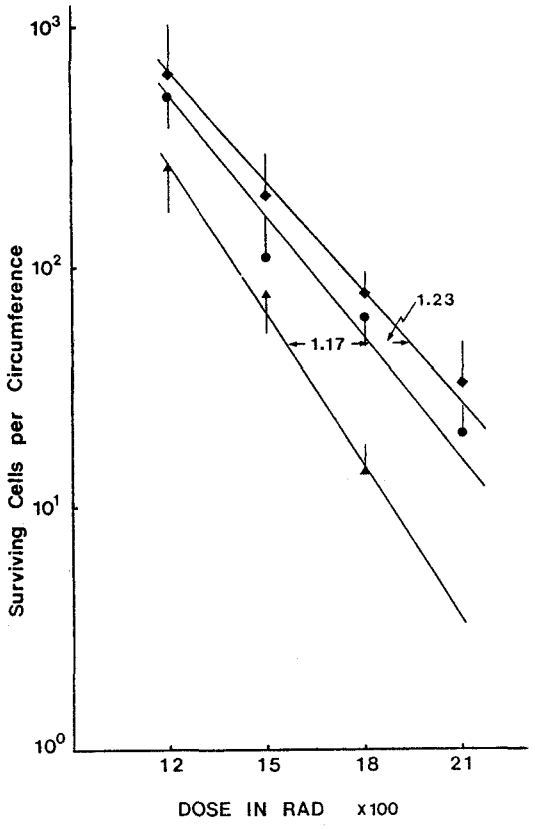

Fig. 2. Dose survival curves of jejunal crypt cells in mice exposed to single doses of radiation 15 min after trithioformaldhyde. Protection factors were estimated at an isoeffect of 30 surviving crypts per circumference.

▲ normal jejunum;
● 15 min after 1.0 g/kg of TTFA;
◆ 15 min after 2.0 g/kg of TTFA.

(Soonchunhyang hospital) at a dose rate of 200 rad/min. Trithioformaldehyde (0.5, 1.0 and 2.0 g/kg) suspended in 5% arabia gum was given intraperitoneally 15 min before irradiation.

Protection of mouse jejunal crypt cells

Groups of three mice were irradiated 1200 to 2100 rad to the whole body using a ^{137}Cs irradiator at a dose rate 573 rad/min. Trithioformaldehyde suspended in 5% arabia gum was administered intraperitoneally 15 min before irradiation. The microscopy assay introduced by Withers and Elkind (1970) was used to assay the survival of crypt epithelial cells in the jejenum of mice exposed to ionizing radiation. At day 3.5 after irradiation, mice were killed, and the jejunum prepared for histological examination.

Three tissue blocks were prepared for each mouse and stained by hematoxylin and eosin. The number of regenerating crypts in the jejunal cross-section was counted. In order to construct radiation survival curves, the number or regenerating crypts was converted to the number of survival cells by applying a Poisson correction for crypts regenerating from more than one stem cell. Lines were fitted to data points by least-sequared regression analysis.

RESULTS

LD_1 of TTFA is over 7.0 g/kg. TTFA was administered intraperitoneally in the mice 15 min prior to irradiation. Total radiation dose in survival test was 800 rad. The basic design and results of the experiment were shown in table 1, with a listing of the total number of mice in each group; the number that survived within 3 days and the number that survived in 10, 15, 20 and 30 days of treatment, respectively.

Fig. 1, shows the histology of normal jejunum, Jejunum exposed to 1500 rad, and jejunum of mice treated with both TTFA and 1500 rad. The average number of jejunal crypts per circumference in control group is 140. The number of regenerated jejunal crypts were 47 and 13 in 1500 and 1800 rad irradiation groups. In mice treated with TTFA (1.0 and 2.0 g/kg), the number of regenerating jejunal crypts after expoure to 1500 rad were 82 and 95, respectively.

Fig. 2, shows the protective effect of different doses of trithioformaldyhyde ranging between 1.0 and 2.0 g/kg ip given to mice 15 min before their exposure to 1200~2100 rad single dose of whole body irradiation. The protection was dose dependent, being more effective as the dose of TTFA increased up to about 3.0 g/kg. The regenerating crypts appeared to be randomly distributed around the jejunal circumference. The lines were fitted by linear regression analysis and protection factors were obtained using the Pike-Alper(1964) method. Protection factors estimated at an isoeffect of 30 crypts per circumference were 1.17 and 1.23 at 1 g/kg and 2 g/kg of TTFA, respectively.

DISCUSSION

A drug which enhances the radioresistance of normal tissues by administered prior to irradiation is termed as radioprotective drugs. Since Patt *et al.* (1949) clarified the radioprotective effect of cysteine, new field was developed in radiobiology. Thereafter, many sulfhydryl-containing compounds were reported as radioprotective drugs. But the clinical application was limitted by the toxicity and short duration of free sulfhydryl group.

Numerous theories have been proposed to account for the action of radioprotectors. These theories range from molecular interaction between radioprotectors and the target molecules, which are damaged by ionizing radiation, to events at the organ level which influence the state of radiosensitivity of the organ. Livesey JC *et al.* (1985) reviewed the mechanisms of action of radioprotectors: radical scavenging; mixed disulfide formation; release of endogeneous radioprotectors; hypoxia; biochemical shock, and hypothermia.

Data presented in this paper show that TTFA, containing three sulfur atom in its cycle, increases the survival number after single doses of radiation (800 rad) and protects the jejunal crypt cells against irradiation. Mice treated with 2.0 g/kg of TTFA show 78% survival ratio at 30 day after irradiation. The protection factors of TTFA (1.0 and 2.0 g/kg) for jejunal crypt cells were 1.17 and 1.23 respectively. Although these protection factor of TTFA is smaller than that of WR-2721 and DDC, we should carry out further studies because the radioprotective effect of TTFA is certain.

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=국문초록=

TTFA의 마우스 공장 소낭선에 대한 방사선 방호작용

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Patt 등이 1949년 처음으로 cysteamine과 glutathione 등이 정상조직의 방사선 내성을 증가시킨다는 사실을 규명한 이후로 많은 방사선 방어제가 연구되어 왔으나, 그들의 독성때문에 임상적 적용이 매우 제한되었다.

본 실험에서는 3개의 sulfur기를 지닌 환상구조의 trithioformaldehyde(TTFA)를 합성하여 방사선 조사 후 생존율에 대한 영향과 마우스 공장 소낭선 세포에 대한 방호 효과를 관찰하였다. TTFA 2.0 g/kg을 방사선 조사 15분 전에 복강내로 투여하고 800 rad를 조사한 후 30일간 관찰했을 때 78%의 생존율을 보였다. 또, 공장 소낭선에 대한 실험에서 TTFA는 1.0 g/kg과 2.0 g/kg에서 공장 소낭선에 대한 protection factor가 각각 1.17와 1.23 임을 나타냈다.