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Serum thymic factor as a radioprotective agent promoting survival after X-irradiation

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Summary. Serum thymic factor (FTS, zinc-free thymulin) protected mice from death after whole-body X-irradiation. It was significantly radioprotective even when administered after irradiation, but it was more effective when administered both before and after irradiation. The protective effect appears to be due to the enhancement of hematologic recovery in the animals.
Key words. Serum thymic factor; thymulin; FTS; radioprotector; radiation protection.

Protection against ionizing radiation can be achieved by so-called chemical radioprotectors, as reviewed recently by Rojas and Denekamp[1], but clinical application of these chemicals has been limited by their toxicity. Another group of radioprotective substances are cytokines. Interleukin 1 (IL-1) increases survival of irradiated animals if it is injected 20 h before irradiation[2] or 3 h after irradiation[3]. Hematopoietic factors like granulocyte-macrophage colony-stimulating factor (GM-CSF) can enhance recovery of damaged hematopoietic tissues in post-radiation therapy, and recombinant GM-CSF has actually been used to treat victims of a radiation accident[4]. Furthermore a substance such as lipopolysaccharide (bacterial endotoxin)[5] probably exerts its radioprotective action by inducing IL-1, CSFs and other cytokines in treated animals.

In the present study, we examined the radioprotective effect of serum thymic factor (FTS) in X-irradiated mice. This factor is a thymic hormone and regulates differentiation and some functions of T lymphocytes[6,7]. Chemically, it is a nonapeptide and its amino acid sequence (pGlu-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-OH) appears to be identical in different animal species[7]. According to Bach and co-workers, FTS requires zinc for its biological activity and the Zn(II)-FTS complex is now called thymulin[8]. Pharmacologically, therapeutic effects, e.g. on multiple sclerosis, have been proposed[9,10]. Because T-cells produce various cytokines able to stimulate macrophages and other cells of the immune system, we expected this T-cell activating hormone to exhibit a radioprotective effect by enhancing defense reactions in the body.

Materials and methods

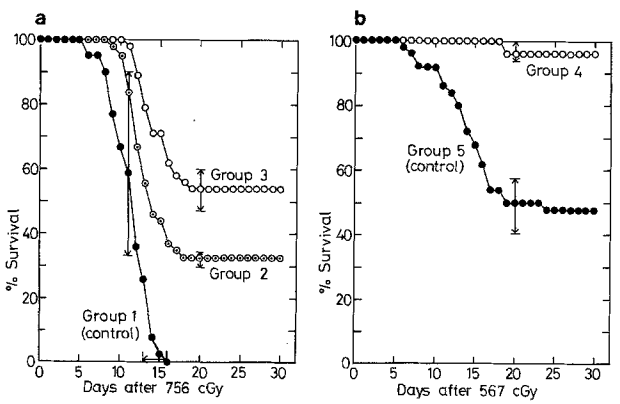
Zinc-free FTS synthesized chemically by the conventional method was dissolved in endotoxin-free saline at 0.5 mg/ml and injected daily in 0.2-ml aliquots (i.e., 100 μg FTS per injection), subcutaneously. This dosage was determined in a previous study in which FTS was given in doses ranging from 0.1 to 1000 μg per injection to establish the optimal dose to protect lethally-irradiated mice (data not shown). X-rays were generated at 200 kVp/20 mA and filtered through 0.5 mm each of Cu and Al. Male 9-week-old mice of the C3H/HeN strain were exposed to 66–70 cGy/min to give a total dose of 567 cGy (half-lethal) or 756 cGy (lethal). The animals were observed for 30 days thereafter to measure the survival rate.

In a separate hematological study, animals were irradiated as above and killed by bleeding under ether anesthesia 7 days after irradiation. Spleen weight and blood leukocyte number were determined in the individual animals. Spleen cells from 5 mice were pooled and cultured at 4×10^5/0.2 ml/well in 96-well flat-bottomed microtiter plates for 66 h at 37 °C in the presence or absence of 0.3 μg/ml concanavalin A. The culture medium was RPMI 1640 containing 5% fetal calf serum, 25 μM 2-mercaptoethanol, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml gentamycin. The cells were labeled with ^{3}H-thymidine (TdR) (0.5 μCi/well) for the last 18 h of cultivation and harvested onto glass-fiber filters. The radioactivity incorporated into the cells was determined in a liquid-scintillation spectrometer.

Results and discussion

Effect of post-radiation injections of FTS in lethally-irradiated mice. Total-body irradiation with 756-cGy X-rays killed all the control mice (Group 1) within 16 days, while 14 mice out of 43 (i.e., 32.6%) survived in Group 2 in which FTS was injected daily for 14 days after irradiation (fig., a).

We previously reported that post-radiation treatment with a 20-kDa fraction of mouse spleen extract increased the number of survivors among lethally X-irradiated mice[11]. Bacterial lipopolysaccharide[5], recombinant IL-1[3], *Acanthopanax senticosus* Harms (Shigoka) extract[12] etc. were also reported to be radioprotective even when administered after irradiation. These substances were considered to act through activation of biological defense system in the body. The use of a chemically defined substance like FTS is more appropriate for clinical application, however.



Radioprotective effects of serum thymic factor (FTS) as demonstrated by repeated daily injections in mice. *a* Lethal irradiation: a series of 3 studies, in which there were 39 control mice given saline twice before and 12 times after irradiation (Group 1); 43 mice given FTS 14 times after irradiation (Group 2); and 48 mice given FTS twice before and 12 times after irradiation (Group 3). *b* Half-lethal irradiation: 3 studies as above using 50 mice (Group 4) which received FTS as in Group 3 and a further 50 mice which received saline (Group 5). Vertical arrows in panels *a* and *b* show the highest and lowest survival rates in the 3 studies, while the horizontal arrow in panel *a* shows the variation of the time when the last animal died in the control groups.

Improved protection by consecutive injections of FTS before and after irradiation. The figure (a) also shows that the animals which received FTS from 2 days before irradiation and then for 12 days thereafter (Group 3) were protected better than the animals in Group 2; namely, 26 mice out of 48 (i.e., 54.1%) were saved from acute radiation death.

The mean survival time of the animals which died within 30 days after irradiation was 11.5 ± 0.4, 13.0 ± 0.4 and 14.4 ± 0.5 days for Group 1 (39 mice), Group 2 (29 mice) and Group 3 (22 mice), respectively. Thus, FTS prolonged the survival time of the animals more effectively when it was used before and after irradiation than when it was used only after irradiation. The mechanism of this life-prolonging effect could of course be different from that of the life-saving effect mentioned above.

Effect of FTS in half-lethally irradiated mice. The radioprotective effect of FTS was confirmed under the condition of half-lethal irradiation (567 cGy). Nearly all animals survived for 30 days when they received 14 consecutive daily injections of FTS, starting 2 days before irradiation (Group 4), while about 50% of the saline-treated animals (Group 5) died within 2 weeks after irradiation (fig., b).

When we compare the survival rate in Group 5 (fig., b) with that in Group 3 (fig., a), we can say that the dose of radiation which killed 50% of mice within 30 days ($LD_{50/30}$) was increased by about 200 cGy by the treatment with FTS. Thus, the dose-reduction factor (DRF) obtained by FTS was above 1.3, a value which is comparable with the values obtained by other groups using a combination of IL-1 and tumor necrosis factor (TNF)[2] or lipopolysaccharide[5].

A single dose of FTS given before or after irradiation. FTS was effective even when only a single dose was given, although the effect was much smaller than when it was given repeatedly.

When FTS was injected in a single dose either one or two days before irradiation (756 Gy), the survival rate was 30% and the mean survival time was prolonged by 2.6 or 3.1 days on average for the animals which died ($p < 0.01$). Administration of FTS 3 days before irradiation, however, had no beneficial effect on the survival rate nor on the survival time.

A single dose of FTS given within 15 min after irradiation (756 Gy) saved 10% of the mice and prolonged the survival time by 3.3 days. When the animals were treated with a single dose of FTS 1, 2 or 3 days after irradiation, the survival time was prolonged by 3.2, 2.5 or 2.0 days (all these values were statistically significant at 1% level) but no animals survived for 30 days in these groups. Thus a single injection of FTS was as effective as repeated injections with regard to the life-prolonging effect but much less effective with regard to the life-saving effect.

Hematological effect of FTS in half-lethally or lethally irradiated mice. A separate experiment showed that FTS prevented the decrease of spleen weight in irradiated mice

Effect of serum thymic factor (FTS) on splenic weight, concanavalin-A responsive spleen cell proliferation and blood leukocyte number in lethally or half-lethally X-irradiated mice.

Treatment[a]	Spleen weight (mg)	^{3}H-TdR uptake[b] by spleen cells ($\times 10^{-3}$ cpm/well)	Peripheral blood leukocyte number ($\times 10^{-3}$/mm^3)
None	116 ± 5[c]	74.8 ± 2.8	4.7 ± 0.4
Saline + 567 cGy	33 ± 4	3.6 ± 2.3	0.4 ± 0.1
FTS + 567 cGy	66 ± 11*	59.0 ± 9.0**	2.7 ± 0.8*
Saline + 756 cGy	38 ± 3	ND	0.3 ± 0.04
FTS + 756 cGy	70 ± 11*	ND	1.2 ± 0.7

[a] The animals were injected daily with saline or FTS twice before and 7 times after irradiation and killed 18 h after the last injection (i.e., 7 days after irradiation). Each group consisted of 5–12 mice. [b] 4 assays from 2 studies. Radioactive thymidine uptake was negligibly small in the absence of concanavalin A (16–23 cpm/well). [c] Means ± SEM. * Significantly different from the saline groups (* $p < 0.05$, ** $p < 0.01$). ND, not done.

(table). Furthermore, spleen cells of the treated animals retained a greater proliferative activity than those of control animals, as evidenced by ^{3}H-thymidine uptake in response to concanavalin A. The results suggest that T-cells competent to respond to the proliferative stimulus had been increased in number in the spleen of FTS-treated mice. In parallel with the increased weight and proliferative activity of spleen, there was a significant increase in the number of leukocytes in the peripheral blood of these animals.

The figures in the table for the protected mice have larger variations (i.e., larger standard errors, SE) than the figures for the unprotected animals. This is a reflection of the fact that FTS increased the number of animals which had recovered from the hematologic disorder, while it left some animals as sick as the unprotected ones. The hematologic picture agreed well with the mortality pattern described above, i.e. that some animals were saved but others were killed even in the FTS-treated group. The precise mechanism of the radioprotective action of FTS is unclear at present. Increase in leukocyte number is presumably not due to a direct effect of FTS on hematopoiesis, but it must contribute greatly to the rescue of mice from radiation-induced death.

Conclusion

FTS has the following advantages over other known radioprotective substances: first, it is a natural peptide hormone; second, its toxicity is very low; third, it can be chemically synthesized; fourth, it is quite stable; fifth, it is effective even when administered after irradiation. The present study raises the possibility of using FTS as a prophylactic or therapeutic drug in cancer radiotherapy as well as after radiation accidents.

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The effect of guanethidine treatment of testicular blood flow and testosterone production in rats

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Summary. Testicular blood flow was measured by means of Xenon-133 clearance in control rats and rats treated with guanethidine for 3 weeks. Plasma and testis testosterone concentrations were also measured, and the effect of hCG-treatment was examined. No difference in testicular blood flow between the control group and the guanethidine-treated group was found. However, in guanethidine-treated rats, plasma and testis testosterone concentrations after hCG-treatment were significantly decreased. The results may indicate that adrenergic nerves are involved in the regulation of Leydig cell function.
Key words. Guanethidine; testis; testicular blood flow; hCG; testosterone.

The important role of the hypothalamus and pituitary in the regulation of testicular function is well established, while little is known about the role of the nervous system. Examination of intratesticular innervation in several spe-