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RADIATION PROTECTION OF MALE FERTILITY IN MOUSE AND RAT BY A COMBINATION OF 5-HYDROXY-L-TRYPTOPHAN AND A THIOL COMPOUND (AET)

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Sperm abnormalities and fall in total sperm count following different doses (4 Gy, 5 Gy and 6 Gy) of whole body gamma irradiation (WBGR) were studied in adult male Swiss strain A mice. The protecting ability of a combination of 5-hydroxy-L-tryptophan (5-HTP, 100 mg/kg) and 2-aminoethyl isothiuronium bromide hydrobromide (AET, 20 mg/kg) was also investigated. Pretreatment with a 5-HTP + AET formulation i.p., 30 min before irradiation modified the fall in sperm counts significantly. Exposures to 4 Gy, 5 Gy and 6 Gy WBGR caused marked increase of sperm abnormalities which could be significantly reduced by pretreatment with 5-HTP-AET. WBGR with 4 Gy, 5 Gy and 6 Gy produced a short period of sterility associated with oligospermia but these abnormalities were corrected by pretreatment with 5-HTP + AET. This finding was supported by breeding experiments in pretreated adult male Sprague-Dawley rats which showed delivery of normal offsprings in drug-protected irradiated groups in contrast to irradiated controls.

Radiation affects male rat fertility (1). The timing of spermatogenesis, as shown by periods of infertility of the treated animals which differs in rats and mice, is also affected by exposure to ionizing radiation (2). Several early studies indicate that in mice certain percentages of sperms show abnormal morphology and that the types and frequencies of abnormalities are genetically controlled (3). Exposure to ionizing radiation leads to increased frequency of morphologically abnormal sperms (4). It has also been reported that following irradiation spermatocytes, spermatids and sperms, present at the time of irradiation, continue their development. However, owing to high sensitivity of late spermatogonial stages, formation of young primary spermatocytes ceases. As a result, progressive disappearence of spermatocytes, spermatids and spermatozoa occurs (5). Some alkylating agents used in chemotherapy of cancer patients have been tested for their effects on the fertility of experimental animals (6).

In our earlier studies we have found that a combination of 5-HTP (100 mg/kg) and AET (20 mg/kg) provides excellent radiation protection in terms of 30 days' survival (7, 8), peripheral blood cell counts (9) and spleen (10). 5-HTP has been reported to provide radioprotection at 200 mg/kg i.p. (11) and AET at 400 mg/kg i.p. (12). However, these doses cannot be used in combination due to the toxicity problem. Separately, 5-HTP in a dose of 100 mg/kg does not render any significant protection and AET in a dose of 20 mg/kg does not protect at all. However, when 5-HTP (100 mg/kg) + AET (20 mg/kg) is administered i.p. radioprotection is significantly enhanced without any additive toxic effects. This drug combination has also been found to provide differential protection to normal tissues (13). Pretreatment with 5-HTP + AET 30 min before 8 Gy WBGR in adult male Sprague-Dawley rats significantly modifies the radiation damage to the testis as evident from increased sialic acid content in testis and 17-ketosteroids in 24-h urine samples (14). In the present study, we have evaluated the protective effect of a combination of 5-HTP (100 mg/kg) with AET (20 mg/kg) when administered

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(i.p.) 30 min before different doses (4 Gy, 5 Gy and 6 Gy) of WBGR on the male fertility in small mammals. In an earlier study, we observed that administration of this combination (i.p.) stimulated formation of superoxide dismutase (SOD)[10]. Hence, we have also studied the effect on zinc concentration in blood and seminal fluid of control and experimental animals.

Material and Methods

AET and 5-HTP were procured from Sigma Chemicals, USA. Male Swiss albino mice (3–3½-month-old and weighing 26 ± 2 g) and adult Sprague Dawley rats of both sexes (200 ± 5 g) were housed at 23 ± 2°C and fed ad libitum. The animals were divided into control and experimental groups of 6 animals each. Experimental groups were injected i.p. with 5-HTP (100 mg/kg) + AET (20 mg/kg) dissolved in distilled water. The volume injected was 0.5 ml/25 g body weight in mouse and 1.0 ml/100 g body weight in male rats. Control groups received an equal volume of distilled water i.p.

After 30 min, control and drug pretreated animals were exposed to 4 Gy, 5 Gy and 6 Gy WBGR, dose rate 2.8–2.6 Gy per min from a ^{60}Co source in Gamma Cell '220' (Atomic Energy of Canada Ltd.). Control and experimental groups of mice were sacrificed by cervical dislocation 35 days (15–18) after WBGR. Epididymis on both sides were dissected out, macerated in normal saline and total sperm counts were made (19). Similarly, the seminal fluid from individual male mice was smeared into 3 separate slides, allowed to dry and later stained with Giemsa stain; sperms were then scored either as normal or abnormal using a Zeiss laboratory microscope. Six hundred (200/slide) sperms were examined from each male.

The effect of 5-HTP (100 mg/kg) + AET (20 mg/kg) per os given consecutively for 7, 10 and 15 days was investigated for testicular function in male Swiss strain A mice (6 animals in each group) as a part of the toxicity study for 5-HTP + AET. Zinc content in both seminal fluid and blood in control and experimental groups was estimated with Atomic Absorption meter.

Male albino Sprague Dawley rats were also exposed to 4 Gy, 5 Gy and 6 Gy WBGR similarly and fertility of irradiated control and drug pretreated irradiated male rats were determined by consecutive weekly pairing of treated males with females of proven fertility 48 days after WBGR (20–21).

Results and Discussion

Exposure to different doses of WBGR caused fall in sperm counts and induced sperm abnormalities (Table 1). Normal sperm counts in control mice were found to be $16.4 \times 10^6 \pm 0.56$ (SE). The numbers decreased to $13.0 \times 10^6 \pm 0.42$ (SE), $10 \times 10^6 \pm 0.38$ (SE) and $6 \times 10^6 \pm 0.38$ following 4 Gy, 5 Gy and 6 Gy WBGR respectively. Pretreatment with a combination of 5-HTP (100 mg/kg) and AET (20 mg/kg) i.p. 30 min before radiation exposures reduced this fall in sperm counts significantly ($p < 0.001$). Radiation also induced sperm abnormalities. Exposures to 4 Gy, 5 Gy and 6 Gy WBGR caused 20%, 25% and 30% sperm abnormalities. Pretreatment with 5-HTP + AET reduced the proportions of these abnormalities significantly ($p < 0.001$, Table 1).

Table 1

Fall in sperm counts and proportions of sperm abnormalities observed after 4 Gy, 5 Gy and 6 Gy total body gamma irradiation and their modification by pretreatment with 5-HTP (100 mg/kg i.p.) + AET (20 mg/kg i.p.) Mean ± SE of 6 mice. $p^* < 0.001$ compared to irradiated controls

Treatment	Sperm counts (10^6 ml^{-1})	Sperm abnormality %
Control	16.4 ± 0.56	
4 Gy	13.0 ± 0.42	20 ± 2
5-HTP + AET + 4 Gy	17.0 ± 0.72*	5 ± 1.5*
5 Gy	10.0 ± 0.56	25 ± 2
5-HTP + AET + 5 Gy	15.6 ± 0.76*	8 ± 2*
6 Gy	6.0 ± 0.38	30 ± 2.5
5-HTP + AET + 6 Gy	12.4 ± 0.82*	10 ± 1.5*

Administration of 5-HTP + AET orally for 7, 10 and 15 days consecutively led to 5%, 8% and 15% sperm abnormalities (Fig. 1). However, considering the increase in the total sperm count that simultaneously was induced (Fig. 1), the increase of sperm abnormalities was ignorable. These results demonstrated that administration of this drug combination, even for longer periods, did not have a significant toxic effect on the testicular function.

Administration of 5-HTP + AET stimulated endogenous zinc concentration in blood and seminal fluid (Figs 2 and 3). Zn^{2+} is an essential constituent of some radioprotective enzymes like superoxide dismutase (SOD) and it can be assumed that increased concentration of zinc has a protective effect on radiation induced damage of the gonads. The

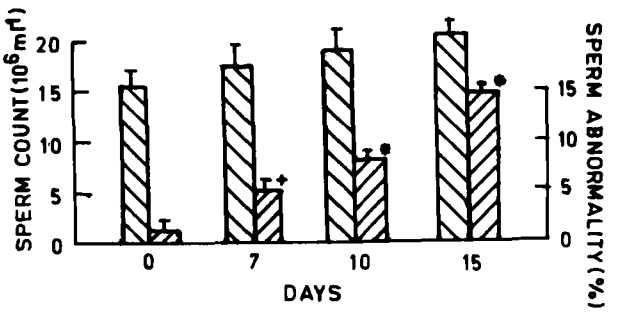

Fig. 1. Effect of 5-HTP + AET (orally) consecutively for 7, 10 and 15 days on the testicular function.
Sperm count ▨, Sperm abnormality ▨
*$p < 0.001$ + $p < 0.05$ compared to controls.

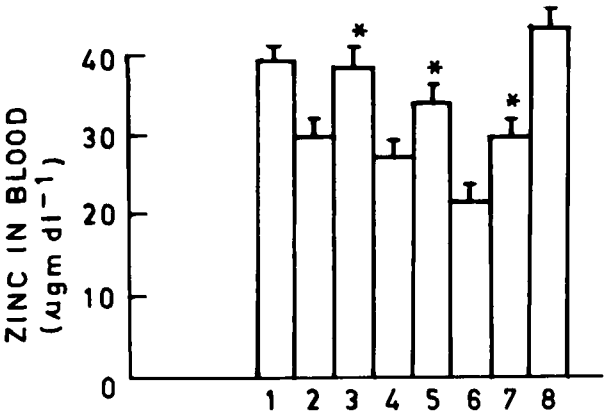

Fig. 2. Zinc content in blood of mice exposed to 4 Gy, 5 Gy and 6 Gy WBGR and its modification by pretreatment with 5-HTP (100 mg/kg i.p.) + AET (20 mg/kg i.p.). 1: Control. 2: 4 Gy. 3: 5-HTP + AET + 4 Gy. 4: 5 Gy. 5: 5-HTP + AET + 5 Gy. 6: 6 Gy. 7: 5-HTP + AET + 6 Gy. 8: 5-HTP + AET, no irradiation. Mean ± SE of 6 mice *p < 0.001, compared to irradiated control.

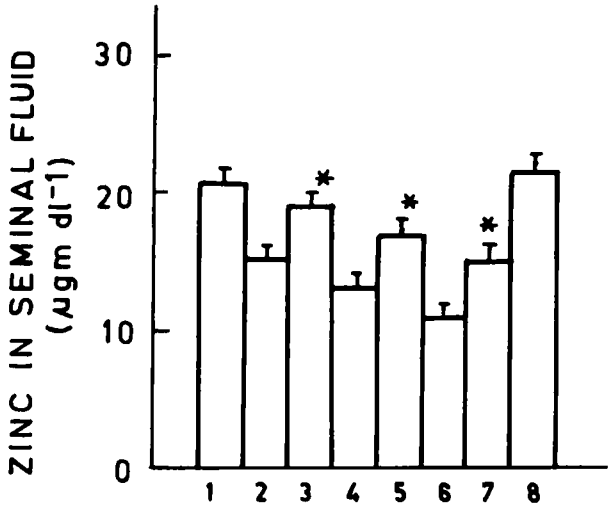

Fig. 3. Zinc content in seminal fluid of mice pretreated with 5 HTP (i.p.) and exposed to 4 Gy, 5 Gy and 6 Gy respectively. Mean ± SE of 6 mice. *p < 0.005, compared to irradiated controls. For explanation of staples 1–7, see legend to Fig. 2. Staple 8: 5-HTP + AET, no irradiation.

presence of metal ion Zn^{2+} exerted a protective effect against inactivation by reducing radicals formed due to radiation exposure (21).

The radioprotective role of 5-HTP + AET was further demonstrated by carrying out breeding experiments on treated males paired with fertile females. The results showed that exposure to 4 Gy WBGR delayed the conception by about 30 days. Exposures to 5 Gy and 6 Gy WBGR inhibited conception for 7 months (observation period) indicating a period of sterility. We are not sure whether these doses of WBGR made the animals permanently sterile (Table 2). However, pretreatment with 5-HTP (100 mg/kg) + AET (20 mg/kg) i.p. 30 min before

Table 2

Effect of total body gamma irradiation and pretreatment with 5-HTP (100 mg/kg i.p.) + AET (20 mg/kg i.p.) studied by pairing of treated male rats with untreated fertile female rats. The animals were set for breeding 48 days after radiation exposure

Treatment	Birth of litters after breeding (days)	No. of litters ± SD
Control	40 ± 5	9 ± 3
4 Gy	70 ± 10	7 ± 2
5-HTP + AET + 4 Gy	60 ± 8	10 ± 3
5 Gy	Nil (up to 7 months)	–
5-HTP + AET + 5 Gy	75 ± 10	8 ± 3
6 Gy	Nil (up to 7 months)	–
5-HTP + AET + 6 Gy	120 ± 12	6 ± 3

radiation exposure resulted in pregnancy and normal delivery of litters (Table 2). No case of abortion or still birth was noted. Body weight, growth pattern and the general condition of the litters were carefully observed and recorded for two months (Fig. 4). The growth pattern of the litters from the drug protected and irradiated groups (5 Gy and 6 Gy WBGR) were comparable to those of the non-irradiated control group.

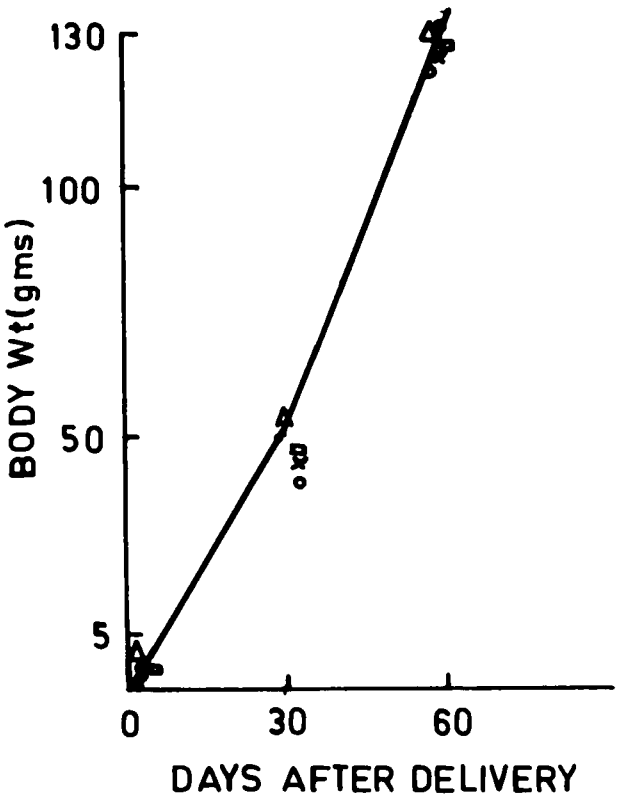

Fig. 4. Development of body weight in litters of treated male rats and untreated fertile female rats.
●– – –● Control; ○– – –○ 4 Gy; △– – –△ 5-HTP + AET + 4 Gy; □– – –□ 5-HTP + AET + 5 Gy; ×– – –× 5-HTP + AET + 6 Gy

In conclusion it may be inferred that the used drug combination rendered significant radioprotection of the male gonadal function against 5 Gy and 6 Gy WBGR.

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