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Reduction in Radiation Lethality by Chemical Mixture and Bone Marrow in Mice[1]

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INTRODUCTION

It has been shown previously (1) that 0.9 mg of serotonin with either 1.2 mg. of 2-aminoethylisothiuronium (AET) or 2.4 mg of 2-mercaptoethylamine (MEA), dose expressed as free base per mouse, afforded 70 to 80 per cent 30-day survival to young CF_1 mice exposed to 1100 r acute whole-body X-radiation; the $LD_{100/30\text{-day}}$ value for these mice being 650 r.

Preliminary studies in this laboratory *(unpublished data)* revealed that giving all three of these agents—serotnin, AET, and MEA in a single injection—produced increased survival to radiation doses above 1100 r.

Isologous bone marrow given post-radiation has been demonstrated to provide survival from supra-lethal radiation (2), and has been shown to enhance protection offered by chemical agents given pre-radiation (3, 4).

The present study was carried out to find increased survival from acute whole-body X-radiation with minimal therapeutic toxicity, using the above three-chemical mixture pre-radiation, and isologous bone marrow post-radiation.

METHODS

Four hundred and forty-two female C_3H mice, 20 to 25 gm, 90-105 days old, were employed. X-rays of 250 KVP with a half-value layer of 1.1 mm Cu were delivered at a rate of approximately 150 r/min. The mice were irradiated in a shallow lucite cage 30 cm in diameter, containing ten sectored compartments. To insure uniform irradiation the cage was placed on a rotating table during exposures. Dosimetry was carried out with a 250-r (Victoreen) thimble chamber placed in a hollow paraffin mouse phantom with phantoms in all other compartments. Mortality was recorded daily. Laboratory chow and water were allowed *ad libitum.*

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The mice were irradiated in four groups as follows: A) control (no treatment), B) marrow only, C) chemical mixture only, containing 0.35 mg serotonin, 0.85 mg AET, and 2.0 mg MEA (free base per mouse), D) chemical mixture plus bone marrow.

The chemical mixture was prepared in saline, under aseptic conditions just prior to injection. Neutralization did not improve results and was not done in these experiments. The mixture in a volume of 0.6 ml was administered intraperitoneally 7 to 10 minutes prior to irradiation.

Isologous marrow was prepared on the day of irradiation from C_3H mice of the same age and sex as the recipients. Marrow "plugs" were flushed through a syringe without a needle, using TC-199[1] as diluent. The suspension was kept in an ice bath or stored at 5°C. All equipment in contact with marrow was siliconized. Heparin was unnecessary as clumping did not occur. Approximately 6×10^6 nucleated cells in 0.5 ml were injected in the tail vein through a No. 27 gauge needle within 4 hours post-irradiation.

RESULTS

The $LD_{50/30}$ value for the control group was approximately 680 r as shown in Table I. Bone marrow given post-radiation increased the value to 1100 r. With chemicals only, 84 per-cent survival was observed at 1400 r with the $LD_{50/30}$ increased to about 1500 r. When the chemical mixture was given pre-radiation and bone marrow post-radiation (Group D) 91 per cent survival occurred at 1700 r, and 52 per cent survival at 2000 r (Table II). Thus, in terms of 30-day survival the chemical mixture gave a dose reduction factor of approximately two (Fig. 1).

Seven deaths were found within 30 minutes of the chemical mixture injection in 212 animals (3.3 per cent). An additional death occurred during marrow injection and was attributed to air embolis. There were thus eight deaths in the 282 treated animals (2.8 per cent). These deaths were not included in the survival

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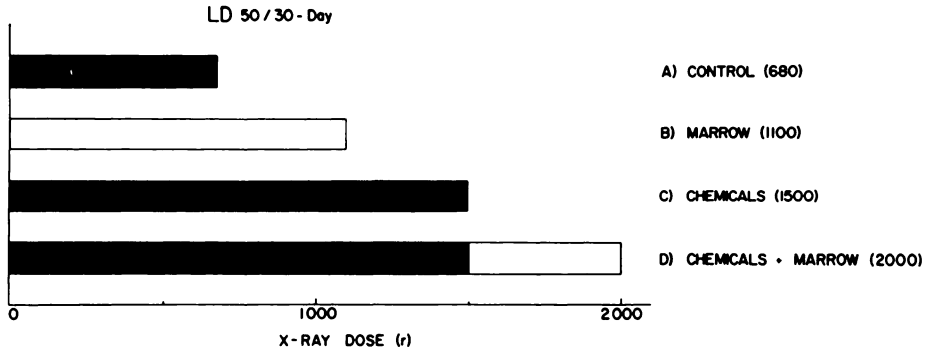

Fig.1. Increase in median lehtal dose on probability plot by chemicals and/or marrow. Comparison of groups A and C, or B and D, shows the dose-reduction factor of 2 for chemicals. The additive effect of marrow is shown in D.

data; no other deaths occurred in the entire experiment until the fourth post-irradiation day, and these were considered to be radiation-induced.

Observation was continued until 90 days post-irradiation. Delayed mortality, that is, death beyond 30 days post-irradiation, appeared in the "chemical only" group (Group C) at 1200 r, but not in the few survivors of the "marrow only" group (Group B) at the same exposure. Delayed death, however, was more obvious in the dose ranges above 1200 r (Fig. 2).

DISCUSSION

In the above experiments 52 per-cent 30-day survival at 2000 r demonstrates the effectiveness of this chemical mixture-marrow combination against the acute lethal response to whole-body X-irradiation. These results compare favorably with those reported by Urso, et. al. (3). Using the combination of 2-mercaptoethylguanidine (MEG) and marrow, they noted an increase of the $LD_{50/30}$ of CAF_1 mice to 1800 r from a control value of 700 r. MEG represents the rearranged product of neutralized AET. In a similar study Burnett and Doherty (4) noted the additive effects of AET, marrow, and streptomycin in (101 x C_3H)F_1 mice.

The significance of the mouse strain used for evaluation is exemplified by the work of Doherty and Burnett (5), who found that at 800 r X-irradiation, 17 to 18 μM AET (2.2 mg free base per mouse) yielded 88-per-cent survival of (101 x C_3H) F_1 mice, but only 33-per-cent survival of C_3H mice.

Toxicity has been a major concern with radioprotective compounds when used in the amount necessary to produce significant protection. Urso (4) noted a 50-per-cent mortality from 9.0 mg AET•Br•HBr (4 mg free base) in the CAF_1 mouse. In female C_3H mice, the LD_{50} for AET is about 5.0 mg free base per mouse (6), and 5.4 mg free base per mouse for MEA (7).

Doses (as free base) up to 1.8 mg serotonin, 2.4 mg AET, and 2.34 mg MEA have been individually given in this laboratory to female C_3H mice without mortality. In these doses none of the agents produce more than 70-per-cent survival at 800 r X-radiation. However, when 0.35 mg serotonin, 0.35 mg AET, and 2.0

TABLE I

30-DAY SURVIVAL OF UNTREATED (GROUP A) FEMALE C_3H MICE AT VARIOUS DOSES OF RADIATION

No. of Mice	*X-Ray Dose (r)*	*30-Day Survival alive/total*	*%*
20	600	15/20	75
30	650	21/30	70
20	700	7/20	35
30	750	5/30	16
30	800	2/30	7
20	850	0/20	0
10	900	0/10	0

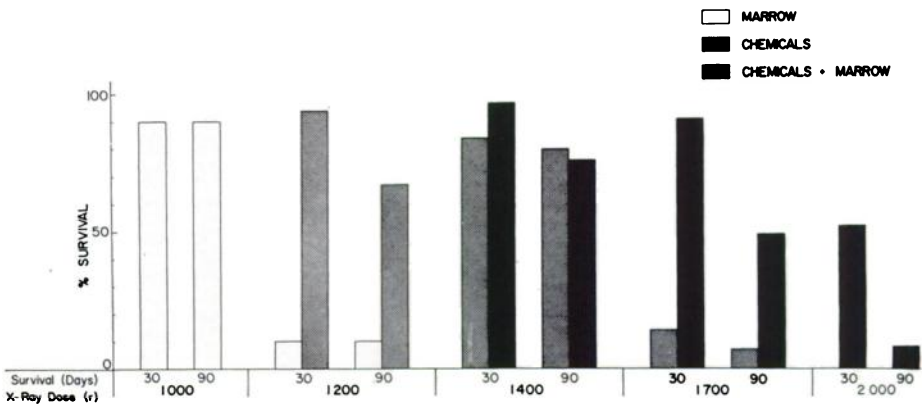

Fig. 2. Comparison of 30 and 90-day survival periods.

mg MEA are combined in a single injection, the results are strikingly different—84 per cent survival at 1400 r (Table III).

The mechanism of action of these compounds remains unknown. Sulfhydryl-containing compounds are thought to have a related mechanism (5, 6, 7); serotonin, not containing sulfhydryl, would thus have a different action. Others (8, 9, 10, 11) regard the profound systemic (anoxic) reaction as possibly being the "protective" factor. Whether a biochemical or physiological reaction is the primary mechanism will probably remain unanswered until radiation injury itself is better understood.

When considering the action of a group of radioprotective agents given

TABLE II

EFFECTS OF CHEMICAL MIXTURE AND/OR MARROW ON 30-DAY SURVIVAL OF FEMALE C_3H MICE EXPOSED TO SUPRA-LETHAL DOSES OF RADIATION

Group	*No. of Mice*	*X-Ray Dose (r)*	*Treatment* *Chem. Mix.*	*Bone Marrow*	*Therapeutic Mortality*	*30-Day Survival* *alive/total*	*%*
	30	1000		+	0	27/30	90
B	20	1100		+	0	11/20	55
	20	1200		+	0	2/20	10
	20	1200	+		2	17/18	94
C	40	1400	+		2	32/38	84
	30	1700	+		1	4/29	14
	30	1400	+	+	0	29/30	97
D	48	1700	+	+	1	43/47	91
	44	2000	+	+	2 (a)	22/42	52

(a) One death attributed to air embolis.

simultaneously, more questions are raised than are answered. Synergistic radioprotective action seems apparent from the fact that the eflect of the mixture is far greater than combined effects of the individual agents (Table III). That these desirable results are not accompanied by synergistic toxic reactions, in terms of drug mortality, is a fortunate occurrence. This suggests that the agents have different mechanisms of action.

Isologous marrow, on the other hand, would act in a reparative rather than a preventive fashion. Fortunately, the action of marrow is retained following chemical protection pre-irradiation. This would logically follow if we assume that chemical protection exerts its effects through "dose reduction." For example, 2000 r, given after chemicals with a dose reduction factor of two, would have the effect on the animal of 1000 r, and at the latter dose marrow is beneficial. Ordinarily, marrow is of no benefit at 2000 r.

The precise meaning of dose-reduction is not entirely clear. As Mole has pointed out (11), when chemical protection is employed radio-resistant members of a group become more resistant, while sensitive members remain so. This results in a statistically significant change in slope of the dose-mortality curve. Isologous marrow produces "all-or-none" results, roughly parallel to the steep dose-mortality curves of radiation alone. From our results when chemicals and marrow are

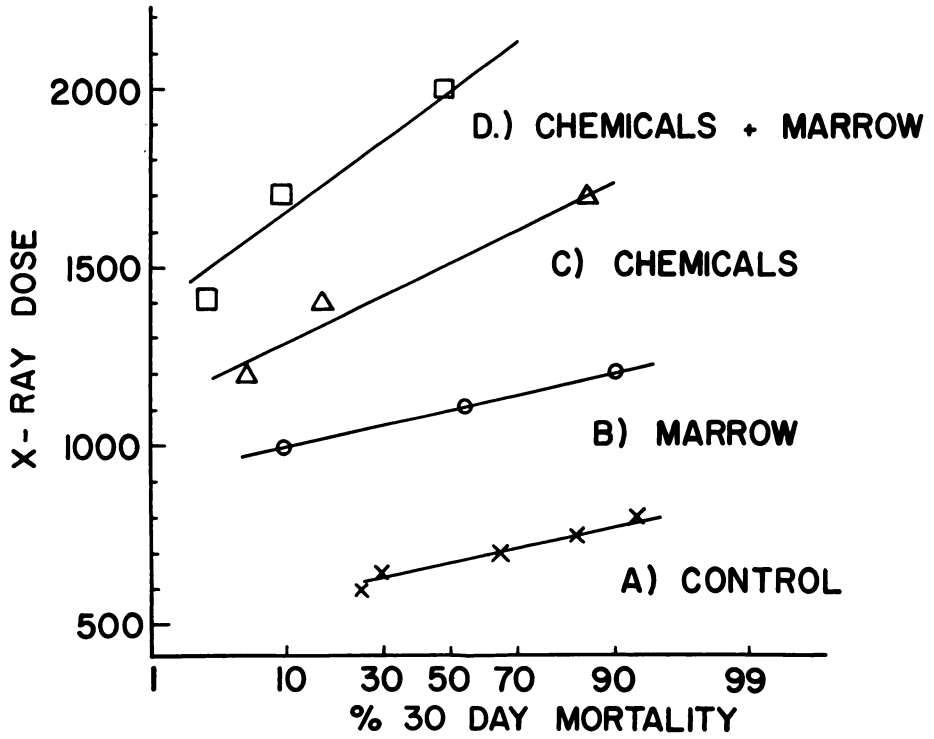

Fig. 3. Dose-mortality curve as influenced by treatment with chemicals and/or marrow. Note change of slope in groups C and D.

combined, the chemical effects predominate yielding a slope similar to chemicals alone (Fig. 3).

Comparison between the $LD_{50/30}$ for controls (680 r) and marrow only (1100 r) reveals a difference of about 400 r. Comparison between the $LD_{50/30}$ for chemicals (1500 r) and chemicals plus marrow (2000 r) shows a difference of about 500 r. The variation of 400 and 500 r is not great; thus, the enhancement of protection by marrow is additive when used with chemicals.

By employing 90-day survival instead of the generally used 30-day survival period, the dose-reduction factor at exposures above 1000 r becomes less than two, as shown in Figure 2. Whether this delayed (beyond 30 days) mortality is dependent on radiation alone, or due to the failure of treatment to protect certain organ systems, is not known.

Nonetheless, we feel that this demonstration of protection to acute radiation injury with minimal therapeutic toxicity by a combination of known effective agents lends itself well to studies of other mammalism systems, including man. As new agents become available and mechanisms of their actions become better understood, increased understanding of the pathogenesis of radiation injury would be the logical result.

CONCLUSIONS

Using a mixture of three radioprotective chemicals—0.35 mg of serotonin, 0.85 mg of AET, and 2.0 mg of MEA (dose expressed as free base per mouse)—the $LD_{50/30\text{-day}}$ exposure value was increased from 680 r to 1500 r X-radiation given to female C_3H mice. Combining this mixture pre-radiation with isologous marrow post-radiation, 91 per-cent 30-day survival at 1700 r and 52 per-cent 30-day survival at 2000 r X-radiation was obtained.

TABLE III

COMPARISON OF RADIOPROTECTIVE CHEMICALS GIVEN INDIVIDUALLY, AND AS A MIXTURE

Chemical 10 min. Pre-Rad	*Mg Free Base/Mouse*	*μM/Mouse*	*X-Ray Dose (r)*	*% 30-Day Survival*	*Dose Reduction in r*
Serotonin	1.8	10	800	50	120
AET	2.4	20	800	70	150
MEA	2.34	30	800	20	75
					345 r (a)
Mixture of:					
Serotonin	0.34	2			
AET	0.85	7	1400	84	>800 r
MEA	2.0	25			

(a) Sum of dose reduction of individual agents.

Seven deaths occurred within 30 minutes of injection of chemicals to 212 animals (3.3 per cent). One additional death was attributed to air embolis at the time of injection. All other deaths were considered radiation induced.

It is suggested that effective protections with reduced toxicity is attained through synergistic chemical action, plus the additive action of marrow. The mechanisms remain unknown.

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