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RADIOBIOLOGY

Shortening of the Span of Life of Rats by 'Myleran'

THERE are few reports on late somatic effects of radiomimetic chemicals, especially those dealing with span of life. In mice and rats the first observations on the effects of single and repeated doses of nitrogen mustards and myleran on span of life are rather conflicting and the dose-response relationship has not been established[1–5]. Following the administration of single doses of a radiomimetic agent the span of life shortening is less effective because the sub-lethal dose range of many of them is narrow. By repeated doses properly spaced to avoid the early mortality, a larger total dose may be administered and in this way a significant reduction of span of life obtained[3]; however, as more variables are introduced inherent to dose fractionation, the interpretation of the dose-response relationship may be complicated. On the other hand, the early mortality in rats associated with bone marrow aplasia induced by single doses of myleran can be successfully reduced by isologous and homologous bone marrow transfusions, and in those rats which survive 30 days the observation of long-term effects can be extended to single doses of myleran two or three times greater than the $LD_{50/30}$ (refs. 6, 7).

This communication reports the relationship between the reduction of span of life in rats and the dose of myleran. White male *L*-strain rats bred here by random mating were used; at the start of the experiment they were 3–4 months old, weighing 140–150 g. A group of rats survived the first 30 days to mid-lethal doses of myleran (12·1, 13·8 and 15·5 mg/kg). Another group is composed of 30-day survivors treated with bone marrow transfusions ($32–128 \times 10^6$ nucleated cells on the 1st, 3rd or 5th day) following administration of increasing doses of myleran (from 15·5 to 48·3 mg/kg). A previous report from this laboratory described experimental methods and results on the 30-day survival[6]. The mean span of life and other criteria of longevity were established from the day of administration of myleran. Simultaneously until natural death a group of normal rats which served as controls has been recorded. Routine autopsies were performed on all animals.

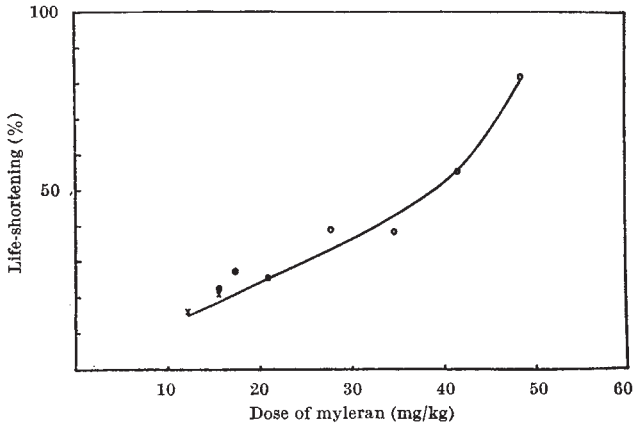

Fig. 1. The relationship between the shortening of span of life of *L*-strain rats and the dose of myleran. ×, rats receiving only myleran; ○, rats treated with bone marrow transfusions after myleran administration; ●, rats treated with bone marrow transfusions, data corrected for the mortality occurring during the 2nd and 3rd month

The results are summarized in Table 1. The mean, median and maximal spans of life at various dose-levels indicate that the long-term survival of rats is inversely related to the dose of myleran.

In the group of rats not treated with bone marrow transfusions, however, observed values for the mean span of life are not significantly different from control values. To demonstrate that the observed differences are statistically significant a greater number of animals had to be used and this fact emphasizes why definitive data at sub-lethal and mid-lethal dose ranges are difficult to obtain[4].

In the group of rats treated with bone marrow transfusions following myleran doses of 15·5 and 17·2 mg/kg the rats presented on the whole a more important reduction of span of life not in relation to dose of myleran used. Among them about 25 per cent died during the second and third month with symptoms indicating a secondary rejection of the bone marrow graft. This particular incidence of mortality does not occur among the rats which received nearest myleran doses without bone marrow treatment or in bone marrow treated rats following administration of higher myleran doses which depress more profoundly the immunological defence reactions of the host. By excepting the latter two dose-levels, the lowest myleran dose that resulted in a statistically significant decrease in span of life was 20·7 mg/kg, at the 0·01 confidence-level.

In Fig. 1, the shortening of life in rats expressed as a percentage of the normal span of life has been plotted against the doses of myleran on the ordinary scales. If

Table 1. LONGEVITY DATA OF *L*-STRAIN RATS SURVIVING 30 DAYS FOLLOWING ADMINISTRATION OF SINGLE DOSES OF MYLERAN WITH OR WITHOUT BONE MARROW TRANSFUSIONS

Experimental conditions	Dose of myleran (mg/kg)	No. of rats	Mean span of life ± *E.S.* (days)	Reduction of mean span of life (%)	Median span of life (days)	Maximum span of life (days)
Controls	—	24	551 ± 41	—	558	998
Myleran	12·1	21	461 ± 34	16·3	482	774
	13·8	12	482 ± 65	12·5	499	789
	15·5	13	436 ± 64	20·9	449	719
Myleran and bone marrow	15·5	106	324 ± 22 (427)*	41·2 (22·5)*	337	852
	17·2	75	303 ± 25 (401)*	45·0 (27·2)*	298	778
	20·7	99	410 ± 21	25·6	442	880
	27·6	119	336 ± 16	39·0	333	700
	34·5	80	340 ± 20	38·3	333	766
	41·4	29	246 ± 33	55·4	250	581
	48·3	2	99	82·0	99	128

* Corrected for the mortality occurring during the 2nd and 3rd month.

we assume that transplantation of bone marrow in rats is ineffective to reduce shortening of span of life, as it has been found in irradiated animals[8], the shortening of span of life by myleran is a non-linear function of the dose with an important reduction of span of life with the increasing dose. It is interesting that a similar dose-response relationship was found in animals exposed to single doses of whole body irradiation[9].

When compared in terms of the $LD_{50/30}$ doses, the ionizing radiation appears a more potent agent for reducing the span of life than myleran. However, the comparison on this basis may be misleading because the sensitivity of rat bone marrow tissue to myleran may be more important than to ionizing radiation and also relatively to the sensitivity of other tissues which could be involved in the shortening of span of life. For example, the sensitivity of bone marrow tissue to myleran, as reflected by the $LD_{50/30}$ doses, is more important in rats than in mice (respectively 14 and 56·7 mg/kg)[6]. If there are no species differences for the shortening of span of life by myleran, it can be predicted that single sub-lethal doses above 20·7 mg/kg of myleran would significantly reduce the span of life in mice.

In addition, routine autopsies of rats showed the presence of lens cataracts and testis atrophies with incidence rate and degree in proportion to myleran dose. The incidence of macroscopic neoplasms was not above the normal level observed in the strain of rats used. Terminal causes of death apparently did not differ from those found in normal controls dying spontaneously; but it is not impossible that systematic histological investigations now in progress would disclose other particular findings, especially in rats which received higher doses of myleran.

It may be concluded that single doses of radiomimetic chemicals share with ionizing radiations the property to reduce the span of life in mammals. It is hoped that further investigations in this field may shed more light on the mechanism of shortening of life by the agents described here and on factors which could modify their delayed somatic effects.

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Distribution and Fate of Soluble Proteins labelled with Iodine-131 from Rat and Rabbit Livers

Soluble proteins of rat and rabbit liver have been labelled[1] with iodine-131 using the chloramine *T* radioiodination procedure[2,3]. In this communication the distribution and fate of liver ^{131}I-proteins in relation to the chloramine *T* and iodine monochloride[4] (ICl) procedures are described.

Soluble proteins were extracted from rat and rabbit liver, were dialysed and labelled with carrier-free iodine-131 ('*IBS*.3', Radiochemical Centre, Amersham) as previously described[1]. ^{131}I-tungstophosphoric acid (TPA, final concentration: 1·7 per cent in 0·68 N hydrochloric acid) soluble radioactivity as percentage of the original radioactivity was measured with carriers sodium iodide and bovine serum proteins.

The distribution of homologous liver ^{131}I-proteins was investigated in male Wistar rats of about 250 g weight having fasted for 26 h. The sample (100–120 μc.) was injected via the femoral vein into the ether-anæsthetized animal and its total body radioactivity was immediately counted. After a 15-min interval blood was withdrawn from the abdominal aorta and perfusion with 1 l. heparinized saline was started via the portal vein. Afterwards several organs were removed, enclosed in thin polythene bags and separately set, in their proper position, inside the thoracic and abdominal cavity of a non-radioactive rat carcass. In identical geometry, the rat was counted in order to assess the percentage of dose retained by the organs. The radioactive rat carcass and an aliquot of blood were also measured.

Results are shown in Table 1. A somewhat lower yield of protein-bound radioactivity was observed using higher amounts of iodine-131 (4 mc.). Raising the amount of chloramine *T* (5–20 mg) determined, after addition of the reducing agent, the appearance of an increasingly intense opalescence in the protein solution. Correspondingly an approximate proportionality between the percentage of dose retained by the liver and the amount of chloramine *T* used became evident, the higher uptake being due to some denatured proteins during the iodination. Heat-denatured[5] serum and liver ^{131}I-proteins gave 81 and 89 per cent retention, whereas normal serum ^{131}I-proteins gave about 1·2 per cent[3].

When liver proteins were labelled with iodine-131 by using the iodine monochloride method[4] (the final reagent

Table 1. Rat Liver Retention of Rat Liver Soluble ^{131}I-Proteins

Labelling techniques	Percentage total ^{131}I-recovery	Percentage of dose after 15 min from injection in: Blood	Liver	Carcass
Chloramine *T* (2 mg)	28·0	22·9 ± 1·3	51·5 ± 0·3	16·7 ± 0·8
Chloramine *T* (5 mg)	40·6	15·5 ± 0·5	61·4 ± 0·9 (+19·2%)	14·7 ± 0·2
Chloramine *T* (10 mg)	44·0	13·4 ± 2·4	66·7 ± 0·3 (+29·5%)	12·7 ± 1·1
Chloramine *T* (20 mg)	43·2	6·8 ± 0·2	74·7 ± 0·2 (+45·0%)	11·1 ± 0·7
Chloramine *T** (20 mg)	43·2	9·3	68·5 (+33·0%)	11·6
Iodine monochloride (126 μg)	4·5	22·1	51·9 (+0·8%)	17·9
Iodine monochloride (210 μg)	6·8	21·9	52·7 (+2·3%)	16·7

Each sample of liver proteins (29·4 mg) was labelled with 4 mc. iodine-131. Percentages of ^{131}I-TPA soluble radioactivity ranged between 3·5 and 6·7 per cent. Carrier liver proteins were added to prevent self-irradiation. About 10 mg of liver ^{131}I-proteins (in 2·0 ml. *p*H 7·6 phosphate buffer) were administered to the rats except one. Figures with ± *S.E.* represent mean of 3 animals. Figures between brackets represent percentages of higher liver retention.

* This rat received 59 mg liver proteins.

Percentage of dose in liver: P (2 mg : 5 mg) < 0·001; P (2 mg : 10 mg) < 0·001; P (2 mg : 20 mg) < 0·001.

Table 2. Rabbit Liver Soluble ^{131}I-Protein Recoveries using the Iodine Monochloride and Chloramine *T* Radioiodination Procedures

Labelling techniques	Opalescence of protein solutions	Percentages ^{131}I-recovery	^{131}I-TPA soluble radioactivity
Iodine monochloride 126 μg	−	12·1	6·3
210 μg (*a*)	−	18·3	4·9
Chloramine *T* 2 mg (*b*)	−	43·8	5·8
Chloramine *T* 5 mg (*c*)	+	45·9	3·7
Chloramine *T* 10 mg (*d*)	+ +	48·5	3·5

The reaction mixture was made up with proteins (30·9 mg) in 0·15 M phosphate buffer, *p*H 7·6, with 5 mc. iodine-131 per each sample. Addition of carrier liver proteins prevented self-irradiation damage. Percentages of ^{131}I-TPA soluble radioactivity were estimated on the eluates.

a, *b*, *c*, and *d* refer to curves shown in Fig. 1.