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KINETICS OF REPAIR OF SUBLETHAL RADIATION INJURIES IN EARLY HEMATOPOIETIC PRECURSORS

T. V. Todriya and O. I. Gan

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Hematopoietic precursor cells, namely splenic colony-forming units (CFUs), possess high ability to repair sublethal radiation injuries (SLRI), as has been demonstrated on Elkind's model of repair for precursors forming colonies in the spleen of a lethally irradiated recipient on the 7th-10th day. The degree of repair of SLRI during fractional irradiation depends on several parameters of radiosensitivity, proliferative activity, proliferation rate, and position of the cells in the cell cycle. For mature CFUs (CFUs at 7-10 days) maximal repair is recorded with a 5-h interval between the first and second fractions of irradiation [11], but by contrast, with the same conditions of fractionation, less mature hematopoietic precursors (CFUs at 11 days) exhibit reduced ability to repair SLRI [1, 2]. It is not yet clear, however, whether reduced ability for early postradiation repair of sublethal injuries is a property possessed by early hematopoietic precursors (CFUs at 11 days), or whether the 5-h interval used between fractions does not permit the completeness of repair of SLRI to be estimated. The present investigation was conducted to shed light on these problems.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice. Repair of radiation injuries was studied on Elkind's model of repair [4], adapted for hematopoietic tissue [11]. Recipient mice were irradiated in a dose of 6 Gy on an IPK γ-ray apparatus (^{137}Cs), with a dose rate of 0.185 Gy/min, after which the irradiated animals (10 mice in each group) were given an injection of $5 \cdot 10^6$ bone marrow cells per mouse. Some of the experimental mice were irradiated 1 h after transplantation of the cells with a single dose of 6 Gy (unfractionated irradiation group — UFI). Other recipient mice were irradiated fractionally, with intervals of 1, 2, 3, 4, 5, and 6 h between two equal doses, each of 3 Gy, of irradiation — the fractional irradiation group (FI). Thus the recipients in all the experiments received a total dose of irradiation of 12 Gy, and the injected cells received 6 Gy (a single dose or in fractions with an interval of 1-6 h). Colonies were counted in the spleens on the 8th day (control) and 11th day

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TABLE 1. Number of 8-Day CFUs (number of colonies per spleen) Formed by Bone Marrow Cells Irradiated in a Single Dose or Fractionally (M ± m)

No. of expt.	UFI	Interval between first and second doses of FI, in h					
		1	2	3	4	5	6
1	9,86±1,47	14,50±3,33 (1,47)	19,17±0,75* (1,94)	15,75±1,03* (1,60)	18,50±4,17 (1,88)	29,57±2,85* (3,01)	26,0±4,06* (2,64)
2	9,83±0,87	12,33±1,38 (1,25)	13,29±2,11 (1,35)	15,40±0,75* (1,57)	17,71±1,06* (1,80)	17,29±2,39* (1,76)	14,45±2,11 (1,47)
3	3,80±0,47	6,11±1,21 (1,61)	10,89±0,98* (2,87)	13,40±1,32* (3,53)	12,00±2,14* (3,16)	13,71±2,20* (3,61)	

Legend. Here and in Table 3, RI shown in parentheses, and estimated as ratio between number of colonies formed during FI to that formed with UFI. Asterisk indicates significant changes in number of colonies relative to values obtained with UFI.

TABLE 2. Dynamics of Changes in RI Depending on Time Interval Between First and Second Fractions of Irradiation (mean data)

Subclass of CFUs	Interval between first and second doses of irradiation, h					
	1	2	3	4	5	6
8-day	1,44±0,74	2,05±0,31*	2,23±0,46	2,28±0,31*	2,79±0,38*	2,05±0,34
11-day	1,04±0,10	1,17±0,20	1,48±0,22	1,43±0,11	1,39±0,17	1,24±0,18

Legend. Asterisk indicates significant differences between RI for 8- and 11-day CFUs, with identical time interval between fractions of irradiation.

TABLE 3. Number of 11-Day CFUs (number of colonies per spleen) Formed by Bone Marrow Cells Irradiated in a Single Dose or Fractionally (M ± m)

No. of expt	UFI	FI interval between first and second doses of irradiation, h					
		1	2	3	4	5	6
1	7,43±0,72	8,00±0,32 (1,08)	6,00±1,08 (0,81)	13,60±1,36* (1,83)	11,29±1,52* (1,52)	14,00±3,24 (1,89)	
2	13,22	16,00±1,73 (1,21)	13,83±1,80 (1,05)	18,43±1,88 (1,39)	15,33±3,39 (1,16)	17,67±2,60 (1,34)	14,25±3,97 (1,08)
3	13,00±1,87	12,60±1,62 (0,97)	14,80±2,22 (1,14)	12,00±1,67 (0,92)	15,75±1,31 (1,21)	15,29±1,54 (1,18)	13,67±0,80 (1,05)
4	8,00±0,58	3,40±1,33 (0,67)	7,30±1,01 (0,91)	12,33±3,18 (1,54)	12,14±0,51* (1,52)	9,00±2,14 (1,13)	12,80±1,58* (1,60)
5	7,08±0,54	8,85±0,79 (1,25)	13,90±0,87* (1,96)	12,18±0,86* (1,72)	12,36±0,91* (1,75)		

and the repair index (RI) was calculated (Table 1). During fractional irradiation, 8-day CFUs are known to form twice as many colonies as bone marrow irradiated only once, if the interval between the first and second doses of irradiation is 5 h [11].

EXPERIMENTAL RESULTS

The 8-day CFUs showed high ability to repair SLRI, and this ability was demonstrated with intervals of 2 to 6 h between the fractions of irradiation. The maximal value of RI was 2.79 ± 0.38 with an interval of 5 h (Table 2). Repetition of these familiar experiments was required in order to compare the degree of repair of SLRI of 11-day CFUs, and to compare the time course of the change in the number of colonies in the case of fractional irradiation compared with a single dose. The 11-day CFUs were able to repair SLRI (Table 3), but significantly less well than 8-day CFUs. On average, the maximal RI was 1.48 ± 0.22, which is roughly half the maximal value of RI for 8-day CFUs (Table 2). A second distinguishing feature is that 11-day CFUs were characterized by rapid repair of SLRI: with an interval of 3 h between fractions of irradiation the maximal number of colonies was recorded compared with a single irradiation of bone marrow. With lengthening of the fractioning time of the dose to 4-6 h, a slow decrease was observed in the values of RI. Differences obtained in the kinetics of the change in RI for 8-

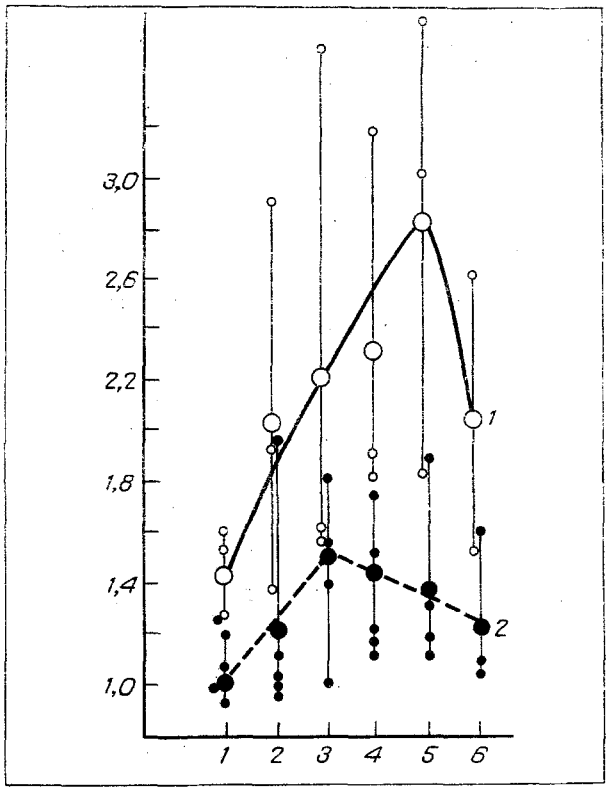

Fig. 1. Changes in RI depending on time interval between fractions of irradiation. Abscissa, interval between fractions of irradiation (in h); ordinate, RI. 1) Trend of change in RI for 8-day CFUs, 2) the same for 11-day CFUs. Large circles — mean values of RI; small circles — values of RI in individual experiments.

and 11-day CFUs are clearly demonstrated in Fig. 1. Despite the quite considerable variations of RI in individual experiments for 8-day CFUs, the curves shown differed significantly from one another (Kolmogorov–Smirnov test, $\lambda^2 = 3.317$, $p < 0.01$). Maximal values of RI for 8-and 11-day CFUs also differed in Student's t test.

The results are evidence that ability to repair SLRI differs in cells at different levels of the hematopoietic precursor hierarchy. Less mature CFUs (11 days) possess ability to repair SLRI that is significantly weaker than the more highly differentiated members of the hematopoietic cell series (8-day CFUs). Previous results indicating reduced ability of 11-day CFUs to repair SLRI with a 5-h interval between two doses of irradiation [1, 2] do really not reflect completely the ability of these precursor cells to repair radiation injuries. For instance, RI with an interval of 3 h between fractions was a little higher than with an interval of 5 h (differences not significant). Nevertheless, with fractionation schedules allowing 2, 4, and 5 h between doses of irradiation, RI for 11-day CFUs was significantly less than RI for 8-day CFUs. Thus besides the qualitative differences described previously [3, 5-10], the two CFUs subpopulations (8- and 11-day precursors) also differ in their degree of repair of SLRI, and also in the temporal parameters of realization of their maximal ability to repair postradiation injuries.

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ULTRASTRUCTURAL MORPHOLOGY OF THE AIR—BLOOD BARRIER AND SURFACTANT IN EXPERIMENTAL PNEUMONIA SUPERPOSED ON ALCOHOL POISONING

A. K. Zagorul'ko and E. E. Fisik

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Inflammatory diseases of the lungs in alcoholics are characterized by a tendency to run a protracted and complicated course, with predominance of suppurative changes resistant to treatment [4-6]. However, there have been only sporadic studies of changes in the ultrastructure of components of the air—blood barrier (ABB) in pneumonia superposed on alcohol poisoning [2, 5]. There have been virtually no investigations into changes in the state of the lung surfactant system (LSS) in pneumonia superposed on alcohol poisoning, although changes in surface activity of LSS are known to play an important role in the pathogenesis of diseases of the bronchopulmonary apparatus.

The aim of this investigation was to study changes in the ultrastructure of components of ABB and the state of surface activity of LSS in animals with experimental pneumonia superposed on alcohol poisoning.

EXPERIMENTAL METHOD

The material for this investigation consisted of the lungs of 21 male and female Wistar albino cats weighing from 290 to 350 g. As the control, the lungs of five healthy rats of both sexes which, like the experimental animals, were decapitated under thiopental anesthesia, served as the control. All the experimental animals were divided into three groups. Group 1 consisted of 7 healthy albino rats in which pneumonia was induced by the method in [1], by transtracheal insertion of a length of sterile fishing line. In the animals of groups 2 and 3, experimental alcohol poisoning was induced by intragastric injection of 50% ethyl alcohol through a tube in a dose of 4 ml/kg body weight in a single dose at 24-hourly intervals for one month. After this period a similar length of sterile fishing line was inserted by the same method as was described above. Later, the albino rats of group 3 continued to receive ethanol by the above schedule, whereas administration of ethanol to animals of group 2 ceased. All animals of groups 2 and 3 were decapitated 21 days after insertion of the sterile thread as described above. Fragments measuring 1 mm^3, free from large vessels and bronchi, were excised from the lungs for electron-microscopic investigation, fixed in 2.5% glutaraldehyde solution in phosphate buffer, and then postfixed in 1% OsO_4 solution. After dehydration the tissue was embedded in a mixture of Epon-812, DDSA, and MNA. Ultrathin sections were stained with lead citrate and examined in the UEMV-100K electron microscope. The state of surface activity of the LSS was studied by physicochemical (determination of surface tension — ST — of the surface-active fraction of lung extracts isolated from animals as described in [7], on Wilhelmy scales) and biochemical (determination of total lipid levels as in [9], phospholipids as in [10]) methods, and thin-layer chromatography of the phospholipids on "Silufol-UV-254" plates (Czechoslovakia) were used.

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