MIGRATION OF HEMATOPOIETIC STEM CELLS IN DOGS

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The rate of migration of bone marrow hematopoietic stem cells (HSC) was studied by two methods in experiments on 54 dogs. In the first case the dogs were irradiated subtotally with an absolutely lethal dose of 550 R, with both knee joints screened. When the screened areas were inactivated by irradiation in a dose of 2000 R 7 days after the first irradiation, the survival rate of the animals was 12.5%, but in the case of inactivation after 14 days, all the dogs survived. In the second case both knee joints were irradiated initially in a dose of 2000 R. After subsequent subtotal irradiation in a dose of 550 R, with the previously irradiated regions screened, all the animals died if the second irradiation was given after 7 days, whereas 20% of the animals survived if the time between irradiations was increased to 31 days. It is concluded that migration of HSC in dogs is much lower in degree than in mice.

KEY WORDS: irradiation; hematopoietic stem cell; migration.

Considerable species differences in the degree of postradiation recovery of the bone marrow are associated with different rates of migration of undamaged hematopoietic stem cells, which are responsible for repair after irradiation in a high dose. It has been shown that after local irradiation the inactivated regions of bone marrow in mice are repopulated quickly and completely, in rats slowly and not completely, but in dogs hardly at all [4, 6]. However, after subtotal irradiation with one knee joint screened, colonization of stem cells from the protected region of bone marrow takes place in dogs also, enabling some of the animals to survive and hematopoiesis to be restored in them in the injured regions of hematopoietic tissue [5]. The rate of migration of hematopoietic stem cells (HSC) is evidently not constant, but depends significantly on the degree of injury to the hematopoietic tissue, the volume of tissue damaged, and the quantity of residual bone marrow.

Methods used to study the rate of migration of HSC include determination of the duration of existence of a region of screened bone marrow in the irradiated animal sufficient to ensure survival [3] and investigation of reimmigration of HSC, i.e., the ability of HSC migrating into a previously damaged part of the bone marrow to remigrate from these regions after subsequent subtotal irradiation [1]. In the present investigation the rate of migration of HSC was studied in dogs by both methods in order to shed further light on species differences in the rates of HSC migration.

EXPERIMENTAL METHOD

Experiments were carried out on 54 dogs, males and females weighing 7-16 kg. For total or subtotal irradiation, the animals, anesthetized with thiopental, were placed in a revolving container and irradiated from three RUM-17 apparatuses, oriented at an angle of 120° to each other. The radiation field was so formed that the coefficient of nonuniformity was 1.15 ± 0.05 [2]. The tube voltage was 180 kV, current 15 mA, filter 0.5 mm Cu + 1.0 mm Al, target-skin distance 75 cm, dose rate 36 R/min, dose 550 R (absolutely lethal dose for whole-body irradiation). In the case of subtotal irradiation the knee joints were screened by a lead sleeve (10 cm wide, 0.5 cm thick). The dose beneath the screen was less than 3% of the total dose. Local irradiation of the knee (field 15×10 cm) was carried out on the RUM-17 apparatus (voltage 180 kV, current 15 mA, filter 0.5 mm Cu + 1.0 mm Al, target-skin distance 40 cm, dose rate 113 R/min. A dose of 2000 R was taken to be that which inactivates bone marrow. The survival rate of the dogs (period of observation 45 days) was counted and the degree of recovery of hematopoiesis was estimated from the peripheral blood leukocyte count.

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TABLE 1. Survival Rate of Dogs Depending on Conditions of Irradiation

Scheme of experiment	Number of dogs	Survival rate, %
Whole-body irradiation (control group 1)	13	8
Subtotal irradiation with both knees screened (control group 2)	11	82
Subtotal irradiation with both knees screened, followed by inactivation of protected regions		
After 7 days	8	12.5
After 14 days	5	100
Inactivation of region of both knees followed by subtotal irradiation with previously irradiated regions screened		
After 7 days	7	0
After 31 days	10	20

EXPERIMENTAL RESULTS

The scheme of the experiments and its results are described in Table 1.

All the dogs of control group 1 died from radiation sickness, which followed a severe course, similar to that described previously [5]. The mean length of survival of the animals was 12 days.

The peripheral blood leukocyte count fell after the first days to reach only $0.6 \times 10^3/mm^3$ by the 12th day. In the dogs of control group 2 a distinct protective effect of screening was observed: nine of the 11 animals survived, the course of their radiation sickness was much milder, and their leukocyte count by the 12th day had fallen to $1.2 \times 10^3/mm^3$, after which it began to rise and approached the normal level on the 45th day of observation.

In the experiments of series I, when the screened regions were inactivated 7 days after subtotal irradiation, this period was found to be too short for survival of the majority of animals: only one of the eight dogs survived. The mean length of survival of the animals which died was 13 days and their peripheral blood picture was the same as in the dogs of control group 1: by the 12th day the leukocyte count had fallen to $0.7 \times 10^3/mm^3$. In the surviving animal the blood picture was similar to that in the animals of control group 2: on the 12th day the leukocyte count was $1.35 \times 10^3/mm^3$, but by the 45th day it had risen to $5.1 \times 10^3/mm^3$, i.e., it was close to normal. With an increase in the period of existence of the screened region of bone marrow in the irradiated dogs to 14 days, the protective effect of screening became clearly manifested: all five animals survived. Their peripheral blood leukocyte count was the same as in the animals of control group 2.

In the experiments of series II, with subtotal irradiation and screening of the two knees irradiated previously, the possibility of reimmigration of HSC was studied. When irradiation followed 7 days after inactivating local irradiation, practically no reimmigration was detected: all seven dogs died, the course of their radiation sickness was severe, the mean duration of survival was 11 days, i.e., this group was indistinguishable from control group 1. However, when the time interval between local and subtotal irradiation was increased, a small protective effect of screening the previously irradiated knees was observed: two of the 10 dogs survived. Moreover, their blood picture was similar to that of the animals of control group 2: by the 12th day the leukocyte count was 2.0×10^3 and $2.2 \times 10^3/mm^3$, i.e., reimmigration of HSC could be deemed to have occurred in these animals.

In the experiments of series I the length of existence of the screened region of bone marrow in the irradiated dog sufficient to produce migration of an adequate number of HSC to ensure survival was determined. Whereas in mice, the presence of a screened region of bone marrow for 3-5 h is sufficient for survival [3], in dogs a screened region of corresponding, or even of larger volume had to be present in the body for 2 weeks in order to ensure maximal survival of the animals. The results of the experiments of series II show that the ability of HSC in dogs to reimmigrate is much less marked than in mice. To increase the level of survival to 20% in mice the time between local and subtotal irradiation need be as little as 3 h, whereas in dogs the minimum is 1 month, in good agreement with data showing the low level of spontaneous repopulation of the bone marrow in dogs after local irradiation [4], despite the presence of HSC in the dogs' peripheral blood [7]. It can thus be concluded from the results of experiments to study migration of bone marrow HSC by two methods

that it is much less intensive than in mice; there is thus a need to study ways and means of intensifying migration of HSC by additional forms of stimulation or by bone marrow autografts.

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GROWTH-STIMULATING ACTION OF SOME NITROSO COMPOUNDS ON ORGAN CULTURES OF MOUSE AND RAT EMBRYONIC LIVER

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The transplacental and direct action of nitrosomethylurea on organ cultures of the liver from 18-20-day CBA and C57BL mouse embryos and of diethylnitrosamine on liver cultures from noninbred rat embryos was studied. The nitroso compounds accelerated adaptation of the explants, increased the viability of the cultures compared with normal, and led to hyperplastic proliferation of the small basophilic cells whose survival rate under both normal and experimental conditions was higher than that of ordinary embryonic hepatocytes. The growth-stimulating effect depended on the object (species, line of animals) and the factor to which it was exposed (carcinogen, mode of administration).

KEY WORDS: nitroso compounds; transplacental carcinogenesis; organ cultures; embryonic liver.

Among the many carcinogens nitroso compounds (NC) are distinguished by their high biological activity and the broad spectrum of the tumors they induce, when these substances are administered in different ways, including by the transplacental route [11, 14]. The possibility of endogenous synthesis of carcinogenic NC from noncarcinogenic precursors, the wide use of NC in various branches of the economy, and their spread in the external environment create conditions for human contact with them [3]. A high sensitivity of the embryo to chemical agents, and to NC in particular, makes the study of various aspects of their action on embryonic tissues with a view to detecting pathological changes one of the utmost urgency.

Previous investigations showed that one possible way of studying chemical carcinogenesis, especially the transplacental kind, is simulation of this process by the organotypical culture of embryonic target tissues [4, 11]. By this means a systematic study could be made of the mechanisms of action of chemical carcinogens on embryonic tissues in animals and man. Species and interlinear differences in the sensitivity of embryonic lungs to the toxic and growth-stimulating action of some NC were discovered previously [5].

The object of this investigation was to compare organ cultures of mouse and rat embryonic liver under normal conditions and following transplacental and direct exposure to nitrosomethylurea (NMU) and diethylnitrosamine (DENA).

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