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RADIATION RESISTANCE IN MICE INCREASED FOLLOWING CHRONIC APPLICATION OF Li_2CO_3

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Lithium salts have been used in the treatment of mental disorders for a long time (HOLLISTER 1978). However, these substances have side effects appearing as disturbances to the immune system (FERNANDEZ & MACSWEEN 1980), induction of neutrophilia (ROTHSTEIN et coll. 1978) and lymphopoenia (PEREZ-CRUET et coll. 1977). It has also been found that lithium increases the production of colony-growth stimulating factors (HARKER et coll. 1977) and the numbers of haemopoietic stem cells determined for the formation of granulocytes and macrophages (TISMAN & HEBERT 1973). Ionizing radiation decreases an organism's resistance to infection. Therefore, the deliberate influencing of haemopoiesis leading to the activation of granulopoiesis can contribute significantly to the organism's recovery from radiation injury and the survival of individuals after irradiation with a lethal dose of roentgen rays.

The influence of chronic feeding of mice on a diet with added Li_2CO_3 on the radiation resistance of haemopoietic stem cells (CFUs) is now reported as well as the survival of mice following whole-body irradiation. Increased radiation resistance was found in mice fed Li_2CO_3, corroborating the activation of haemopoietic stem cells.

Material and Methods

The experiments were performed on female mice of the semi-inbred strain H, 12 weeks old at the beginning of the experiments. The animals were kept in cages of 20, fed on a Larsen diet and an admixture of Li_2CO_3 (1.5 mg Li_2CO_3 per g mixture). Water and food were given ad libitum.

Whole-body irradiation was administered using the apparatus TUR with the following settings: 180 kV, 15 mA, filtration 0.5 mm A1+0.5 mm Cu, exposure rate 0.478 $Gy \times min^{-1}$.

Haematologic methods. The number of endogenous colonies of haemopoietic tissue macroscopically visible on the parietal surface of the spleen was determined on day 9 after irradiation (TILL & MCCULLOCH 1961). The mice were killed, the spleens removed, weighed and fixed in Bouin's solution. The number of colonies was determined after 3 h fixation.

The number of CFUs in the bone marrow and spleen was determined by a transplantation method (TILL & MCCULLOCH). The suspension of bone marrow cells (6–8 femurs) and spleen cells (3–5 spleens) was prepared in M-199 medium at the temperature of melting ice. The number of cells in suspension was assayed on a Coulter Counter, model Fn, and following appropriate dilution the cells were injected into the lateral tail vein of 10 isogenous recipients irradiated with a whole-body dose of 6.7 Gy 2 to 3 h before transplantation.The mice were killed on day 9 after transplantation and the number of macroscopic colonies was assayed as in the case of the endogenous colonies. The background endo-

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genous colonies was 0.2×0.1. The number of CFUs in the tissue was calculated from the number of CFUs in the injected volume of cells and the mean cellularity of the spleen or bone marrow.

The cellularity of the bone marrow was determined in the femur, stripped of muscle tissue. After the epiphyses had been cut off an injection needle was introduced into the diaphysis, the bone was immersed in 10 ml physiologic solution stabilized with a phosphate buffer, and the bone was thoroughly rinsed by means of several strokes of the piston. The number of cells was assayed on a Coulter Counter, model Fn. For each interval of measurement 5 to 8 animals were used.

The number of leukocytes in a blood sample withdrawn following incision of the tail vein was assayed on a Coulter Counter, model Fn. For each interval of measurement 5 mice were used.

Proliferative activity of CFUs. The volume of the fraction of the cell population of CFUs in the S-phase of the cell cycle was determined on the basis of the sensitivity f the CFUs to a large dose (1000 $mg \times kg^{-1}$) of hydroxyurea (Aldrich-Europe, Jensen Pharmaceutical Lab., Belgium), which brings about the death of cells in the S-phase of their cycle (BHUYAN et coll. 1973, VASSORT et coll. 1973). A single injection of hydroxyurea was given 90 min before withdrawal of the bone marrow and spleen. Hydroxyurea was dissolved in a physiologic solution immediately before injection and was applied intraperitoneally in quantities of 0.3 ml.

The control group was injected intraperitoneally with physiologic solution. The differences in the number of CFUs in the femur and the animals to which hydroxyurea had been applied shows the fraction of CFUs in the S-phase at the time of injection.

The number of mobilized granulocytes was determined in venous blood 7 h after the intraperitoneal injection of endotoxin (Lipopolysaccharid B.S. typhosa Difco) in a dose of 20 μg/mouse (SMITH et coll. 1961). Each group contained 10 mice. The lithium concentration in the serum was determined by flame photometry in a three day interval during Li_2CO_3 feeding.

Statistic evaluation of differences in the number of endogenous and exogenous CFUs was performed with the aid of a t-test, and the survival of animals was evaluated using a chi-square test. The regression lines of CFU survival were calculated by the least squares method.

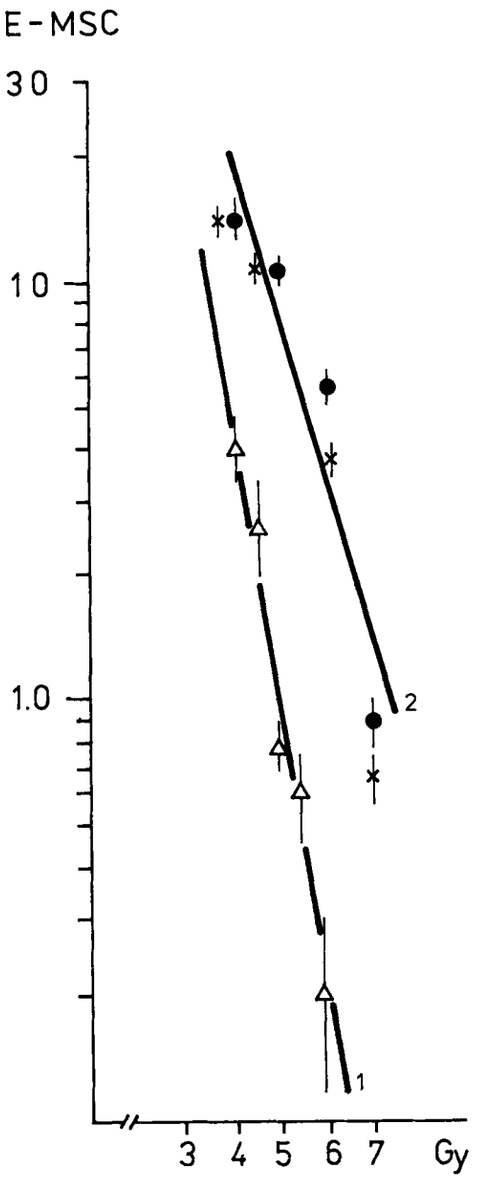

Fig. 1. Regression lines of the dependence of the number of macroscopic colonies of haemopoietic tissue on the surface of the spleen (E-MSC) on radiation dose. Dose in Gy (0.478 Gy× min^{-1}) and mean number of E-MSC ± SE. 1: Control group, irradiated only. 2: Experimental group, 24 h (•).

Results

The mean daily consumption of food per 25 g mouse was 5 g; this quantity included 7.5 mg Li_2CO_3, which amounts to a consumption of 0.52 mg Li/day. During 3-day feeding the concentration of Li in the serum reached a value of 0.5 mmol/l, remaining at this level until the end of feeding.

Fig. 1 shows the number of endogenous macro-

Table 1

Influence of Li_2CO_3 application on number of CFUs in the spleen and femur of mice. Ten animals in each group. Hydroxyurea measured 90 min after hydroxyurea injection

Groups	Spleen				Femur			
	Cellularity $\times 10^7$	CFUs/ 10^6	CFUs/ spleen	Hydroxy-urea (per cent)	Cellularity $\times 10^7$	CFUs/ 10^5	CFUs/ femur	Hydroxy-urea (per cent)
Control	10±1.2	4.4±0.9	440±12*	100	3.7±0.4	6.0±1.2	2 214±136	100
Hydroxyurea	10±0.4	3.5±0.8	352±16	80	3.2±0.3	5.5±0.8	1 787± 89	80.7
Day 1 after Li	11.7±1.4	5.3±0.9	621±24**	100	4.0±0.6	6.7±0.7	2 664±148	100
Hydroxyurea	9.5±0.5	2.6±0.3	243±18	39.1	3.2±0.5	4.9±1.1	1 564±130	58.71
Day 3 after Li	15.0±2.1	4.4±0.5	656±32**	100	2.9±0.7	6.0±1.4	1 754±150	100
Hydroxyurea	9.1±1.3	3.4±0.8	307±33	46.8	2.7±0.4	4.5±0.8	1 203±124	68.6

* versus ** $p<0.01$.

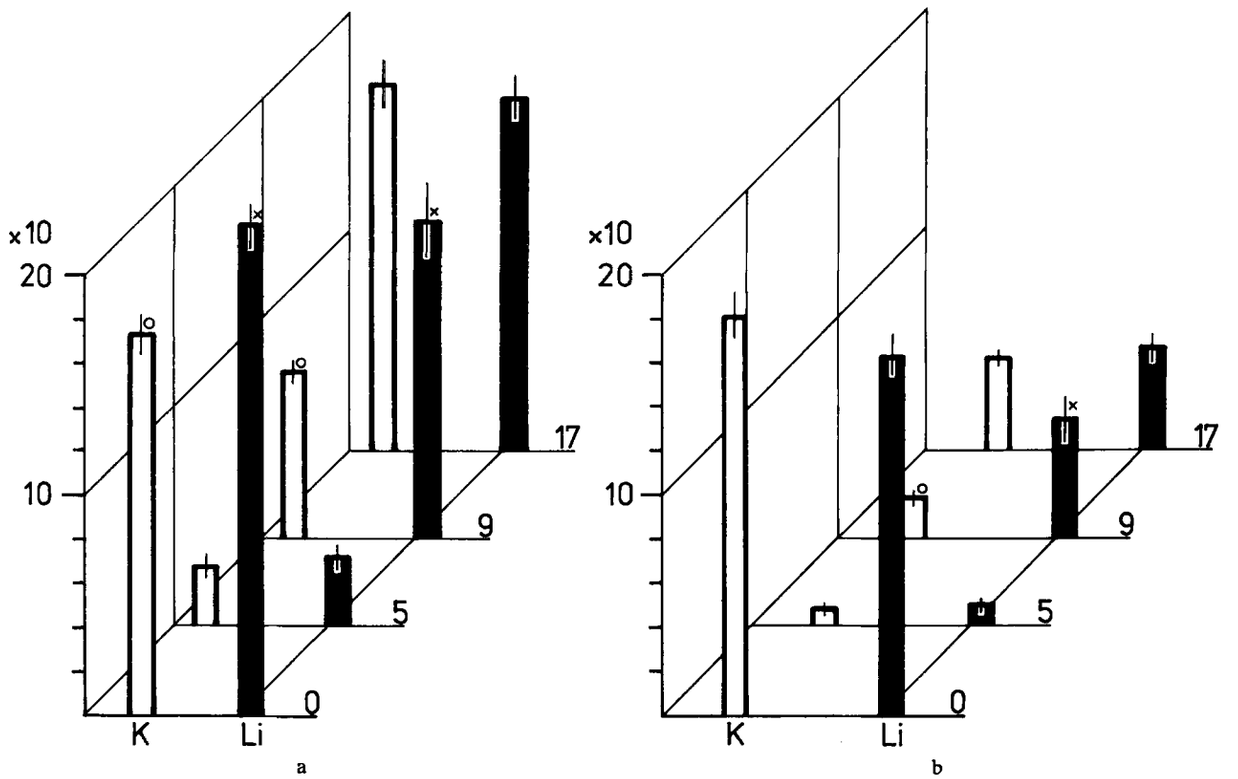

Fig. 2. Recovery of damage of haemopoiesis following irradiation with a dose of 5.0 Gy (0.478 Gy×min^{-1}) the first day after Li_2CO_3 application. a) Cellularity of femur. Mean values of cellularity of bone marrow ($\times 10^6$). b) Leukocytes of peripheral blood. Mean values of lymphocyte ($\times 10^3$). K: Intact group, control. Li: First day after 21-day application of Li_2CO_3. ○ versus × $p<0.01$.

scopic colonies of haemopoietic tissue on the parietal surface of the spleen on day 9 after the irradiation. The lines of dependence of the number of spleen colonies on the radiation dose have a value b− of the gradient of the regression line of −0.652 × in the control group and D_0 reaches a value of 0.8 Gy, while on the first and third days after the administration of Li_2CO_3 the value of b− falls to −0.386 and D_0 increases to 1.2 Gy. The mean numbers of colonies are statistically significantly higher

Table 2

Survival of mice to day 30 after lethal irradiation

Group	No. of mice	Survived	Died	Per cent survival	Mean survival time*	p**
Exposure: 7.5 Gy						
Control	20	8	12	40	9	
After 21-day Li feeding						
On day 1	19	17	2	89	17	0.01
On day 3	20	19	1	95	6	0.01
Exposure: 8.2 Gy						
Control	20	2	18	10	8.5	
After 21-day Li feeding						
On day 1	21	10	11	48	14.9	
On day 3	21	11	10	52	16	

* Animals dying before day 30.

** Statistical significance compared with control group.

($p<0.01$) in the experimental groups throughout the whole range of the exposures used than in the controls.

The number of CFUs in the spleen and bone marrow (femur) determined by the transplantation method (TILL & MCCULLOCH) are summarized in Table 1. On day 1 after Li application the mean values of the number of CFUs in the spleen were increased by 40 per cent ($p<0.01$) and on day 3 the increase amounted to 50 per cent over the number of CFUs in the spleen of the control group. In the bone marrow of the femur an increase in the number of CFUs in the experimental group was found only on day 1 after Li application.

At an interval of 90 min following the injection of hydroxyurea the number of CFU in the spleen of the experimental group on day 1 after Li application was 41 per cent lower than that in the control group, and on day 3 after the application of Li the difference in the number of CFUs in the spleen amounted to 33 per cent. In comparison with the control group the number of CFUs in the femur of mice after the injection of hydroxyurea on day 1 after Li application was decreased by 20 per cent and on day 3 by 11 per cent.

The number of nucleate cells in the femur on day 1 after Li application was higher ($p<0.01$) than in the control group. Following whole-body irradiation with 5.0 Gy the number of nucleate cells in the femur on day 9 was higher in the group affected by Li application than in the intact group ($p<0.01$; Fig. 2a).

The numbers of leukocytes in the peripheral blood were lower (180 ± 12) in the control group than in the experimental group (520 ± 90) on day 9 after irradiaiton (Fig. 2b). At 7 h after the injection of 20 μg endotoxin the number of leukocytes (granulocytes) rose to 500 ± 120 in controls and to 2240 ± 280 in animals affected by Li_2CO_3 application. The differences between the two groups are statistically significant ($p<0.01$).

Irradiation with a lethal exposure of 7.5 Gy on days 1 and 3 after the end of the 21-day feeding with Li_2CO_3 produced a survival rate on average 53 per cent higher than when animals were fed on a normal diet (Table 2). With an increased exposure of 8.2 Gy the difference in survival of animals to day 30 after irradiation was 38 and 42 per cent, respectively.

Discussion

The results show that the application of Li_2CO_3 to the diet increases the radiation resistance of mice to a lethal exposure of radiation and modifies the radiation resistance of haemopoietic stem cells.

The increase observed in the number of CFUs in the spleen and bone marrow of mice following chronic application of Li_2CO_3 corroborates the data of other authors, who found an activation of haemopoiesis in mice following application of a single dose of Li salts (GALLICCHIO & CHEN 1980). The higher number of CFUs and the increased percentage of CFUs in the DNA-synthesis may be one of the causes of the observed increase, of radiation resist-

ance of haemopoietic stem cells and the higher number of endogenous spleen colonies in animals to which Li_2CO_3 had been administered. Similar results were observed following the activation of haemopoiesis in mice by the chronic application of dried thyroid gland (VACEK et coll. 1978).

Increased numbers of pluripotent haemopoietic stem cells in animals administered Li may contribute to the repopulation of haemopoietic tissues devastated by the effect of ionizing radiation and accelerate the recovery of the cellularity of bone marrow and also of peripheral blood cell elements.

The increased numbers of mobilized granulocytes found in irradiated animals following the injection of endotoxin (SMITH et coll.) under the influence of Li_2CO_3 application may demonstrate the expression of a granulopoietic effect of Li (BARRETT 1980). Since the number of granulocytes produced is of particular importance for overcoming the critical effects of bone marrow syndrome in radiation sickness (BOND et coll. 1965), a granulopoietic effect of Li_2CO_3 might be one of the important factors contributing to an increase in the radiation resistance of experimental animals.

The mechanism of the effect of Li on haemopoietic stem cells presupposes an indirect effect through an increase in the production of colony stimulating factor (CSF), to which macrophages, lymphocytes and epithelial cells contribute (HARKER, BARRETT). The activation of CSF formation after Li_2CO_3 application in vivo is manifested in an increased number of determined haemopoietic stem cells (CFUc) for the production of granulocytes, while the number of erythroid haemopoietic stem cells (CFUe, BFUe) is decreased (GALLICCHIO & CHEN 1981).

The biochemical mechanism of the effect of Li on haemopoietic cells has not yet been satisfactorily explained. It has been found that Li inhibits the activity of the adenylcyclase of cells (SINGER & ROTENBERG 1973), and it could well be that the influence of Li on haemopoietic stem cells is mediated through cyclic monoadenosine phosphate (BARRETT).

SUMMARY

In experiments on strain H mice the increased radiation resistance of mice was analysed after three weeks' feeding with a diet including Li given as lithium carbonicum. The concentration of Li in the serum during the first three days of feeding was increased to 0.5 mmol/l and remained at that level to the end of feeding. The application of Li increased the overall number of stem cells in the spleen by 80 per cent compared with the control group. D_0 of the line of dependence of the number of endogenous colonies on radiation dose increased following Li application by 1.2 Gy compared with controls. The proliferation activity of haemopoietic stem cells observed 90 min after injection of hydroxyurea was, after 21 days feeding with a mixture containing Li, increased by 200 per cent. The results support the idea that the increased radiation resistance of mice following feeding with Li salts before irradiation may be due to the increased content and resistance of the haemopoietic stem cells, as well as activation of granulopoiesis.

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