MECHANISM OF ACTION OF BENZENE ON HEMATOPOIESIS (INVESTIGATION OF HEMATOPOIETIC STEM CELLS)

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The effect of benzene on colony-forming activity of the bone marrow and spleen was studied in four groups of experiments. In benzene hypoplasia of hematopoiesis, fewer colony-forming units were found in the hematopoietic organs although there was no change in the distribution of their cell types. Incubation of normal bone marrow cells with benzene did not reduce the efficiency of colony formation. Injection of benzene into lethally irradiated mice after transplantation of a suspension of normal bone marrow cells into the animals caused a sharp decrease in colony production, with inhibition chiefly of the formation of granulocytic colonies. These observations suggest that during benzene poisoning the properties of the microenvironment of the hematopoietic stem cell are modified.

KEY WORDS: hematopoiesis; action of benzene; hematopoietic stem cell.

Many aspects of the mechanism of action of benzene on hematopoiesis still remain unexplained. The view that in this condition the hematopoietic stem cell is damaged [7, 9] has not been verified experimentally.

Data on the effect of benzene on colony-forming activity of the bone marrow and spleen are given below.

EXPERIMENTAL METHOD

Colony formation was studied in four groups of experiments on CBA mice of the same age and sex. In the experiments of group 1 the number of colony-forming units (CFU) was determined in the bone marrow and spleen of mice with benzene poisoning, caused by subcutaneous injection of benzene in a dose of 2 ml/kg body weight on alternate days for 3-5 weeks. In the experiments of group 2 the effect of benzene on bone marrow CFU was studied after incubation of a suspension of normal bone marrow cells in homologous serum, to which benzene was added in a final concentration of 100 mg %, for 45 min at 37°C. In the experiments of group 3 the effect of benzene was studied on colony formation in vivo after injection in a dose of 2 ml/kg on the second, fourth, and sixth days after transplantation of a suspension of normal bone marrow cells into lethally irradiated mice. In group 4 the colony-forming activity of normal bone marrow was determined after injection into lethally irradiated recipient mice in which benzene poisoning had been produced.

To determine the number of CFU, cell suspensions were made up in medium No. 199 and injected intravenously into lethally irradiated (1000 rad) mice in a dose of 10^5 bone marrow cells or 10^6 spleen cells. The conditions of irradiation were: RUM-13 apparatus, voltage 180 kV, current 10 mA, dose rate 16-18 R/min, filters Cu 0.5 mm and Al 1 mm. On the eighth day after injection of the cells the recipient mice were killed, the spleen was removed and fixed in Bouin's fluid, and the macroscopically visible colonies were counted. The number of endogenous colonies did not exceed 0.2 per mouse. The experimental arrangements and method of assessing colony formation on the whole followed the usual procedure [4, 11]. The cell type of the colonies was determined histologically.

EXPERIMENTAL RESULTS

Benzene poisoning was accompanied by a decrease in the number of leukocytes, erythrocytes, and platelets in the peripheral blood and a decrease in the number of bone marrow and spleen cells (Table 1), as was

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TABLE 1. Number of Cells and Number of CFU in Bone Marrow and Spleen during Benzene Poisoning (M ± m)

Animals	Number of animals	Bone marrow			Spleen		
		number of cells in femur, millions	number of CFU per 10^5 cells	number of CFU per femur	number of cells, millions	number of CFU per 10^6 sec	number of CFU per spleen
Control	10	14,5±1,0	7,3±1,1 (20)	1060±150	389±50	7,0±1,0 (20)	2720±410
Benzene	9	7,2±0,6*	7,3±1,0 (18)	520±70*	33±10*	6,3±0,6	210±70*

Legend. Number of recipients shown in parentheses. For results marked with asterisk, $P \leq 0.01$.

TABLE 2. Effect of Benzene on Colony Formation in vivo

Experimental conditions	Number of animals	Number of CFU per 10^5 cells $M \pm m$	Types of colonies, %			
			E	G	M	mixed
Without administration of benzene	10	7,6±0,9	70	15	10	5
With administration of benzene	20	1,2±0,2 $P<0,001$	83	4	8	5

Legend. E) erythroid, G) granulocytic, M) megakaryocytic.

TABLE 3. Colony Formation in Lethally Irradiated Recipient Mice with Benzene Poisoning

Experimental conditions	Number of animals	Number of CFU per 10^5 cells $M \pm m$	Types of colonies, %			
			E	G	M	mixed
Without preliminary administration of benzene	10	7,1±1,1	70	15	10	5
Benzene poisoning	18	7,2±0,8	81	5	6	8

observed previously [3, 6]. The efficiency of colony formation, expressed as the number of CFU per 10^5 injected karyocytes [11], was virtually unchanged in benzene poisoning. However, allowing for the reduction in the number of bone marrow and spleen cells, the absolute number of CFU was considerably reduced in benzene poisoning. On histological investigation no significant changes were found in the distribution of the types of colonies formed.

The results show that during inhibition of hematopoiesis caused by benzene poisoning, as in hypoplasias of other origin [10, 12], the number of stem cells in the hematopoietic organs is reduced. The mechanism of this decrease may vary: It may occur either through changes in the hematopoietic stem cells themselves or through changes in their microenvironment. To test the first of these possibilities the effect of benzene was studied on the colony-forming activity of bone marrow cells incubated in vitro with benzene in a concentration of 100 mg % (experiments of group 2). This concentration is close to the possible maximum in the hematopoietic organs of man and animals with benzene poisoning [13]. The number of colonies growing under these circumstances was virtually unchanged: the efficiency of colony formation was 7.8 ± 0.5 per 10^5 injected cells (7.3 ± 1.0 in the control). Consequently, it can be postulated that a direct harmful effect of benzene on the hematopoietic stem cells is hardly likely to occur even if the maximal possible concentration of benzene is present in vivo.

After injection of benzene into lethally irradiated recipient mice following previous transplantation of a suspension of normal bone marrow cells (experiments of group 3), a sharp decrease in the number of colonies was observed (Table 2). The relative proportions of the different colonies also were altered: The formation of granulocytic colonies was sharply reduced whereas the proportion of erythroid colonies was correspondingly increased. Since marked poisoning could not arise as the result of three injections of benzene, the results in Table 2 probably indicate that the direct harmful action of benzene or its metabolites in this case, as in the experiments of group 2, was unlikely. The observed decrease in the number of colonies and also the change in their cytological distribution were most probably connected with the effect of benzene on processes in the inducing microenvironment of the hematopoietic stem cell. This hypothesis was confirmed to some extent also during the study of colony formation in lethally irradiated mice, in which benzene poisoning was produced before injection of normal bone marrow (Table 3). The efficiency of colony formation under these circumstances was not reduced, but the distribution of cell types of the resulting colonies was altered; the proportions of granulocytic and megakaryocytic colonies were reduced and the proportion of erythroid colonies correspondingly increased. The change in the cytological distribution of the colonies thus observed could probably also be attributed to changes in the properties of the inducing microenvironment. The results of the last two groups of experiments likewise still do not rule out the possibility of changes in committed stem cells as a result either of direct injury to them by benzene or of the microenvironment. This view is supported by the change in the ability of the stem cells to differentiate, as reflected in changes in the relative percentages of the various colonies formed. It is interesting to note that the higher sensitivity of granulocytopoiesis than of erythropoiesis to the harmful action of benzene was demonstrated by the writers previously by different methods [3, 6].

The reticulum cells of hematopoietic organs [4] are known to be an important microenvironmental factor determining the ability of the stem cells to differentiate [5]. In this connection it is interesting that the relative number of reticulum cells is increased in benzene poisoning [2, 3, 14], sometimes up to the stage of reactive reticulosis [1]. The importance of this phenomenon is insufficiently clear. The view that differentiation of reticulum into hematopoietic cells is delayed in benzene poisoning [1, 14] must now be regarded as obsolete, for it has been shown that the reticulum cells of hematopoietic organs do not change into hematopoietic cells [8]. The results described above suggest a certain connection between changes in the reticulum cells and in the hematopoietic stem cells during benzene poisoning.

LITERATURE CITED

1. B. V. Aleshin, Sh. A. Burshtein, and B. I. Chernyak, in: Alimentary-Toxic Aleukia [in Russian], Orenburg (1947), pp. 125-144.
2. L. A. Zorina, "Chronic benzene poisoning," Author's Abstract of Doctoral Dissertation, Moscow (1971).
3. V. N. Frash, in: Experimental Investigations into the Mechanisms of Hematopoiesis [in Russian], Sverdlovsk (1971), pp. 125-130.
4. I. L. Chertkov, L. N. Lemenova, and O. A. Mendelevich, Probl. Gematol., No. 1, 3 (1972).
5. V. N. Shvets, L. S. Seslavina, and V. V. Shikhodyrov, Byull. Éksp. Biol. Med., No. 6, 89 (1972).
6. A. P. Yastrebov and V. N. Frash, Pat. Fiziol., No. 2, 76 (1975).
7. H. E. Bock and K. Wiede, Folia Haemat. (Leipzig), 42, 7 (1930).
8. R. W. Caffrey, N. B. Everett, and W. O. Riecke, Anat. Rec., 155, 41 (1966).
9. T. Ferguson, W. H. Harvey, and T. D.Hamilton, J. Hyg. (London), 33, 547 (1933).
10. E. F. Saunders, D. Amato, and M. H. Freedman, Blood, 44, 913 (1974).
11. E. A. McCulloch, L. Siminovitch, J. E. Till, et al., Blood, 26, 399 (1965).
12. A. Morley and J. Blacke, Aust. J. Exp. Biol. Med. Sci., 52, 909 (1974).
13. H. H. Schrenk, W. P. Yant, S. J. Pearce, et al., J. Indust. Hyg., 23, 20 (1941).
14. B. Steinberg, Blood, 4, 550 (1949).