ACTION OF DIPYRIDAMOLE ON MEGAKARYOCYTOPOIESIS IN REGENERATING AND STATIC BONE MARROW CELL POPULATIONS

I. N. Ivansenko, Yu. A. Blyudzin, D. N. Chernyakova, V. A. Almazov, and G. E. Arkad'eva

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Relations between hematopoietic and stromal tissues of the bone marrow in experimental animals receiving dipyridamole (DP) were studied by the writers previously [4, 5]. Blocking of platelet aggregation by DP, leading to a decrease in release of platelet growth factor (PGF), causes inhibition of the proliferative activity of connective-tissue cells [1]. We previously observed for the first time a phenomenon of accumulation of pathologically changed, disintegrating megakaryocytes (MKC) in mouse bone marrow under the influence of DP, against a background of karyorrhexis and karyolysis of these cells [4]. It was also noted that a lipid-containing film, not present in medium containing bone marrow cells from control animals, was present on the surface of a medium containing a suspension of bone marrow cells from animals receiving DP. Prostaglandins, which are involved in lipid metabolism (and synthesis) of cells can be formed from polyunsaturated aliphatic acids, such as arachidonic [11]. They are formed during injury to the cells, which disturbs the cytoplasmic membrane or causes destruction of the cells [6, 8].

The aim of the present investigation was an electron-microscopic study of MKC in regenerating and static bone marrow populations from animals receiving DP and physiological saline, and also to undertake a biochemical determination of the effect of DP on the composition and distribution of fatty acids of total lipids in the same tissues by heterotropic bone marrow transplantation.

EXPERIMENTAL METHOD

Experiments were carried out on 180 male (C57BL × CBA)F_1 mice weighing 18-20 g. To obtain a regenerating bone marrow population (RMP) a bone marrow fragment, taken from the medullary cavity of the mouse femur, was transplanted beneath the connective-tissue capsule of the kidney of syngeneic recipients under hexobarbital anesthesia [10]. With effect from the day of transplantation of bone marrow, the experimental animals were given an intraperitoneal injection of DP in a dose of 30 mg/kg daily for 50 days. Control animals received injections of physiological saline. The femoral bone marrow and RMP were sampled on the 7th, 19th, 30th, and 50th days of the experiment for histologic, electron-microscopic, and biochemical investigations. For the histologic study part of the material from the control and experimental animals was fixed in Bouin's fluid and sections were stained with hematoxylin-eosin and azure II-eosin. For electron-microscopic investigation a suspension of bone marrow cells from the femur and RMP was resuspended in medium 199, centrifuged, fixed in Karnovsky's fluid, and embedded in Epon; sections cut on an ultramicrotome were stained with uranyl acetate and lead citrate. MKC were studied in the JEM-100 CX electron microscope.

For the biochemical investigations bone marrow of the femur and RMP of the experimental and control animals were homogenized in chloroform–methanol mixture and then subjected to methanolysis [7, 9]. All subsequent operations connected with removal of methyl esters of fatty acids, and of the test lipids and their preparation

Academician I. P. Pavlov First St. Petersburg Medical Institute. A. A. U. Institute of Physiology. Institute of Pulmonology, St. Petersburg. (Presented by Academician of the Russian Academy of Medical Sciences Yu. A. Romanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 12, pp. 611-613, December, 1992. Original article submitted March 27, 1992.

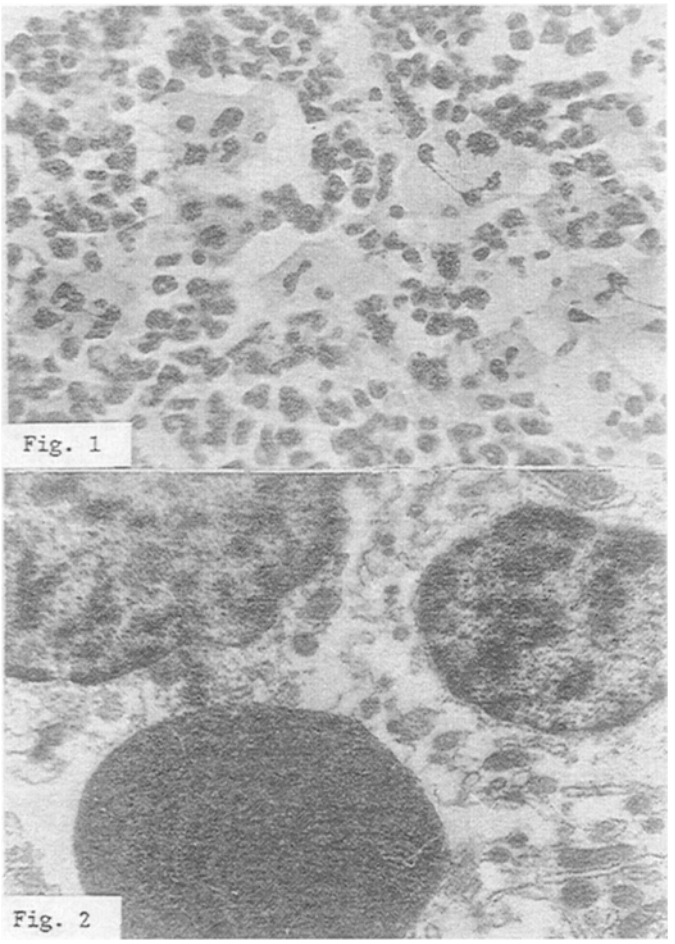

Fig. 1. Accumulation of pathologically changed megakaryocytes in bone narrow of mice receiving dipyridamole. Hematoxylin-eosin. 70×.

Fig. 2. Giant lipid-containing granules in megakaryocytes ln bone marrow of mice receiving dipyridamole. 35,000×.

for gas chromatographic analysis followed the usual procedure. The investigation was conducted on a Tsvet-101 chromatograph with flame-ionizator. Peaks of the acids on the chromatogram were identified against their standards and the values of the relative retention time, which were calculated against the palmitic acid peak [2]. The content of each acid was expressed as a percentage of the total of the acids found. The quantitative characteristic was the area of the peak occupied by the acid on the chromatogram. The results obtained were subjected to statistical analysis [3].

EXPERIMENTAL RESULTS

Heterotopic bone marrow transplantation beneath the connective-tissue capsule of the kidney of syngeneic recipients led after 30 days to the formation of a normally functioning RMP with osteogenic and hematopoietic tissues. In response to injection to DP the osteogenic component of the regenerating hematopoietic organ was present in only 30% of cases compared with the control.

Morphologic changes taking place in the regenerating and static bone marrow cell populations under the influence of DP are evidence of considerable changes in mass of the bony capsule and the cell content of RMP. Whereas under normal conditions, for instance, the ratio of the mass of the osteogenic tissue and its cell composi-

TABLE 1. Fatty Acid Composition of Total Lipids in Static (femur) and Regenerating (focus of heterotopic hematopoiesis) Bone Marrow Populations of Experimental Animals (in 70 of total acids)

Fatty acids		Static bone marrow cell population		Regenerating bone marrow cell population	
		control n = 5	Dipyridamole n = 6	control n = 5	dipyridamole n = 8
Myristic	14:0	2,22±0,27	1,76±0,52	1,40±0,19	3,07±0,36+
Palmitic	16:0	19,93±1,19	15,94±2,40+	14,84±0,48	18,60±1,84+
Palmitoleic	16:1	5,27±0,20	5,15±0,62	5,64±0,35	4,43±0,44
Stearic	18:0	11,93±0,59	12,54±0,61	12,27±0,57	9,25±1,18
Oleic	18:1	24,18±1,14	24,57±2,22	17,18±1,79	25,39±2,68+
Linoleic	18:2	12,80±1,71	13,17±0,81	13,19±1,21	13,10±1,12
Arachidonic	20:4	11,42±0,57	11,40±1,10	16,69±0,86	11,54±0,67+
Eicosapentaenic	20:5	3,53±0,22	2,02±0,44	6,11±0,54	2,36±0,32+
Docosatetraenic	22:4	1,78±0,33	2,68±0,67	1,79±0,38	1,76±0,48
Docosahexaenic	22:6	3,21±0,43	7,70±0,98+	3,24±0,40	4,05±0,53
Remaining acids		4,64	3,08	7,67	6,43
Saturation index		153	176	186	163

Legend. In fatty acids column numbers before colon indicate number of carbon atoms in fatty acid, numbers after colon indicate number of double bonds; +) $p < 0.05$.

tion on the 19th, 30th, and 50th days of the investigation amounted to 1:6, 1:3, and 1:5, after administration of DP the ratio changed abruptly to 1:13, 1:10, and 1:11 respectively. All this took place against the background of high mitotic activity of the hematopoietic cells. Stromal bone marrow stem cells were responsible for these changes [4]. By the 50th day of the investigation, when a state of dynamic equilibrium had become established, the degree of suppression of release of platelet growth factor (PRG) from platelet granules in response to injection of DP became comparable with the degree of intramedullary destruction of MKC. Moreover, this state of equilibrium was reached more rapidly in the static bone-marrow population than in the regenerating population. It was also observed that under the influence of DP excessive accumulation and destruction of MKC appeared in the bone marrow (Fig. 1).

Electron-microscopic investigation of these MKC showed that as early as on the 7th day of injection of DP, and rising to a peak on the 30th day of the experiment, there was an increase in the number and size of the giant lipid-containing granules in the cytoplasm of these MKC (Fig. 2); this evidently leads to rupture of the cytoplasmic membrane of MKC and to the leakage of the contents of the cytoplasm of these cells into the intercellular space. Similar data on the effect of inhibitors of metabolism of arachidonic acid, a precursor of prostaglandins, on the outflow of intracellular enzymes from the skeletal muscles as a result of experimental injury, were obtained by other investigators [6, 8]. The data given in this paper show that administration of DP for 30 days led to a significant decrease in the content of polyunsaturated arachidonic and docosahexaenic acids in lipids of the regenerating bone marrow and to an increase in the content of saturated oleic acid (Table 1).

The results are thus evidence that administration of DP for 30 days modifies the fatty acid profile of the bone marrow lipids. For instance, under the influence of DP the content of unsaturated fatty acids in the regenerating bone marrow population decreases, whereas that of saturated fatty acids, on the contrary, increases; in the static bone-marrow population, however, the percentage content of unsaturated acids increases, whereas that of saturated acids decreases.

REFERENCES

1. V. A. Almazov, M. V. Blagosklonnyi, and A. Yu. Zaritskii, Ter. Arkh., **56**, No. 7, 144 (1956).

2. H. P. Burchfield and E. E. Storrs, Biochemical Applications of Gas Chromatography, Academic Press, New York (1962).
3. E. V. Gubler, Computational Methods of Analysis and Diagnosis of Pathological Processes [in Russian], Leningrad (1978).
4. I. N. Ivasenko and G. E. Arkad'eva, Arkh. Anat., **100**, No. 4, 74 (1991).
5. I. N. Ivasenko, O. V. Klestova, G. E. Arkad'eva, and V. A. Almazov, Byull. Éksp. Biol. Med., No. 7, 98 (1990).
6. W. E. Brocklehurst, Proc. R. Soc. Med., **64**, No. 1, 4 (1971).
7. J. Folch, M. Lees, and G. N. Sloane-Stanley, J. Biol. Chem., **226**, No. 1, 497 (1957).
8. M. Jackom, A. J. M. Wagenmakers, and R. H. T. Edwards, J. Biochem., **241**, No. 2, 403 (1987).
9. J. Peicker, J. Am. Oil Chem. Soc., **41**, No. 1, 87 (1964).
10. M. Tawassoli, A. Mamiatis, and W. Gosly, Proc. Soc. Exp. Biol. (New York), **133**, 878 (1970).
11. H. B. White, G. Gally, and R. Paoletti, J. Neurochem., **18**, No. 7, 1337 (1971).