INFLUENCE OF A HETEROGENEOUS HIGH-POLYMER DNA UPON ERYTHROPOIESIS

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Iutensive investigations of the influence of nuclear acids upon regeneration of bone-marrow tissue after radiation have been carried out recently. The radiation causes destructive changes in tissues with high DNA-concentration; nuclear DNA is a primary locus of ion-radiation action (1, 2). However, certain authors report the influence of exogenic nuclear acids upon haemopoiesis of non-radiated animals with applied homologous DNA (3, 4, 6).

The objective of the present study is to investigate the eventual changes of some indexes of peripheral blood after application of heterologous native high-polymer DNA.

Materials and methods

DNA was isolated (separated) from human spleen after splenectomy by using the MUP-method of Britten, modified by G. Markov and G. Ivanov (1974). The preparations had molecule weight $8{,}0\times10^5$—$12{,}0\times10^5$ D and 0,5% protein admixture. Their concentration was determined by using the diphenylamine method (7), whereas the protein amount was measured by the method of Lowry (9). 24 hours after Fe^{59}-injection the animals were killed and blood-samples were read on gamma-scintillating counter NK-107 (Hungary). The indexes of red blood were determined by routine methods.

The study covered 2 seria of experimental animals. Female white mice (race «Vistar»), weight 135—145 g were used. The animals of first series were injected with a single dose of 1 mg heterologous DNA intraperitoneally, whereas those of the second one — with 3 mg heterologous DNA (in 3 ml 0,14 M NaCl). The controls were injected with equal amount of saline solution.

The red blood content was investigated each 2 days until the end of the second week after injection, followed by determination of Fe^{59} incorporation in erythrocytes. The latter is a complex process, therefore we had controls of each experimental group.

All data were analysed by using the criterion of Student.

Results

Both experimental seria had no statistical deviations in erythrocyte number, Hb-concentration, hematocrit-level, etc. for the studied period. However, the change-dynamics in number of reticulocytes of the investigated animals (compared to controls) was interesting.

The reticulocyte-number of animals treated with 1 mg DNA, 6 days after injection, was higher (52,49% and $p<0{,}05$); most expressed reticulocytosis — on the 8th day (121,11% and $p<0{,}025$). Two days later (10th day) it was decreas-

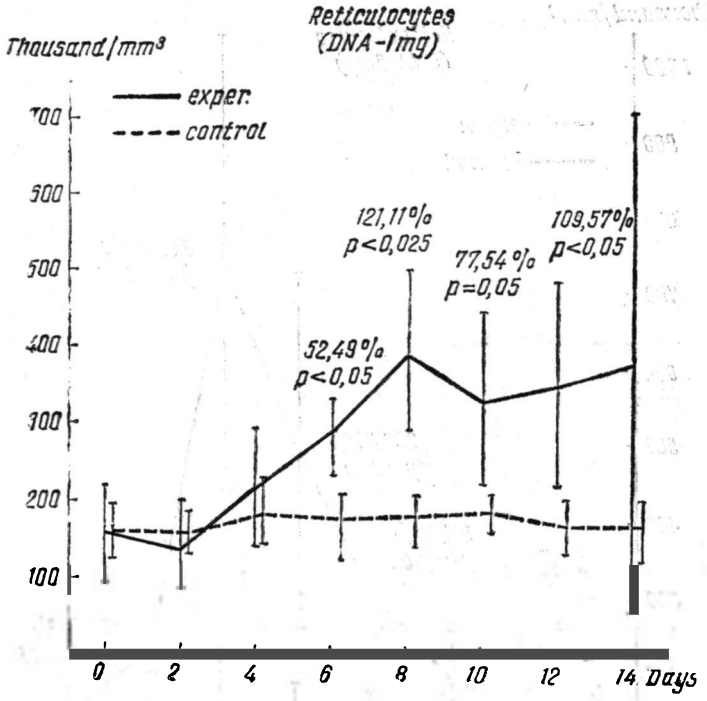

Fig. 1

ed to 77,54% (p=0,05) but the level was comparatively high until the end of the experiment (fig. 1).

The reticulocytes of the second group animal (3 mg DNA) considerably increased in number after the 6th day (101,31% and p<0,05); on the 10th day it was 348,21% (p<0,025) and after the 14th day the values tended to normalize (fig. 2).

The percent determination of Fe^{59}-incorporation in erythrocytes of both group animals (on 14th day) shew higher values: with 1 mg DNA dose it was 25,77% (p>0,05) higher than the controls (fig. 3), whereas with 3 mg DNA dose it was 334,31% (p<0,05) higher.

Discussion

The data prove that high polymer heterologous DNA stimulates the erythropoiesis of experimental animals. The reticulocytosis, being a feature of erythroid proliferative activity of bone-marrow, is most considerable 8—10 days after injection. It coincides with the pronormoblast transformation into an acidophile normoblast (5—6 days) and the period of its final development: 36 hours. The mechanism of this stimulating effect upon erythropoiesis can be explained by the applied DNA and activated enzyme systems, connected with its destruction, as well as with haemoglobin biosynthesis.

Many authors (2, 5, 6) establish that the labelled exogenic H^3-DNA can not be detected up to 6 hours after injection but the activity of desoxyribo

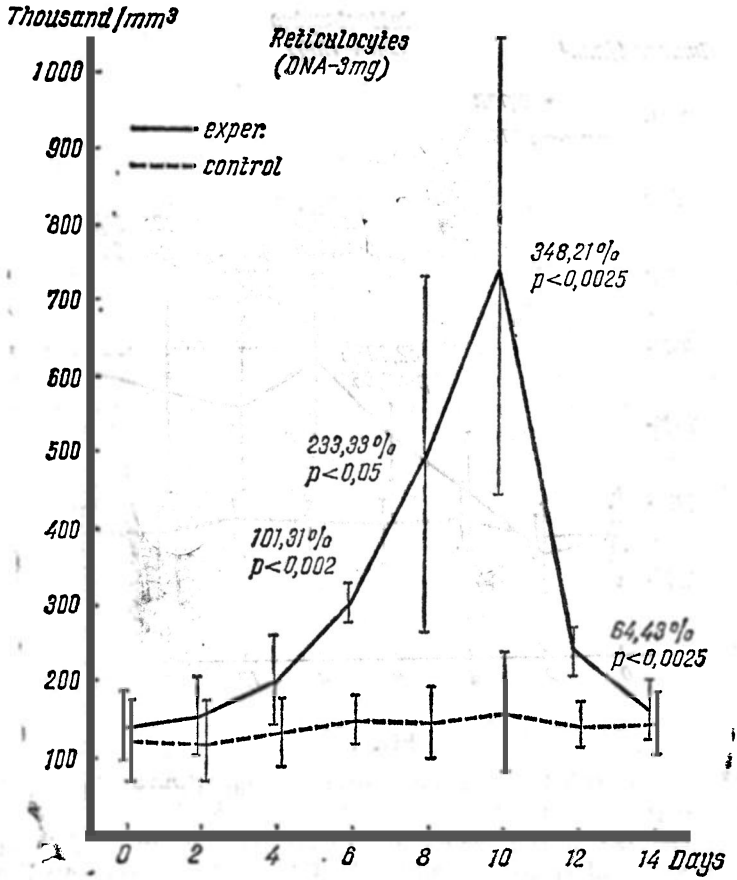
Thousand/mm³
Reticulocytes
(DNA-3mg)
exper.
control
1000
900
800
700
600
500
400
300
200
100
348,21%
p<0,0025
233,33%
p<0,05
101,31%
p<0,002
64,43%
p<0,0025
0
2
4
6
8
10
12
14 Days

Fig. 2

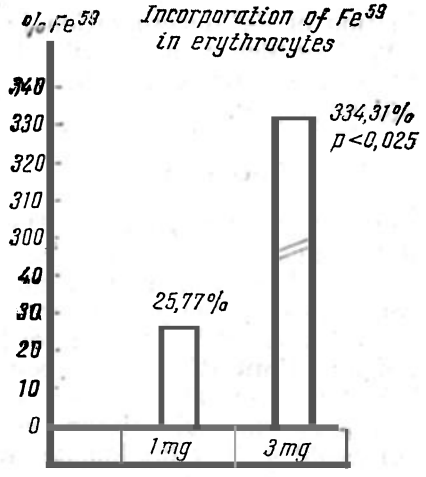
% Fe⁵⁹
Incorporation of Fe⁵⁹
in erythrocytes
340
330
320
310
300
40
30
20
10
0
334,31%
p<0,025
25,77%
1mg
3mg

Fig. 3

nucleases is simultaneously increased: desoxyribonuclease I is activated 1 hour after injection, while desoxyribonuclease II (with highest tissue concentration and proliferative ability) is activated after 6 hours. The asparat-carbamyltransferase and desoxyribonucleopolymerase are activated 24 hours later. It is suggested that DNA-fragments (a product of activated desoxyribonucleases) transported to rapidly proliferating tissues are capable of being plastic material and also inducers of enzyme-desoxyribonucleokinases, thus controlling the DNA-synthesis.

According to the principles of enzyme kinetics the various dynamics of reticulocytosis can be explained by the different stage of activation of aforementioned enzymes due to the substrate concentration; in our experiments the exogenic DNA is a limitting factor. Its low concentration (1 mg dose) tends to a weaker activation of desoxyribonucleokinases and slower incorporation of DNA-fragments during replication (s-period of cell cycle). With a dose of 3 mg DNA it is possible that higher percent of reticulocytosis and quick decrease to normal values after 10 days is a result of total consuming of cell nucleotide reserve and enzyme retroinhibition. According to Duces (1968) and Rogacheva (1970) it can be explained also by the decreased number of precursor-cells for erythropoiesis. Ruissina (1976) reports that 3 mg exogenic homologous DNA stimulates the proliferative activity of bone-marrow; H^3-timidine incorporation in new-synthesized DNA cells is highest at the 24^{th} hour and can not be detected after the 72^{nd} hour. Nakao (1968) establishes a peak of DNA-synthesis at the 48^{th} hour and higher activity of the ferment systems for Hb-synthesis — at the 52^{nd} hour after stimulation. All these deviations are a direct result of the influence of heterologous DNA upon proliferative activity. Indirect influence of erythropoietic system is also minded. It can be suggested by the dose-depending percent of Fe^{59}-incorporation in erythrocytes on the 15^{th} day when, it is presumed, that the direct effect of the applied DNA and reticulocytosis is already diminished. The data of Burger (1971) and Rodgers (1972) support the aforementioned opinion; the exogenic stimulation of erythropoietin-synthesis and the considerable amount of nucleotides and bases after DNA-destruction are those to influence cell processes.

The following conclusions are a result of our analysis:

1) The applying of heterologous high-polymer DNA (doses 1 or 3 mg) stimulates the erythropoiesis.

2) Both doses influence the reticulocyte number (increased 8—10 days later). The higher the dose is, the more considerable the influence is.

3) The dose of 3 mg increases the percent of Fe^{59}-incorporation in erythrocytes (334,31%), whereas 1 mg increases it to only 25,77%.

4) It is very probable that exogenic DNA has an indirect influence added by the erythropoietin-system.

REFERENCES

1. Белоус, А. М., В. П. Годин, Е. Я. Панков. Экзогенные нуклеиновые кислоты и востановительные процессы, М., 1974, 117. — 2. Мазурик, В. К. *Радиобиология,* **IV**, 1966, 3, 482—483. — 3. Рогачева, С. А., Г. Г. Русинова, Э. Г. Шарова. *Цитология,* **XII**, 1970, 7, 917. — 4. Рысина, Т. Н. *Бюл. Эксп. Биол.,* 1976, 5, 533. — 5. Рысина, Т. Н., Р. Е. Либинзон, Э. Г. Ша-

рова. *Биохимия,* 1971, 534. — 6. Braun, W., W. Firshen. *Bact. Rev.,* 1967, 31, 83. — 7. Burton, K. *J. Bioch.,* **62,** 1956, 2, 315—317. — 8. Duces, P. P. *Ann. N. Y. Acad. Sci.,* 1968, 149, 437—449. — 9. Lowry, O. H. et al. *J. Biol. Chem.,* 1951, 193, 265—269. — 10. Laytha, L. G., R. Oliver. Ciba Found. Symp. Haemopoiesis. London, 1960, 289. — 11. Markov, G. G., I. G. Ivanov. *Anal. Biochem.,* 1974, 59, 555—563. — 12. Nakao, K., S. Sassa. *Ann. N. Y. Acad. Sci.,* 1968, 149, 224—230. — 13. Rodgers, G. M., W. I. George, I. W. Fisher. *Proc. Soc. Exp. Biol.,* Med., 1972, 40, 977—979.

ВЛИЯНИЕ ГЕТЕРОЛОЖНОЙ ВЫСОКО ПОЛИМЕРНОЙ ДНК НА ЭРИТРОПОЭЗ

Р. Падолски, Д. Даскалов

РЕЗЮМЕ

Исследовано влияние гетероложной высокополимерной ДНК на эритропоэз. Авторами рассматриваются детально методы Britten в модификации Marcov и Ivanov для получения ДНК, метод Lowry, позволяющий количественное определение белка и другие методы, как и их применение. Были использованы самки белых мышей породы «Вистар». Анализируется влияние ДНК на ряд систем организма.