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INFLUENCE OF PIRACETAM ON ERYTHROPOIESIS IN RATS

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The effects of piracetam (2 x 200 mg/kg b. w.) injected intraperitoneally for three days on erythropoiesis and some functional characteristics of erythrocytes were studied in Wistar rats. A significant increase of reticulocytes in relative (by 122,03 %, $p < 0,01$) and in absolute counts (by 109,29 %, $p < 0,01$), an increase of ^{59}Fe incorporation in newly-formed erythrocytes (by 13,80 %) and a significant rise of erythroblasts as followed: total counts (by 59,39 %, $p < 0,01$), proerythroblasts (by 52,87 %, $p < 0,05$), and orthochromatic erythroblasts (by 54,25 %, $p< 0,01$) were observed in the piracetam-treated rats. It was accepted that piracetam stimulated erythroid proliferation and differentiation. Erythroid deformability enhanced by 14,69 % ($p < 0,01$) but spontaneous haemolysis of erythrocytes reduced by 16,95 % ($p < 0,025$). Thus it could be suggested that piracetam, along with its stimulatory effect on erythropoiesis, improves some of the most important functional characteristics of erythrocytes such as deformability and oxidative resistance.

Key-words: Piracetam, erythropoiesis, erythrocyte deformability, spontaneous haemolysis, aluminium

The positive influence of piracetam on learning and memory and its antihypoxic effects in nervous system are well-known (11,12). Recently, its effects outside the nervous system were reported as an increase of bilirubin and cholesterol in bile and uric acid in plasma (5). Erythrocyte filterability "in vitro" in patients with epilepsy ameliorated after piracetam treatment was also described (4).

Having in mind the influence of piracetam on metabolic processes in the nervous system suc as an increased synthesis and utilization of ATP, phospholipids and elevated extraction ratio of oxygen and glucose (13) as well as the above mentioned positive effect on erythrocyte filterability, we are inclined to suggest that piracetam influences positively not only on erythrocyte functions, but on erythropoiesis rates as well.

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Aim of our study was to examine the influence of piracetam in "in vivo" experiments on erythropoiesis and some functional characteristics of mature erythrocytes, as far as these problems remained understudied.

MATERIAL AND METHODS

The experiment was carried out on 136 Wistar rats weighing 170-180 g. The experimental group (n=70) was treated with piracetam (Pyramem - "Pharm.") in a dose of 200 mg/kg b. w. twice daily, intraperitoneally, for 3 days. The controls (n=66) were injected with saline. The parameters of erythropoiesis, including ^{59}Fe incorporation in newly-formed erythrocytes were determined by methods described elsewhere (2). Bone marrow smears for a cytologic study were stained by Pappenheim's method. Index of erythroblast maturation was calculated. Erythrocyte deformability was assessed according to Tannert and Lux method (1981) in our modification (2) and spontaneous haemolysis of erythrocytes - by Jager's method (1968). Differences between groups were tested using Student-Fisher *t*-test and considered significant when the p-value was less than 0,05.

RESULTS AND DISCUSSION

Data are presented on Table 1 and Figures 1 through 3. No considerable changes were observed in erythrocyte counts, haemoglobin and haematocrit so the results were not mentioned.

Table 1

Changes in reticulocyte counts and ^{59}Fe incorporation in newly-formed erythrocytes after 3 -day piracetam treatment (2 x 200 mg/kg b. w.) in rats

Parameters	N	Experiment	N	Control	%
Reticulocytes – "per mille"	40	66,85 ± 10,78	37	30,10 ± 4,35	+ 122,03 ($p < 0,01$)
Reticulocytes $10^9/l$	35	431,42 ± 62,90	32	206,13 ± 31,93	+ 109,29 ($p < 0,01$)
Incorporation of ^{59}Fe in newly-formed erythrocytes - %	33	27,52 ± 1,25	34	24,18 ± 1,69	+ 13,80 ($p > 0,05$)

Data are presented as means ± SEM. Percent deviation calculated against the controls. + increase, N – number of animals per group

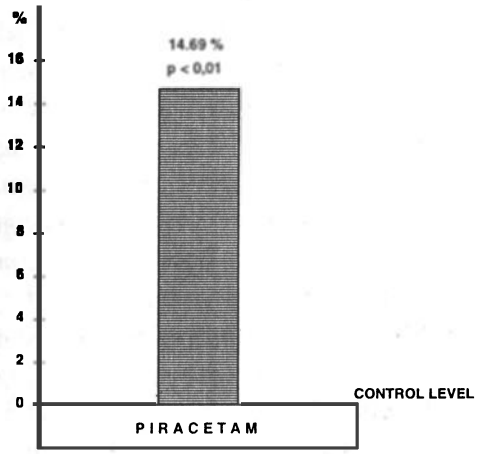

Fig. 1. Changes in erythrocyte deformability after 3-day piracetam treatment (200 mg/kg b. w. twice daily) in rats. Data are presented as percent deviation from the controls which are taken to be zero.

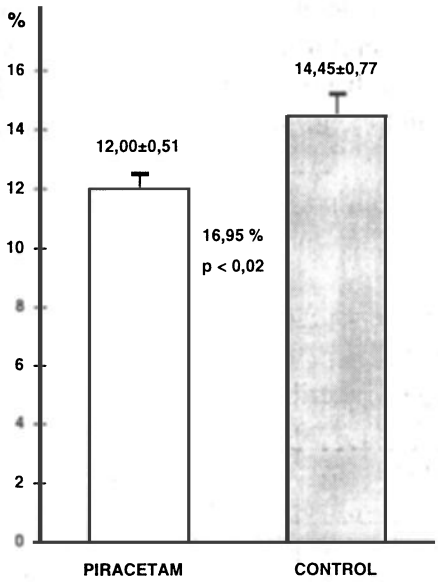

Fig. 2. Spontaneous haemolysis of erythrocytes after 3- day piracetam treatment (2 x 200 mg/kg b. w.)
- piracetam treated (n=15)
- saline treated (n=15), controls
Data are presented as means ± SEM

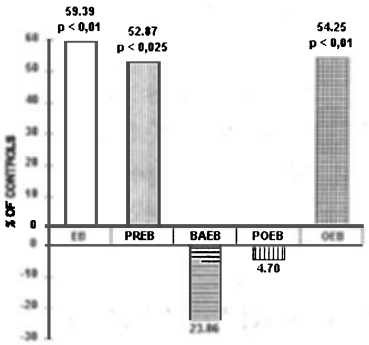

Fig. 3. Erythroblast shifts influenced by piracetam.
Data are presented as percent deviation from the controls. (+) - increase, (-) - decrease

***EB** - erythroblasts, **PREB** - proerythroblasts, **BAEB** - basophil erythroblasts, **POCB** - polychromatic erythroblasts, **OEB** - orthochromatic erythroblasts*

From Table 1 it can be seen that piracetam treatment significantly increases the reticulocyte counts - in relative (by 122,03 %; $p < 0,01$) and absolute (by 109,29 %; $p < 0,01$) values as compared to the controls. ^{59}Fe incorporation in newly-formed erythrocytes also shows a tendency to increase by 13,80 % ($p < 0,05$). The data we have obtained point to the suggestion that piracetam treatment stimulates erythroid regeneration and haemoglobin formation. The established significant increase of the total number erythroblasts in bone marrow by 59,39 % ($p < 0,01$) (Fig. 3) supports this suggestion as well. The increase of PREB in bone marrow by 52,87 % ($p < 0,025$) may be

considered as a manifestation of enhanced transition of unrecognizable erythroid precursors to the erythroblast section of this cell line under the influence of piracetam. The tendency towards lower values of POEB and BAEB along with the significant increase of OEB by 54,25 % ($p < 0,01$) could be interpreted as a result of the direct stimulatory effect exerted by piracetam on proliferation and, predominantly, on differentiation and maturation of erythroblasts. The raised index of maturation of erythroblasts by 10,18 % ($p < 0,01$) observed in the experimental group reflecting the increased number of mature erythroblasts supports this suggestion, too. It is possible that the direct positive metabolic influence of piracetam (well-known for other cell systems (13) on erythroid bone marrow cells, to account for this stimulation of erythropoiesis. The positive influence of piracetam on adrenergic neurotransmission should not be excluded as well (14). Undoubted evidence exists that the activated adrenergic system has stimulatory effects on erythropoiesis (8).

Additional information about the properties of mature erythrocytes influenced by piracetam under normal conditions could be obtained from determination of some functional characteristics of mature erythroblasts. In vitro studies show that low doses of piracetam treatment (12 mg/kg) in patients with epilepsy improve the aggravated erythrocyte filterability (4). In our "in vivo" experiment with higher doses of piracetam, erythrocyte deformability significantly increases by 14,69 % ($p < 0,01$) (Fig. 1). The stimulation of metabolic processes in erythroid cells (3,7) and the raise of reticulocyte counts in blood (6) could explain that increase. The fact that piracetam reduces phosphorylation of membrane proteins should be also considered (11). Obviously, the raised reticulocyte counts under piracetam influence and the stimulated metabolic processes in erythroid cells determine a better deformability. As a result erythrocytes show improved functional properties for transition trough critical places of microcirculation and delivery of oxygen to the tissues. Erythrocyte spontaneous haemolysis after piracetam treatment was decreased by 16,95 % ($p < 0,025$) (Fig. 2). A possible explanation may be that piracetam ameliorates metabolic processes in erythroid cells and the stability of erythrocyte membranes. Hence, the erythrocyte becomes more resistant to the influence of haemolytic factors. In this respect, the increase of reticulocytes which are known to be more resistant to haemolytic influences should be considered as well. Data exist about a

direct suppression of lipid peroxidation in rat brain tissue by piracetam (1) which also must be taken into account. It may be suggested that erythrocyte resistance against lipid peroxidation products is increased as well.

In conclusion, our data show that piracetam not only stimulates erythropoiesis, but also improves the most important functional characteristics of erythrocytes such as deformability and oxidative resistance. It is supposed that piracetam may be used as a therapeutic means in cases with haemorheologic disturbances accompanied by increased erythrocyte rigidity and formation of oxygen radicals.

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Влияние на пирацетам върху еритропоезата у плъхове

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Резюме: Влиянието на пирацетам (2 x 200 mg/kg телесна маса, i. p. за 3 дни) върху еритропоезата и някои функционални характеристики на еритроцитите е проучено

при бели плъхове от порода Wistar. У третираните с пирацетам плъхове се наблюдава значимо повишение на ретикулоцитите "про мили" със 122,03 % ($p < 0,01$), в абсолютни стойности - със 109,29 % ($p < 0,01$), на включеното ^{59}Fe в новообразуваните еритроцити - с 13,80 %, както и значимо увеличение на еритробластите: общия им брой - с 59,39 % ($p < 0,01$), проеритробластите - с 52,87 % ($p < 0,05$) и оксифилните еритробласти - с 54,25 % ($p < 0,01$). Приема се, че пирацетамът стимулира еритроидната пролиферация и диференциация. Еритроцитната деформабилност показва увеличение с 14,69 %, а спонтанната хемолиза на еритроцитите - по-ниски стойности с 16,95 % ($p < 0,025$). Следователно пирацетамът не само стимулира еритропоезата, но и подобрява едни от най-важните функционални свойства на еритроцита - неговата деформабилност и прекисна устойчивост.