STUDY OF THE EFFECTS OF NON-SELECTIVE BETA-ADRENERGIC TREATMENT ON PLASMA THROMBOCYTOPOIETIN ACTIVITY IN RATS IN CONDITIONS OF AN ACUTE THROMBOCYTOPENIA

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Key-words: thrombocytes — ^{75}selenomethionine incorporation — β-adrenergic agents—acute thrombocytopenia

The relation between the count of peripheral blood thrombocytes and plasma thrombocytopoietin activity is long ago established. It is known that normally, thrombocytopenia is attended by thrombocytopoietin production increase (8, 10) whereas any thrombocytosis — by its decrease (6, 13).

Our previous studies show that thrombocytopoietin production in rats in normal conditions depends also on beta-adrenergic receptors (1, 3). On the basis of that we make it our object to studying to what extent non-selective β-adrenergic effects are related to plasma thrombocytopoietin activity in conditions of an acute thrombocytopenia. This could enable more profound conclusions concerning the importance of non-selective β-adrenergic influences and β-adrenoceptors, respectively, on thrombocytopoietin production.

Material and methods

Plasma required for thrombocytopoietin activity determination was obtained from 53 male rats of Wistar breed with 180—220 g body weight divided into three groups and injected as followed: Ist group — with Isoprenaline (IP) hydrochloride (a non-selective β-adrenostimulator) at dosis 2×3 mg/kg b. w.; IInd group — with Propranolol (PR) hydrochloride (a non-selective β-adrenoblocker) at dosis 2×5 mg/kg b. w., and IIIrd group — controls — with saline in the same amount. Injections were made intraperitoneally every 12 hours. 3 h after first application all the rats were injected with thromboplastin (TP) after the scheme of Kelemen et al. (7) in our modification, namely: We used TP produced by the Research Institute of Hematology and Transfusion — Sofia in our study. The first injection was done in the tail vein in vol. $0.5.10^{-6}m^3$ but the second one after 40 min intraperitoneally in vol. $1.10^{-6}m^3$. Thrombocyte count was determined according to the method of Feissly et Ludin (4) at the beginning and the end of the assay. Animals were bleeded by means of aortic cannulation on the 20th hour after first TP injection. Separated plasma was stored at —8° C for 10 days. We judged of plasma thrombocytopoietin activity from the changes of thrombocyte count and from ^{75}selenomethionine (^{75}Se-M) incorporation in newly formed thrombocytes in recipients-mice under the influence of material tested. We used 51 white male non-thoroughbred mice with 20—30 g b. w. divided into 3 groups. The animals from the first group were injected with plasma from donors treated with IP+TP, those from the second one — with plasma from donors treated with PR+TP, and the mice from the third group were controls (injected with plasma from donors treated with saline+TP). We tested the plasma after Penington's method in our modification (12). It included the following changes:

a) In the place of male mice C 57 we used white mice — male non-thoroughbred mice with initial thrombocyte count — 200—380.10^9/l;

b) We injected the specimens in two successive days s. c. at dosis 1.10^{-6}m³ each as contrasted with Penington's method (12) who applied a dosis not higher than 0.5.10^{-6}m³ but injected the mice with antithrombocyte serum one week before;

c) We injected the isotope (^{75}Se-M) intraperitoneally at dosis 2 μCi per mouse 1 h after second plasma injection but not on the next day as it was according to Penington.

We took 0.5.10^{-6}m³ blood for estimation of the percentage of ^{75}Se-M incorporated in newly formed thrombocytes on the 72nd hour by using cardiac puncture under ether narcosis. We took the determination of isotope incorporation on the 72nd h (in contrast to Penington who determined it on the 24th h) because this period coincided with the peak of thrombocyte count and of percentage of ^{75}Se-M incorporated in newly formed thrombocytes. Thrombocyte separation was done according to Penington's method (11) but radiometrics — on the apparatus N/K 350 — Hungary. Data processing was done by the methods of variation statistics.

Results and discussion

It can be seen on fig. 1 that thrombocyte count decreases at the end of the assay with 18.96 per cent ($p<0.002$) as compared with the initial one in mice from the first group but it increases with 95.09 per cent ($p<0.001$) in mice from the

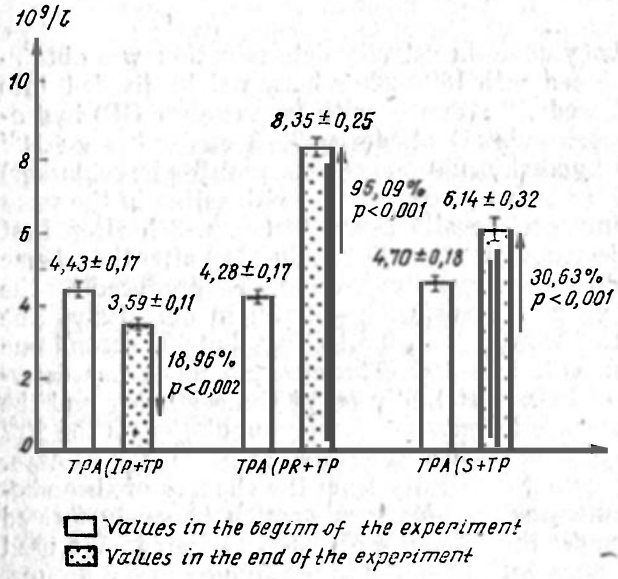

Fig. 1. Thrombocytopoietin activity of donor's plasma — rats treated with β-adrenergic agents and thromboplastin determined by investigation of the changes of thrombocyte count in recipients-mice. Data are presented as $x \pm Sx$. Percentage difference is calculated towards initial values

second group. Thrombocytes increase with 30.63 per cent ($p<0.001$) in control mice. ^{75}Se-M incorporation in thrombocytes (fig. 2) in experimental and control animals both shows that there is a reduction with 53.48 per cent ($p<0.001$) in the first group and an increase with 754.1 per cent ($p<0.001$) in the second one.

It is first of all notable that these two indexes undergo unidirectional and significant changes in the animals from all groups studied. There is a reduction of thrombocyte number and ^{75}Se-M incorporation in the first group. This allows us to accept that there is a thrombocytopoiesis suppression in recipients probably due to the reduced plasma thrombocytopoietin activity. In our opinion it results from the realized negative feed-back mechanism (7) in IP+TP treated donors as far as we previously reported that IP alone induced a significant thrombocyte count increase (2). The fact that IP+TP treatment of donors (9) does not cause thrombocyte countan chges probably also contributes to the low plasma thrombocytopoietin activity in the practical example. It is possible that the significantly increased megakaryocyte count in these experimental conditions has a definite importance for the inhibition of thrombocytopoietin biogenesis, i. e. in the mechanism of negative feed-back, too (9) thus stimulating the production of thrombocytopoiesis inhibitor. All that could reduce total plasma thrombocytopoietin activity illustrated by the diminution of thrombocyte count and ^{75}Se-M incorporation in newly formed thrombocytes in recipients-mice.

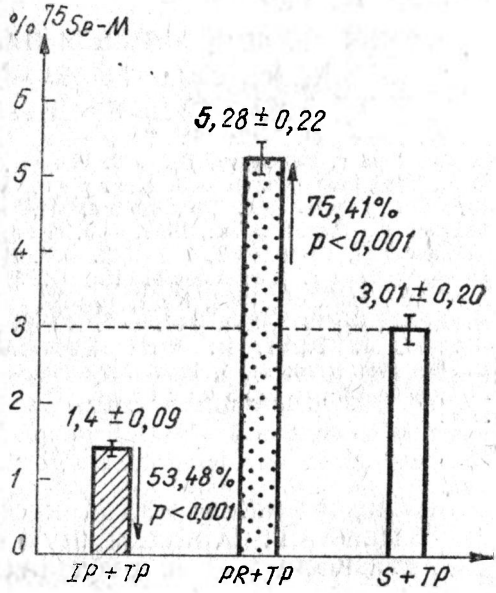

Fig. 2. Thrombocytopoietin activity of donor's plasma — rats treated with β-adrenergic agents and thromboplastin determined by the percentage of incorporated ^{75}SeM in newly-formed thrombocytes in recipients-mice. Data are presented as $\bar{x} \pm Sx$. Percentage difference is calculated towards the controls

The significant increase of thrombocyte count and ^{75}Se-M incorporation in newly formed thrombocytes in animals tested with plasma from PR+TP treated donors is a sure sign of an enhanced thrombocytopoietin activity. This conclusion confirms directly our previous investigations that in such donors there is a strongly expressed thrombocyte count reduction, a significant young form count increase, and a considerable decrease of the number of mature forms of the megakaryocyte line (9). We suppose that the increased plasma thrombocytopoietin activity results not only from the thrombocytopenic thromboplastin action (5, 7) but also mainly from the capacity of the non-selective β-adrenoblocker PR to reduce significantly independently thrombocyte count in so far as plasma from donors injected with saline+TP induces a less expressed thrombocyte count increase (30.63 per cent). It allows us to suggest that there is a summation of two influences reducing thrombocyte count — that of PR and that of TP. Thus, in the mechanism of negative feed-back thrombocytopoietin biosynthesis can be stimulated.

Our results obtained allow us to presume that non-selective β-adrenergic influences modify additionally plasma thrombocytopoietin activity in condition of an acute thrombocytopenia. β-adrenostimulation with IP reduces but β-adrenoblokade with PR increases considerably plasma thrombocytopoietin activity.

It seems most probably that beta-adrenoceptors play a regulatory role in thrombocytopoietin production. Therefore, modulation effect of β-adrenoceptors on plasma thrombocytopoietin activity is present in conditions of an acute thrombocytopenia, too.

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ИССЛЕДОВАНИЕ ЭФФЕКТОВ НЕСЕЛЕКТИВНОГО БЕТА-АДРЕНЕРГИЧЕСКОГО ВОЗДЕЙСТВИЯ НА ПЛАЗМЕННУЮ ТРОМБОЦИГОПОЭТИНОВУЮ АКТИВНОСТЦ У КРЫС В УСЛОВИЯХ ОСТРОЙ ТРОМБОЦИТОПЕНИИ

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РЕЗЮМЕ

Исследованы изменения числа тромбоцитов и включение ^{75}селенометионина (^{75}Se-M) в новообразованные тромбоциты у реципиентов-мышей под влиянием плазмы доноров-крыс.

Устанавливается, что у реципиентов, которым вводилась плазма доноров, инъецированных изопреналин гидрохлоридом (неселективным бета-стимулятором) в сочетании с тромбопластином, число тромбоцитов уменьшается на 18,96 % ($p<0,002$), а включение ^{75}селенометионина приводит к уменьшению числа тромбоцитов на 53,48 % ($p<0,001$). Реципиенты, тестированные плазмой доноров, инъецированных пропранолол гидрохлоридом (неселективным бета-адреноблокером) в сочетании с тромбопластином, характеризуются наличием значимого увеличения как числа тромбоцитов (95,09 %, ($p<0,001$), так и включения ^{75}Se-M — 75,41 % ($p<0,001$). Контрольная группа реципиентов, тестированных плазмой доноров, инъецированных физиологической сывороткой в сочетании с тромбопластином, показывает увеличение числа тромбоцитов на 30,63 % ($p<0,001$).

Автор считает, что бета-адренергические воздействия дополнительно изменяют тромбоцитопоэтиновую активность плазмы в условиях острой тромбоцитопении, причем бета-адреностимуляция изопреналином вызывает понижение, а бета-адреноблокада пропранололом — сильное повышение плазменной тромбоцитопоэтиновой активности.