CHANGES OF THROMBOCYTOPOIESIS IN RATS AFTER NON-SELECTINE BETA-ADRENERGIC TREATMENT IN CONDITIONS OF AN ACUTE THROMBOCYTOPENIA

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In our previous studies we established that in normal conditions rat thrombocytopoiesis and plasma thrombocytopoietin activity depended directly on the functional state of beta-adrenergic receptors (7, 8). The aim of the present work is to determine the extent to which non-selective beta-adrenergic actions influence thrombocyte and megakaryocyte number in thromboplastin induced acute thrombocytopenia. It will permit us to establish the role of beta-adrenergic receptors in thrombocytopoiesis regulation in extreme conditions.

Material and methods

The study covered 53 white male rats of Wistar breed with 180—200 g body weight divided into three groups. Ist — injected with Isoprenaline (IP) hydrochloride (a non-selective beta-adrenostimulator) at dosis 2×3 mg/kg b. w.; IInd — with Propranolol (PR) hydrochloride (a non-selective beta-adrenoblocker) at dosis 2×5 mg/kg b. w., and IIIrd — with saline (PS) in the same amount (controls). The experiment duration was 24 h and the injections with adrenergic agents and PS twice every 12 h intraperitoneally. 3 h after first injection thromboplastin (TP) was injected after the scheme of Kelemen et al. (1963) in our modification. TP produced by the Research Institute of Hematology and Transfusion — Sofia was used in our study. The first injection was done i. v. 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 after the method of Feissly-Lüdin (cited after 9) at the beginning and the end of the assay as well as megakaryocyte line cells of 33 animals only by using bone-marrow smears prepared after the routine method (9). Megakaryocyte classification was done after Levine et al. (1982). The data were processed by the methods of variation statistics.

Results and discussion

The comparison of the changes of thrombocyte count shows that it increases insignificantly (0,75 per cent) in IP+TR treated animals, it decreases with 51,97 per cent ($p < 0{,}001$) in PR+TR treated ones but with 30,96 per cent ($p < 0{,}002$) in PS+TR treated ones (see fig. 1). The changes of bone-marrow megakaryocytes (see table 1). show that IP+TR treatment causes a total megakaryocyte increase with 50 per cent ($p < 0{,}001$). Ist and IInd stage megakaryocytes decreased with 49 per cent ($p < 0{,}001$) and 3,20 per cent, respectively, but IIIrd and IVth stage ones increased with 161 per cent ($p < 0{,}001$) and 263 per cent ($p < 0{,}001$), respectively. In PR+

TR treated animals megakaryocytes increased totally with 44 per cent ($p < 0{,}001$) (I^{st} stage with 52 per cent ($p < 0{,}001$); II^{nd} one with 71 per cent ($p < 0{,}001$), and III^{rd} one with 39 per cent) while IV^{th} stage megakaryocytes decreased with 54 per cent.

It is evident that IP+TR injection induces an insignificant thrombocyte and a strongly expressed megakaryocyte increase (totally on the account of more matured forms — III^{rd} and fourth stages) while I^{st} stage megakaryocytes are significantly reduced. It is known that TR injection induces thrombocyte diminution and a parallel plasma thrombocytopoietin activity increase up to 24^{th} hour (14) which is one of the reasons for megakaryocyte increase and reduced thrombocyte count restoration. The comparison of IP+TR treated thrombocytes with the other two groups shows that only they have reached their initial levels at the end of the experiment. We suppose that IP and non-selective beta-adrenostimulation respectively, is an important factor for thrombocyte number restitution in extreme conditions. Probably, IP is related with the strongly expressed increase of III^{rd} and IV^{th} stage megakaryocytes, too, and sets the pattern for the significant I^{st} stage megakaryocytes reduction. We established analogous changes in these cells under IP influence also in normal conditions of the animals (7). We accept that most probably IP exerts a direct stimulating effect upon beta-adrenergic receptors of stem cells and the cells of megakaryocyte line as far as such an effect was demonstrated in other blood cell systems (1, 2, 11). It is known that IP stimulates directly beta-adrenergic receptors of early precursors and unipotent stem cells as well as of more matured erythroid elements (2, 11) and causes an increased DNA synthesis, incorporation of cells into cell cycle and shortening of the latter. In our opinion, IP induces similar changes also in megakaryocyte line which is expressed in a strong megakaryocyte number increase accompanied by mature forms prevalence and in thrombocyte count augmentation. It is likely that IP acting on different processes (carbohydrate and fat metabolism, Leydig cell steroidogenesis, ^{75}Se-methionine incorporation, etc. — 7, 13, 16, 18) stimulates additionally the processes of proliferation and differentiation resulting in mature cellular forms increase. IP total effect can be also related with the possible suppression of thrombosthenin production which reduces thrombocyte count (4, 5). Thrombocyte reduction with 51,97 per cent and significant total increase of megakaryocytes under PR+TR treatment allows us to accept that there is megakaryocytopoiesis stimulation with suppression of thrombocyte formation. It is evident that megakaryocyte increase is mainly on the account of these of the first two stages and, partially, of the third one, while mature megakaryocytes (IV^{th} stage) are reduced. We assume that thrombocyte count diminution is due to the action of some factors. As already outlined, injected TR had a strong thrombocytopenic effect accompanied by a rapid plasma thrombocytopoietin activity increase (14). According to the fact that thrombocyte reduction in these animals

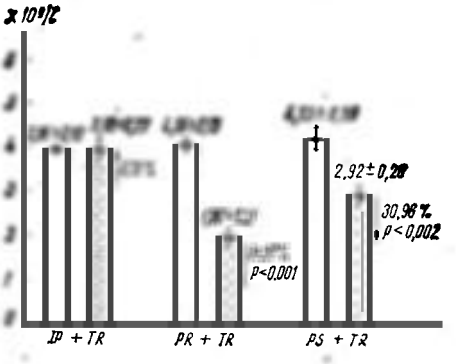

Fig. 1. Changes of thrombocyte count under the influence of IP, PR and PS in an acute thrombocytopenia after thromboplastin injection. Data are presented as x and Sx. The difference in per cent is calculated towards initial values accepted for 100 per cent

Table 1

Influence of propranolol and isoprenaline on bone marrow megakaryocytes of male rats in thrombopenic reduced acute thrombocytopenia

Injected agents	I[st]	% difference	II[nd]	% difference	III[rd]	% difference	IV[th]	% difference	Total index	% difference
	Megakaryocytes — stages									
PP+TP	1,81 ±0,28	−49 p<0,001	3 ±0,32	−3,2	5,5 ±0,35	+161 p<0,001	5,09 ±0,16	+263 p<0,001	15,36 ±0,35	+50 p<0,001
PR+TP	5,50 ±0,24	+52 p<0,001	5,30 ±0,39	+71 p<0,001	2,93 ±0,24	+39	0,91 ±0,21	−56	14,66 ±0,30	+4 p<0,001
PS+TP (control)	3,60 ±0,17	—	3,10 ±0,19	—	2,10 ±0,33	—	1,40 ±0,17	—	10,20 ±0,34	—

Data are presented as an arithmetical mean for the whole groups and their standard deviations.
Percentage difference is calculated for the values of control groups.
Sign (+) means an increase and sign (−) a decrease

is stronger expressed than this of the control ones (with 30,96 per cent) we can suppose that PR possesses also an expressed thrombocytopenic effect. This conclusion is confirmed by literature data (3, 15) and our own investigations (7). It is also probably that PR induces thrombosthenin increase. Bone marrow megakaryocyte increase is most probably a result of a fulfilled negative feed-back mechanism brought into play by thrombocyte reduction (4, 14). The changes of different stage megakaryocytes can be due to PR action. It suppresses carbohydrate metabolism, serotonin passage through thrombocyte membrane, ^{75}Se-methionine incorporation into thrombocyte precursors, erythroid mitoses, etc. (2, 7, 10, 12). It is possible that just by means of direct suppression of stem cell and megakaryocyte metabolism the processes of differentiation in them can be delayed which is then followed by a strong young megakaryocyte accumulation and thrombocyte diminution. This PR effect confirms our previous studies on animals in normal conditions (7). The aforementioned changes can be the result of disturbed thrombocytopoietin realization on the background of beta-adrenoblockade. However, the question to what extent the functional state of both stem cell and megakaryocyte beta-adrenergic receptors influences plasma thrombocytopoietin activity realization and proliferation and differentiation of these cells requires further investigations.

We can conclude that IP beta-adrenoceptor stimulation has a positive effect on thrombocyte regeneration in an acute TR-induced thrombocytopenia while beta-adrenoblockade aggravates thrombocytopenia and worsens regeneration.

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ИЗМЕНЕНИЯ ТРОМБОЦИТОПОЭЗА У КРЫС ПРИ НЕСЕЛЕКТИВНЫХ БЕТА-АДРЭНЕРГИЧЕСКИХ ВОЗДЕЙСТВИЯХ В УСЛОВИЯХ ОСТРОЙ ТРОМБОЦИТОПЕНИИ

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РЕЗЮМЕ

В результате 24-ех часового эксперимента на крысах самцах устанавливается, что применение изопреналина (неселективного бета-адрэностимулятора) + тромбопластин вызывает незначительное увеличение тромбоцитов, в то время как число мегакариоцитов возрастает на 50 % ($p<0,001$), при увеличении числа мегакариоцитов III стадии на 16 % ($p<0,001$) и IV стадии — на 263 % ($p<0,001$); наблюдается также уменьшение их числа в I стадии. Введение пропранолола (неселективного бета-адреноблокера) + тромбопластин вызывает понижение числа тромбоцитов на 51,97 % ($p<0,001$), что сопровождается увеличением числа мегакариоцитов на 44 % ($p<0,001$). Это происходит за счет молодых форм мегакариоцитов — I и II стадии и частично III стадии. У контрольной группы животных, которым вводилась физиологическая сыворотка + тромбопластин, устанавливается уменьшение числа тромбоцитов на 30,96 % ($p<0,001$).

Автором сделан вывод, что регуляция тромбоцитопоэза при острой тромбоцитопении зависит от функционального состояния бета-адрэнергических рецепторов.