INFLUENCE OF COLCHICINE ON THROMBOCYTOPOIESIS AND PLASMA THROMBOCYTOPOIETIN ACTIVITY IN RATS

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Key-words: colchicine — thrombocytopoiesis — bone-marrow megakaryocytes — plasma thrombocytopoietin.

It is known that colchicine treatment induces specific complexing together with tubulin cellular molecules (3) which inhibits their assembling into microtubules (MT) (7). This exerts a negative effect on mitotic processes and functions of numerous cellular systems (4, 7). Treatment with colchicine in strong doses results in leukocytosis, thrombocytosis and bone-marrow applasia when blood system is concerned (7). Concerning thrombocytes themselves, colchicine in MT destructing doses inhibits slightly the retraction of fibrin coagulate and the liberation of serotonin as well as the aggregation of thrombocytes (12, 14).

Recently, Handagama et al. (9) have observed thrombocytopoiesis inhibition after microtubule destruction by colchicine.

There are no data in the literature available about the influence of the changes determined by colchicine on plasma thrombocytopoietin (TP) activity. This fact as well as the controversies between some data cited insisted us to study thrombocytopoiesis, bone-marrow megakaryocytes (MKC) and plasma TP activity after 3-day low-dose colchicine treatment in rats.

Material and methods

Our observation was carried out on 69 white male rats of Wistar breed with body weight of 190—200 g and 20 white male recipient mice with body weight of 25—30 g. Animals were divided into an experimental and a control group. Experimental rats were treated with colchicine at dosis of 0.5 mg/kg b. w. daily intraperitoneally for 3 days but the control animals were administered a physiological saline. Thrombocyte count was estimated after Lisichkov's phasic-contrast method (9). 75-Selenomethionine (^{75}Se-M) incorporation into newly-formed thrombocytes (i. e. the most significant index of the state of thrombocytopoiesis) as well as plasma TP activity was assessed after Penington's method in our own modification (Negrev and Ganchev, 15). Bone-marrow megakaryocytogram was estimated after the method of Levine et al. (10). The data were processed by the method of variation analysis.

Results and discussion

Our data obtained are presented on fig. 1 and 2 and on table 1. On fig. 1-a one can see that 3-day colchicine treatment reduces significantly thrombocyte count in comparison with that of the control animals. ^{75}Se-M incorporation

into newly-formed thrombocytes is significantly inhibited, too (fig. 1-b). Bone-marrow MKC analysis reveals their diminution not only as a total number but also as number in the single stages of their development (I^{st}, II^{nd}, III^{rd}, and especially IV^{th} stage). Plasma TP activity tested on 20 recipient mice by means of two parameters (thrombocyte count and percentage of incorporated ^{75}Sa-M) under the influence of assessed plasma from animals treated with colchicine, on the one hand, and with saline, on the other hand, is statisticallyl significantly higher in colchicine-treated animals' plasma than in the contro one (fig. 2-a, b).

An additional study by using ß- and τ-lumicolchicine in 27 male rats does not show any significant differences in thrombocyte count and especially in the percentage of incorporated ^{75}Se-M in comparison with that of saline-treated controls. This enables saline application as a reliable control when experiments with colchicine in this system are concerned. Our data abou hrombocyte count reduction are in concordance with those of other authors (9) and contrary to Dustin's results (7). The considerable thrombocytopoiesis inhibition after colchicine treatment indicated mainly by means of reduced thrombocyte count, percentage of ^{75}Se-M incorporated into newly-formed thrombocytes as well as of general megakaryocytopoiesis suppression proves that both dosis and time duration of the experiment selected by us are inhibitory effective concerning thrombocytopoiesis. It stresses that MKC count decreases significantly in general and in the single stages, especially in the fourth one (by 57.14 per cent). All differences were statistically reliably at $p<0.001$. It is known that during the last stage of megakaryocytopoiesis the greatest number of MKC are liberated followed by the other stages (5). It is probably the main reason for peripheral thrombocyte count reduction and for diminution of the percentage of ^{75}Se-M incorporated in thrombocyted when colchicine-treated rats are concerned.

In regard to the increased plasma TP activity (TP is considered a basic specific thrombocytopoiesis regulator — 11) after colchicine treatment established in our study it has to be noted that the missing literature data do not allow us to perform a comparative analysis. It is considered proved that negative feed-back mechanisms participate at TP biogenesis (2). The reduction of thrombocyte count and of the percentage of ^{75}Se-M incorporated into newly-formed thrombocytes as well as of MKC number in all stages (and especially in the third and fourth one) are most probably those factors in feed-back mechanisms which induce TP biogenesis increase in our study.

In our opinion, MT destruction after colchicine treatment results probably in supperssion of proliferation as well as in inhibition of MKC differentiation. Thus it does not influence directly negatively upon structures responsible for TP biogenesis. MKC count reduction, especially in the first stage, can be due additionally to an inhibited transition of immediate MKC precursors to this cellular fund that is again a concequence of antitubular colchicine effect.

It is possible that after colchicine treatment other well-known inhibitory and side effects of colchicine participate at the inhibition of bone-marrow megakaryocytopoiesis and thrombocytopoiesis, respectively (6, 8, 13).

We can conclude that our data prove the negative influence of MT destruction after colchicine treatment on megakaryocytopoiesis and thrombocytopoiesis. However, colchicine treatment does not restrict plasma TP biogenesis but induces by means of suppressed megakaryocytopoiesis and thrombocytopoiesis, respectively, its significant increase.

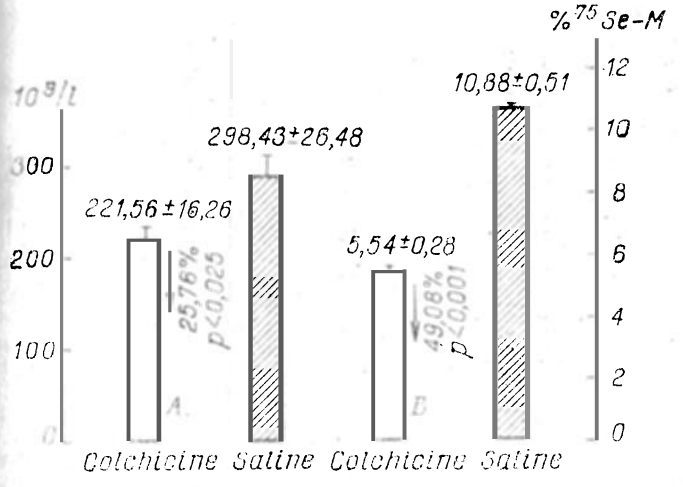

Fig. 1. Thrombocyte number and percentage of incorporated ^{75}Selenomethionine (^{75}Se-M) in newly-formed thrombocytes after colchicine treatment in rats.
A-thrombocytes
Colchicine — n = 16
PS — n = 16
B — percentage of incorporated ^{75}Se-M
Colchicine — n = 8
PS — n = 8
Data are presented as $x \pm Sx$.

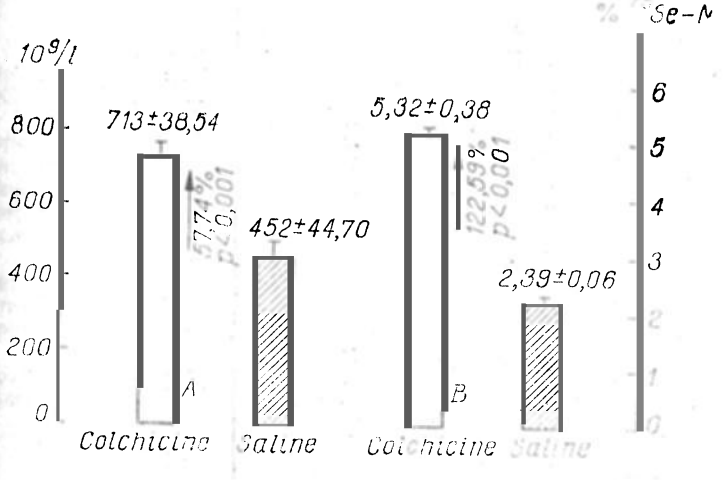

Fig. 2. Plasma thrombocytopoietin activity estimated by the changes of thrombocyte number and of percentage of incorporated ^{75}Se-M in newly-formed thrombocytes of recipient mice.
A — thrombocytes
Colchicine — treated with plasma from colchicine-injected rats; n = 8.
PS — treated with control plasma; n = 8.
B — percentage of incorporated ^{75}Se-M.
Colchicine — treated with plasma from colchicine-injected rats; n = 10.
PS — treated with control plasma; n = 10.

Table 1

Effect of 7-day Colchicine treatment (in dose of 0.5 mg/kg) on bone-marrow megakaryocytogram in rats

	Megakaryocytes — stages									
Treatment	Ist	% difference	IInd	% difference	IIIrd	% difference	IVth	% difference	Total	% difference
Colchicine = 3	3.37±0.26	—42.58 p<0.001	2.00±0	—44.75 p<0.001	1.50±0.18	—42.74 p<0.001	0.75±0.16	—57.14 p<0.001	7.62±0.37	—45.06 p<0.001
Controls = 8	5.87±0.22	—	3.62±0.26	—	2.62±0.18	—	1.75±0.16	—	13.87±0.39	—

Data are presented as $\bar{x}\pm S_{\bar{x}}$ to a total of 1000 mylokaryocytes. Sign (—) means reduction. Percentage difference is calculated towards control values. n = number of animals in the group.

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ВЛИЯНИЕ КОЛХИЦИНА НА ТРОМБОЦИТОПОЭЗ И ТРОМБОЦИТОПОЭТИНОВУЮ АКТИВНОСТЬ ПЛАЗМЫ У КРЫС

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РЕЗЮМЕ

Изучается влияние колхицина как антитубулярный агент на показатели тромбоцитопоэза и тромбоцитопоэтиновую активность плазмы у крыс. Колхицин применялся в дозе 0,5 мг/кг телесного веса в день, в течение трех дней.

Устанавливается, что колхицин статистически значимо уменьшает число тромбоцитов, процент включенного ^{75} селенометионина в новообразовавшихся тромбоцитах и клетках мегакариоцитиного ряда, в особенности в их зрелых формах. Плазменная тромбоцитопоэтиновая активность, которая определялась на мышах-реципиентах посредством изменения числа тромбоцитов и процента включенного ^{75}селенметионина, показывает сильное увеличение у крыс, которым вводился колхицин.

Авторы полагают, что микротубулярная деструкция после введения колхицина, ингибируя мегакариоцитопоэз и тромбоцитопоэз у крыс, наверное, по принципу обратной связи, стимулирует биогенез тромбоцитопоэтина — специфического гуморального регулятора тромбоцитопоэза.

Делается заключение, что микротубулярная деструкция не оказывает отрицательного воздействия на структуры и механизмы, ответственные за тромбоцитопоэтиновый биогенез при указанной дозе и длительности введения колхицина в нашем опыта.