INFLUENCE OF COLCHICINE ON THROMBOCYTOPOIESIS AND PLASMA THROMBOCYTOPOIETIN ACTIVITY IN RATS

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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 aplasia 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 thrombopoietin (TP) activity. This fact as well as the controversies between 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 (75Se-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. 75Se-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\textsuperscript{st}, II\textsuperscript{nd}, III\textsuperscript{rd}, and especially IV\textsuperscript{th} stage). Plasma TP activity tested on 20 recipient mice by means of two parameters (thrombocyte count and percentage of incorporated \textsuperscript{75}Se-M) under the influence of assessed plasma from animals treated with colchicine, on the one hand, and with saline, on the other hand, is statistically
significantly higher in colchicine-treated animals' plasma than in the con
tro one (fig. 2-a, b).

An additional study by using \(\beta\) - and \(\gamma\)-lumicolchicine in 27 male rats does not show any significant differences in thrombocyte count and especially in the percentage of incorporated \textsuperscript{75}Se-M in comparison with that of saline-tre
ated controls. This enables saline application as a reliable control when expe
riments with colchicine in this system are concerned. Our data abou
thrombocyte count reduction are in concordance with those of other au
thors (9) and contrary to Dustin's results (7). The considerable throm
bocytopoiesis inhibition after colchicine treatment indicated mainly by means
of reduced thrombocyte count, percentage of \textsuperscript{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 \textsuperscript{75}Se-M incorporated in thrombocytes when colchicine-tre
ated rats are concerned.

In regard to the increased plasma TP activity (TP is considered a basic
specific thrombocytopoiesis regulator — 11) after colchicine treatment establi
shed 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 \textsuperscript{75}Se-M incorporated into newly-formed thrombo
cytes as well as of MKC number in all stages (and especially in the third and fourth one) are most probably those factors in feedback mechanisms which in
duce TP biogenesis increase in our study.

In our opinion, MT destruction after colchicine treatment results probably in suppression 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 consequence 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 me
gakaryocytopoiesis and thrombocytopoiesis, respectively (6, 8, 13).

We can conclude that our data prove the negative influence of MT destruc
tion after colchicine treatment on megakaryocytopoiesis and thrombocytopoi
esis. However, colchicine treatment does not restrict plasma TP biogenesis but induces by means of suppressed megakaryocytopoiesis and thrombocytopoi
esis, 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
Saline — n = 16
PS — percentage of incorporated $^{75}$Se-M
Colchicine — n = 8
Saline — n = 8
Data are presented as $\bar{x} \pm Sx$.

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.
Saline — treated with control plasma; n = 8.
PS — percentage of incorporated $^{75}$Se-M.
Colchicine — treated with plasma from colchicine-injected rats; n = 10.
Saline — 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

<table>
<thead>
<tr>
<th>Megakaryocytes — stages</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>Total</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>% difference</td>
<td>% difference</td>
<td>% difference</td>
<td>% difference</td>
<td>% difference</td>
<td></td>
</tr>
<tr>
<td>Colchicine</td>
<td>3.37±0.25</td>
<td>-12.58</td>
<td>2.00±0</td>
<td>-44.75</td>
<td>1.52±0.18</td>
<td>-42.74</td>
</tr>
<tr>
<td>Controls</td>
<td>5.87±0.22</td>
<td>3.62±0.25</td>
<td>2.62±0.18</td>
<td>1.75±0.16</td>
<td>13.87±0.39</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

Data are presented as $x \pm S_x$ to a total of 1000 myelokaryocytes. Sign (—) means reduction. Percentage difference is calculated towards control values. $n$ = number of animals in the group.
ВЛИЯНИЕ КОЛХИЦИНА НА ТРОМБОЦИТОПОЭЗ И ТРОМБОЦИТОПОЭТИНОВ У КРЫС

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РЕЗЮМЕ

Изучается влияние колхицина как антитубулярный агент на показатели тромбоцитопоэза и тромбоцитопоэтиную 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 в новообразовавшихся тромбоцитах и клетках мегакариоцитарного ряда, в особенности в их зрелых формах. Плазменная тромбоцитопоэтичная активность, которая определялась на мышах-реципиентах посредством изменения числа тромбоцитов и процентов включенного в них селенметионина, показывает сильное увеличение у крыс, которым вводился колхицин.

Авторы полагают, что микротубулярная деструкция после введения колхицина, ингибируя мегакариоцитопоз и тромбоцитопоз у крыс, указывает на принципиальную связь, стимулирующую биогенез тромбоцитопоэза — специфического гуморального регулятора тромбоцитопоза.

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