PHARMACOLOGY

DECREASE OF THE ACTION OF SARCOLYSIN ON THE HEMATOPOIESIS OF RABBITS BY PRELIMINARY COLCHICINE ADMINISTRATION

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We discovered the phenomenon of interference between colchicine and some chlorethylamines in experiments which were directed at another goal. Engaged for several years in the combined chemotherapy of tumors, we attempted to increase the effectiveness of chlorethylamines with colchicine. As is known, colchicine is related to the group of mitotic poisons and, apparently, it is capable of slowing the growth of some malignant tumors by virtue of its property of blocking mitosis. The positive action of this alkaloid in the experimental chemotherapy of tumors was noted by a number of authors [1, 2,5, 6, 12, 13]. However, even from the papers mentioned, it is possible to observe that colchicine's inhibitory effect on tumor growth occurs only at toxic doses, which are considerably greater than the doses sufficient for blocking mitosis. Therefore, the application of colchicine as an anti-tumor remedy is severely limited.

On the other hand, indications exist [7, 9] that in a number of cases colchicine in comparatively small doses, apparently, has the property of increasing the sensitvity of tumor tissue to the subsequent action of x-ray therapy. In connection with the fact that colchicine, when administered in doses causing depression of tumor growth, has a considerable toxic effect, while in small doses it is capable of increasing the effectiveness of x-rays, and in connection with a certain similarity in the biological action of x-rays and chlorethylamines, we used small doses of colchicine in our experiments on combined chemotherapy, administering them before the administration of preparations of the chlorethylamine group.

One of the first preparations in this group which was tested in combination with colchicine was lymphoquine [phenyl-di-(2-chlorethyl)amine], which was investigated in detail in our laboratory by V. P. Konoplev and A. Ya. Krashilina.

However, even in the first experiments on sarcoma 45, it was found that colchicine, administered in dosages of 0.6-0.06 mg/kg 24 hours prior to the administration lymphoquine, did not increase but, on the contary, decreased the anti-timor effect of the latter. Thus, the lymphoquine preparation inhibited the growth of this tumor by 71%, while the inhibitory effect of the combination of the same dose of it with colchicine, with the same intervals between injections, was only 49-51%.

In this connection, it can be suggested that colchicine makes tumor tissue, or possibly all the proliferating tissues of the organism, less sensitive to chlorethylamines by some means. From this point of view, we thought it interesting to discover whether colchicine lowers the depressant action of chlorethylamines on hematopoiesis also.

Special experiments were set up with rabbits in order to clarify this problem.

The most active anti-tumor agent among the chlorethylamine group of preparations is a chlorethylamine derivative of the irreplaceable amino acid phenylalanine [dl-p-di (2-chlorethyl)aminophenylalanine] or sarcolysin. This preparation, according to the data of V. I. Trusheikina [4], causes the complete resorption of some strains of experimental neoplasms. At the same time, as the investigations of G. L. Zhdanov [3] showed, sarcolysin has a depressant action, as do all the chlorethylamines, on the normal proliferating tissues also, such as the lymphoid organs, bone marrow and the mucosa of the small intestine.

Naturally, we attempted to discover the possibility of removing the depressant effect of chlorethylamines on hematopoiesis by means of colchicine, using this most active preparation, especially since no decrease in the effectiveness of sarcolysin was found in experiments on the anti-tumor activity of the combination of cholchicine and sarcolysin, set up analogously with experiments with limphoquine on sarcoma 45. For this purpose, the effect of preliminary administration of colchicine on the leucopenic effect caused by a single administration of a toxid dose of sarcolysin was studied.

EXPERIMENTAL METHODS

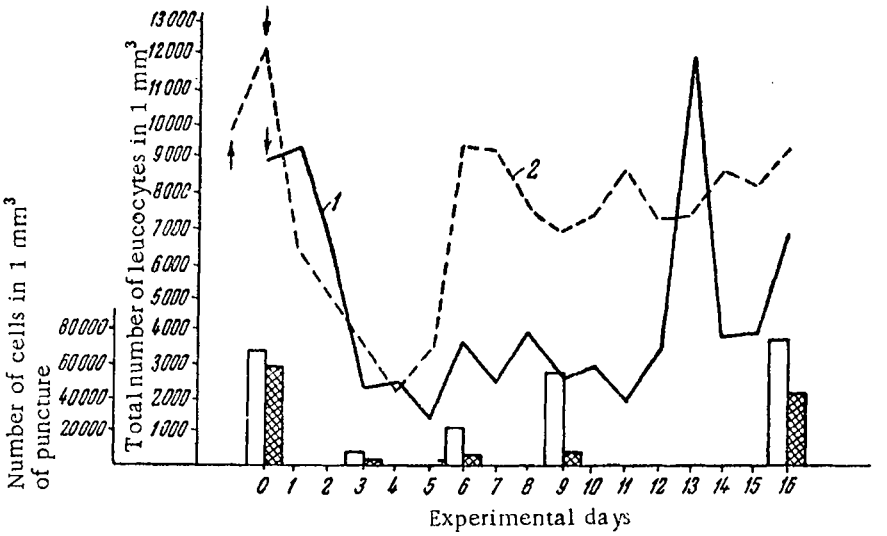
The experiments were carried out on 20 rabbits. To one group of rabbits, 2.5-3 kg in weight, sarcolysin was administered intravenously in a dosage of 10 ml/kg (which killed the majority of the animals and caused prolonged depression of hematopoiesis among all the survivors). To the other group, colchicine in a dosage of 0.6 mg/kg was administered 24 hours prior to the injection of the same dose of sarcolysin, also intravenously. Before the administration of the preparations and then every 24 hours,after, the total number of leucocytes in the peripheral blood was investigated. In addition, the number of cells in 1 mm^3 of bone marrow puncture was determined before the administration and then on the 3rd, 6th, 9th and 16th day after its administration.

EXPERIMENTAL RESULTS

Out of 9 rabbits receiving only sarcolysin, 7 animals with symptoms of severe leucopenia died on the 4th-9th day after the administration of the preparation, while only 4 rabbits died out of 9 to which colchicine had been administered first. Thus, colchicine somewhat decreased the total toxic effect of sarcolysin.

As is evident from the graph which is presented, severe leucopenia developed in rabbits of both groups, i.e., both among those receiving sarcolysin only and among those receiving sarcolysin and colchicine, on the 3-4th day – the total number of leucocytes fell to 1,500-2,000 on the average. But as soon as on the 5th day, differences are observed in the reaction of the hematopioetic system of rabbits the two groups: in rabbits which had been administered sarcolysin alone the number of leucocytes fell for two more days, then after increasing to 2,500-3,500, it remained at this level until the 13th day; the number of leucocytes among rabbits which were subjected to the preliminary injection of colchicine had begun to increase on the 5th day. Five days after the administration of sarcolysin the leucocytes number of all five surviving rabbits in the second group reached normal values. Examination of the bone marrow also indicated more rapid blood regeneration among the rabbits of this group. As is evident from the figure (the columns at the bottom), the number of cells in a 1 mm^3 puncture of the bone marrow on the 3rd day was almost equally low among both groups of animals. However, while aplasia of the bone marrow remained steady 8 days later among rabbits of the first group (the number of cells in a puncture was equal to 8,000 on the average), the bone marrow of the rabbits in the second group (colchicine plus sarcolysin) was almost completely regenerated by this time – the number of cells in a puncture reached 57,000. Only on the 16th day did the number of cells in a bone marrow puncture of rabbits receiving only sarcolysin increase to 46,500, but it was still less than that of the rabbits which had received colchicine first. It should be pointed out that an experiment, which we set up especially, with the injection of colchicine alone in a dosage of 0.6 mg/kg showed that this dosage of colchicine has no noticeable effect on the rabbits' blood.

Thus, the intereference between colchicine and sarcolysin was evident under the described experimental conditions as a decrease in the general toxic effect of sarcolysin and, even more obviously, as a weakening of the depressant effect of sarcolysin on hematopoiesis. It is typical that the protective action of colchicine consisted, not of a decrease in leucopenia during the first four days after the administration of sarcolysin, but of speeding the regeneration of the hematic system.



Changes in the total number of leucocytes in the peripheral blood of rabbits which received only sarcolysin (1) and of rabbits which received colchicine first (2).

Columns represent the number of cells in a puncture of the rabbits (cross-hatched – with administration of sarcolysin only, white – with preliminary administration of colchicine). Arrows indicate ↓ – administration of sarcolysin, ↑ – administration of colchicine.

It is possible that the reason for such interference can be explained by a difference in the action of these two agents on the cell. Colchicine acts in such a way that it blocks part of the bone marrow cells during metaphase and in this way, hinders or, at least, slows their transition into the last phase of cell division and into the postmitotic stage, while the chlorethylamines, as many authors have proved [8,10,11,14], harm the cells which are in the postmitotic phase the most, i.e., in the initial stage of interkinesis, when the synthesis of nucleoproteins is going on intensively.

On the basis of the above, it can be hypothesized that colchicine, by keeping part of the young bone marrow cells in the metaphase stage which is insensitive to chlorethylamines, decreases the number of cells which are most susceptible to sarcolysin. The young bone marrow cells which are saved in this way ensure the more rapid regeneration of hematopoiesis.

Further investigations are necessary in order to confirm this hypothesis, as well as to clarify the questions which have arisen, for example, regarding the practical possibility of using colchicine to decrease the depressant effect of sarcolysin on the hematic system.

SUMMARY

Prior injection of colchicine (24 hours before) reduces the action of phenyl-di-(2 chlorethyl)amine upon sarcoma in rats (45 animals) and the action of dl-p-di (2 chlorethyl) aminophenylalanine upon hematopoiesis in rabbits. Preventive action of colchicine consisted not in decrease of leucopenia within the first four days but in a faster regeneration of blood system. A possible mechanism of this action is discussed.

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