EFFECT OF VARIOUS SUBSTANCES ON COLCHICINE UPTAKE BY CELLS SENSITIVE AND RESISTANT TO IT

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UDC 615.277.3.033.018.1+616-008. 949.4:615.277.3

The effect of various substances on the uptake of [^{3}H]colchicine by L and L-53 cells, resistant to colcemid and colchicine was investigated. Vinblastin, to which L-53 cells possess cross resistance, increases accumulation of labeled colchicine in L cells threefold, and in L-53 cells fivefold. Substances lowering the ATP level in the cells (oligomycin, etc.) increase the uptake of colchicine by L and L-53 cells by 2-4 times. Colchicine uptake by resistant cells is increased in the presence of these substances more than in sensitive L cells. Lumicolchicine, a structural analog of colchicine which does not bind with tubulin, the protein of the microtubules, stimulates colchicine uptake approximately equally by L and L-53 cells.

KEY WORDS: Resistance; colchicine; vinblastin; permeability; uptake.

Resistance of mammalian somatic cells to drugs frequently arises as the result of changes in permeability of the plasma membrane of the cells, reducing the uptake of the cytotoxic agent by the cells [1, 3, 5, 8]. Investigation of the character of changes in the membrane which lie at the basis of drug resistance of cells to various substances is made difficult above all by the fact that transport of most of these substances within and out of the cells has received little study.

The object of this investigation was to study some aspects of colchicine transport in L cells and also in cells of the L-53 subline [2], which are resistant to colcemid and cross-resistant to colchicine and vinblastin.

EXPERIMENTAL METHOD

The conditions of culture of the L and L-53 cells were described previously [2]. Lumicolchicine was obtained by irradiating 25 mM of an aqueous solution of colchicine (from Merck, West Germany) with light from a mercury vapor lamp through a UFS-6 filter (transmission maximum $\lambda = 360$ nm). The preparation had the usual absorption spectrum for lumicolchicine in the ultraviolet region [10]. Vinblastin (from Richter, Hungary), oligomycin (from Serva, West Germany), sodium azide (from Sigma, USA), and 2,4-dinitrophenol potassium arsenate, sodium cyanide, and sodium fluoride (from Reakhim, USSR) also were used. [^{3}H]Colchicine with a specific activity of 2-3 Ci/mmole was obtained from the Radiochemical Centre, Amersham, England.

The uptake of [^{3}H]colchicine by the cells was determined by the method described previously [1] and the effect of the various substances on its uptake was studied by adding them to the cultures together with labeled colchicine. Vinblastin and lumicolchicine, dissolved in culture medium, were added for 1 h. Under the influence of substances lowering the ATP level of the cells colchicine was taken up for 20 min in physiological saline made up with phosphate buffer, pH 7.3

EXPERIMENTAL RESULTS

The study of the effect of vinblastin and lumicolchicine on the uptake of [^{3}H]colchicine showed that both substances considerably increased uptake in sensitive and resistant cells. The results of one such experiment

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 0007-4888/79/8809-1062$07.50 © 1980 Plenum Publishing Corporation

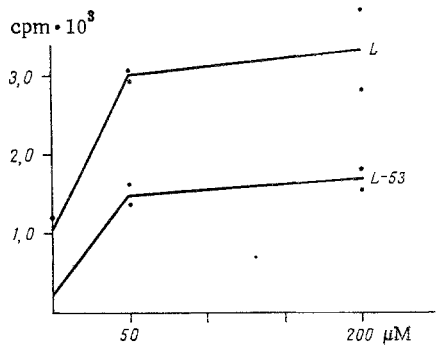

Fig. 1. Effect of vinblastin on uptake of [³H]-colchicine by L and L-53 cells. Here and in other figures, each point corresponds to one flask. Abscissa, vinblastin concentration (in μM); ordinate, here and in Figs. 2 and 3, quantity of radioactivity in cells (in cpm • 10^3).

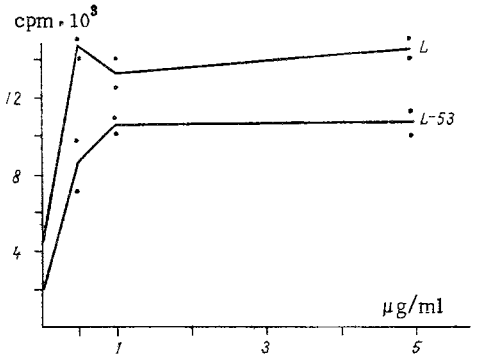

Fig. 2. Effect of oligomycin on uptake of [³H]colchicine by L and L-53 cells. Abscissa, oligomycin concentration (in μg/ml).

are illustrated in Fig. 1. In the presence of vinblastin, the uptake increased relatively more rapidly in L-53 cells (about eightfold), as a result of which the amount of colchicine which entered the resistant cells was greater than the amount of the mitostatic taken up by untreated sensitive cells. Lumicolchicine, an isomer of colchicine which does not bind with tubulin, approximately doubled the uptake of colchicine into L and L-53 cells.

Tests were carried out to discover whether vinblastin increases the membrane permeability nonspecifically, by increasing the entrance of many different substances into the cell. The results showed that even high concentrations of the alkaloid (10 and 50 μM) not only did not increase, but actually reduced by 15-20% the uptake of [³H]2-deoxy-D-glucose by L and L-53 cells.

The effect of metabolic inhibitors lowering the ATP level in the cells on the uptake of [³H]colchicine was studied in five experiments. Uncouplers of respiration and oxidative phosphorylation (2,4-dinitrophenol and trichlorocarbonylcyanide phenylhydrazone), and also oligomycin, sodium cyanide, sodium azide, and potassium arsenate, were studied. All these substances increased the uptake of labeled colchicine in L-53 cells by 2-4 times. Sodium azide and oligomycin had the strongest action. The results of one experiment to study the effect of oligomycin on L and L-53 cells are shown in Fig. 2.

If the increase in the uptake of colchicine under the influence of metabolic inhibitors is specific and is due to the fall in the ATP concentration in the cells, an increase in the ATP level (on account of glycolysis, for example) ought to abolish the effect of the inhibitor. In fact, 10-50 mM glucose abolished the increase in the colchicine uptake by the cells caused by sodium azide (Fig. 3).

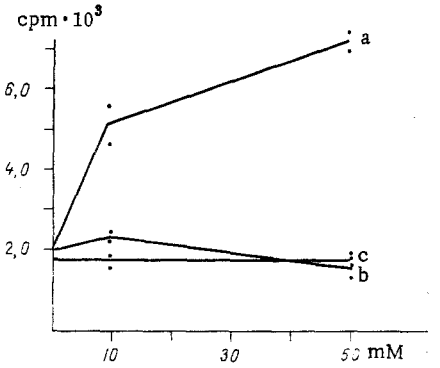

Fig. 3. Effect of glucose on uptake of [^{3}H]colchicine by L cells in the presence of sodium azide. a, b, c) Glucose concentrations 0, 10, and 50 mM, respectively. Abscissa, sodium azide concentration (in mM).

The investigation showed that substances lowering the ATP concentration in the cells, notably oligomycin, and also vinblastin and lumicolchicine, increase the uptake of [^{3}H]colchicine by L and L-53 cells. The simplest explanation of the increase in uptake under the influence of inhibitors of cell energy metabolism, in the writers' view, is that colchicine enters the cell by passive transport (possibly by diffusion) and is eliminated from the cell by means of an active carrier. Inhibition of the carrier in the absence of ATP leads to a decrease in the elimination of colchicine and, ultimately, to an increase in the accumulation of colchicine in the cells. Similar results have been obtained by CHO cells [4, 7]. However, the authors cited explain them by the presence of active obstruction to the inflow of colchicine into the cells. In the present writers' view, there are no grounds for postulating such an unusual mechanism. The increase in colchicine accumulation in the presence of vinblastin and lumicolchicine could be explained by various causes. First, it might be supposed that all these substances stimulate diffusion of colchicine inside the cells; second, these preparations could inhibit the active discharge of colchicine from the cells; and third, these substances could affect the binding of colchicine with tubulin. This last suggestion can be ruled out, for it has been shown that neither vinblastin nor lumicolchicine has any effect on this process [11, 12].

It seems a more likely explanation that vinblastin and lumicolchicine inhibit colchicine transport from the cells into the medium specifically or nonspecifically. Since cells resistant to colchicine are usually cross-resistant to vinblastin [9], there may perhaps be common pathways for the transport of both these substances through the membrane. If there is in fact a common carrier for them, which transports the substances through the membrane from the cell into the medium, the increase in colchicine accumulation in the presence of vinblastin could explained by competitive inhibition of the carrier.

By studying the character of colchicine transport through the cell membrane, it is possible to come nearer to the understanding of the mechanism of cell resistance due to changes in membrane permeability. Clearly these changes can be expressed either as difficulty in transport (most likely by diffusion) of various substances inside resistant cells or by an increase in the outward transport of colchicine from such cells. The results of the present investigation suggests that the second alternative is most probable.

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SURFACE ORIGIN OF ERYTHROCYTIC CHALONE in vitro IN EXPERIMENTAL POLYCYTHEMIA

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UDC 616.155.191.5-092.9-07:616.419-018.1-092.4-076.5

Tests on surviving cultures of bone marrow cells from rats with experimental polycythemia showed that the chalone activity of erythrocytic chalone is considerably reduced in the presence of phytohemagglutinin (PHA). The chalone depresses the agglutinating activity of PHA on bone marrow cells. Adsorption of the chalone preparation on immobilized PHA leads to disappearance of electrophoretically recorded PAS-positive bands from it and a sharp decrease in the intensity of the PAS-negative band. Experiments with preliminary incubation on rat erythrocytes before isolation of the chalone preparation suggest that in the course of its isolation two polypeptides are selectively liberated into the medium, one of them being PAS-positive. It is suggested that erythrocyte surface membrane proteins are components of the chalone. The possible mechanism of liberation of chalones from the surface of the cells and the particular features of their action on cells are discussed.

KEY WORDS: erythrocytic chalone; bone marrow cells.

Much attention is currently being paid to chalones, endogenous regulators of cell division [4, 5, 12]. The suggestion that they may be components of the surface of normal cells appears helpful toward the understanding both of the character of their liberation and the mechanism of their action.

The results of an investigation of erythrocytic chalone are described below.

EXPERIMENTAL METHOD

A surviving culture of albino rat bone marrow cells [2] was used. The "polycythemia in vitro" model [1] was used to obtain erythrocytic chalone (EC).

Before incubation the erythrocytes were washed 3 times with physiological saline in order to rule out any possible contamination by serum proteins.

As proof of the membrane origin of EC their ability to bind with phytohemagglutinin (PHA) was used. It was assumed that if EC contains PHA receptors, its inhibitory activity toward bone marrow cells ought to be reduced in the presence of lectin. Conversely, on the addition of N-acetyl-D-galactosamine (NAGA) to the PHA + EC complex the inhibitory effect ought to increase because of the blocking of PHA by specific hapten.

To test these hypotheses the following series of experiments were carried out: 1) control – intact cells, 2) cells with the addition of NAGA, 3) cells with PHA, 4) cells with EC, 5) cells with PHA + EC, 6) cells with PHA + EC + NAGA. The PHA concentration in the experiments varied from 10 to 400 μg/ml medium. The concentrations of the other substances are given in the caption to Fig. 2. Colchicine was added to the Eagle's medium with the cells 15 min before incubation began. The bone marrow cells were fixed 2 h later and stained by the Romanovsky–Giemsa method. To estimate the mitotic activity of the erythron [1] the stathmokinetic index among the proliferating cells was calculated. The numerical results were subjected to statistical analysis by Student's test. When high concentrations of PHA (400 μg/ml) were used the character of action of the various factors was judged by the "delay of agglutination" criterion, which was determined by counting the number of agglutinates formed in 20 fields of vision of the microscope (magnification 900×).

Department of Pathological Physiology, Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 345-348, September, 1979. Original article submitted January 19, 1979.

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