LITERATURE CITED

1. G. I. Bezin, B. B. Moroz, O. O. Romashko, and R. V. Petrov, Byull. Éksp. Biol. Med., No. 10, 494 (1978).
2. G. P. Burgman, E. P. Yurishchev, V. S. Snigirev, and Kh. B. Aide, Problems in the Pathogenesis and Treatment of Head Injury [in Russian], Moscow (1978), p. 60.
3. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, Stress and the Blood System [in Russian], Moscow (1983).
4. Yu. V. Zotov and V. V. Shchedrenok, Surgery of Traumatic Intracranial Hematomas and Foci of Pulping of the Brain [in Russian], Leningrad (1984).
5. E. A. Korneva, Vestn. Akad. Med. Nauk SSSR, No. 3, 63 (1985).
6. E. A. Korneva and V. A. Shekoyan, Regulation of Protective Functions of the Body [in Russian], Leningrad (1982).
7. V. K. Kulagin and V. I. Aleksandrov, Byull. Éksp. Biol. Med., No. 5, 45 (1982).
8. V. A. Lesnikov and E. N. Isaeva, Abstracts of Proceedings of the 4th All-Union Symposium on Regulation of Immune Homeostasis [in Russian], Leningrad (1986), p. 12.
9. A. Ya. Lyubina, L. P. Il'icheva, T. V. Katasonova, and S. A. Perosova, Clinical Laboratory Investigations [in Russian], Moscow (1984).
10. A. E. Pereverzev, Hematopoietic Colony-Forming Cells and Physical Stress Factors [in Russian], Leningrad (1986).
11. R. V. Petrov and R. M. Khaitov, Radiobiologiya, No. 1, 69 (1972).
12. V. M. Ugryumov, Severe Closed Head Injury [in Russian], Leningrad (1974).
13. V. P. Shakhov, A. M. Dygai, A. V. Mikhlenko, et al., Patol. Fiziol., No. 5, 24 (1986).
14. E. V. Shmidt and I. V. Gannushkina, Vestn. Akad. Med. Nauk SSSR, No. 12, 47 (1984).

CHANGES IN AMINO ACID METABOLISM IN DJUNGARIAN HAMSTER FIBROBLAST CELL CULTURE BECOMING RESISTANT TO COLCHICINE

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UDC 616-006.04-018.1-02:615.277.3]-07:616-006-008.934.66

KEY WORDS: colchicine; fibroblasts; amino acids.

Multiple drug resistance (MDR) is one of the best studied forms of resistance of tumor cells to cytostatics. The MDR phenomenon is characterized by reduced accumulation of the cytostatic in tumor cells, its active transport from the cell, the presence of P glycoprotein on the cytoplasmic membrane of resistant cells, and abolition of resistance by Ca^{++} antagonists [1, 2]. However, despite our greater knowledge of the mechanisms of MDR, in practice its overcoming is attended by unsolved problems. The use of Ca^{++} antagonists leads to selection of cells with a higher level of resistance [3, 4]. The use of antibodies against P glycoprotein of resistant cells is not accompanied by the destruction of this cell population, for the number of antigenic determinants on the cell falls below the threshold level [5]. Attempts to find differences in metabolism of sensitive and resistant cells have proved unsuccessful [6].

In the present investigation an attempt was made to find differences only in consumption of low-molecular-weight sources of nitrogen (amino acids) from the incubation medium. This approach is preferable, for analysis is restricted to a small class of compounds that are essential for life and activity of the cells.

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All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Blokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 10, pp. 484-485, October, 1989. Original article submitted November 18, 1988.

 0007-4888/89/0010-1488 $12.50

TABLE 1. Concentrations of Amino Acids in Medium after Incubation of Cells (in μmoles/ml)

Amino acids	DM	5.1 cells with colchicine	5.1 cells without colchicine	Control
Aspartic acid	0,026±0,002	0,006±0,002	0,025±0,004	ABS
Threonine	0,15±0,01	0,17±0,02	0,16±0,01	0,23±0,02
Glutamic acid	0,19±0,01	0,28±0,03	0,24±0,03	0,21±0,01
Glycine	0,036±0,002	0,027±0,007	0,02±0,002	ABS
Alanine	0,19±0,007	0,11±0,02	0,19±0,01	ABS
Valine	0,12±0,01	0,17±0,02	0,15±0,01	0,16±0,02
Methionine	0,02±0,001	0,03±0,002	0,03±0,001	0,03±0,002
Isoleucine	0,08±0,01	0,13±0,01	0,09±0,01	0,15±0,01
Leucine	0,1±0,01	0,16±0,02	0,13±0,01	0,16±0,01
Tyrosine	0,06±0,01	0,08±0,01	0,07±0,01	0,08±0,01
Phenylalanine	0,06±0,01	0,08±0,01	0,07±0,01	0,08±0,01
Lysine	0,2±0,03	0,26±0,04	0,24±0,03	0,2±0,05
Arginine	ABS	0,18±0,02	0,15±0,02	0,17±0,03

Legend. Cells were incubated under conditions described previously [7]. DM) Strain of Djungarian hamster fibroblasts transformed by SV-40 virus; 5.1) strain of Djungarian hamster fibroblasts obtained from DM cells by induction of resistance to colchicine: 5.1 with colchicine) 5.1 cells were incubated in medium in presence of colchicine (5 μg/ml); 5.1 without colchicine) cells resistant to the cytostatic, incubated without colchicine; control) incubation medium without cells; ABS) amino acid absent in incubation medium.

EXPERIMENTAL METHOD

Experiments were carried out on Djungarian hamster fibroblasts sensitive and resistant to the action of colchicine [7]. Amino acids were determined on a KLA-3B amino acid analyzer ("Hitachi") by the method described previously [8].

EXPERIMENTAL RESULTS

The concentration of amino acids in the medium after incubation of sensitive cells (strain DM), of resistant cells with the addition of colchicine (strain 5.1 with colchicine), and resistant cells without addition of the cytostatic to the incubation medium (5.1 without colchicine), and control samples of medium alone, is given in Table 1. The number of cells in the different groups did not differ significantly. There was a significant difference in the aspartic acid concentration in the medium used to incubate the DM cells and 5.1 cells with colchicine. However, this difference in the aspartic acid concentration disappeared if the cytostatic was removed from the incubation medium of the resistant cells. The glutamic acid and glutamine concentrations in the incubation medium of the resistant cells with colchicine was higher (in this case these two compounds were not separated). Concentrations of methionine, arginine, valine, isoleucine, and leucine also were higher than in the incubation medium of the sensitive cells. Concentrations of these amino acids after incubation of the resistant cells were the same as concentrations of the amino acids in the control specimens, in which the cells were not incubated.

Thus resistant cells, on incubation with the cytostatic, do not utilize low-molecular-weight sources of nitrogen from the incubation medium. Another interesting feature must be noted: after incubation with both sensitive and resistant cells the incubation medium became richer in amino acids. Amino acids such as aspartic acid, glycine, and alanine "appeared" in it. Even assuming that "dynamic equilibrium" was observed in the incubation medium, i.e., amino acids could both enter the cell and leave it, or become converted from one to another, this enrichment of the medium can be explained on the grounds that the cells utilize another source of nitrogen in the medium, namely proteins. It can be stated on the basis of the number of cells in the incubation medium and the quantity of

amino acids and ammonia excreted that not more than 5% of protein was utilized from the incubation medium. This low utilization of proteins explains why this effect of utilization of macromolecules has not been studied in connection with the MDR phenomenon. Utilization of protein by tumor and normal cells has been known for a long time [4]. However, the results given above enable it to be postulated for the first time that the ratio of utilization of high- and low-molecular-weight compounds by the cell may play a leading role in the development of resistance to the exclusively low-molecular-weight antitumor compounds used at the present time.

The phenomenon of preferential assimilation of macromolecules by resistant cells, discovered in this investigation, requires further study. Probably at low levels of resistance it is reversible, and resistant cells, on incubation without the cytostatic, may assimilate certain amino acids from the medium. As regards the concentration of amino acids in the medium after incubation of the cells this group occupies an intermediate position between DM cells and 5.1 cells with colchicine.

Incidentally, in the present investigation differences in metabolism of cells sensitive and resistant to the action of the cytostatic were found for the first time. Under these circumstances, it can be determined from the concentration of amino acids in the medium which strain of cells (sensitive, resistant with cytostatic, or resistant without cytostatic) was incubated. The technique suggested above may prove useful for the study of other strains of resistant and sensitive cells.

LITERATURE CITED

1. A. V. Gudkov and B. P. Kopnin, Genetika, 19, No. 7, 1045 (1983).
2. A. R. Kushelev, Abstracts of Proceedings of the First All-Union Conference on Chromatography [in Russian], Moscow (1983), pp. 119-120.
3. B. A. Chabner and M. M. Gottesman, J. Natl. Cancer Inst., 80, No. 6, 391 (1988).
4. G. C. Easty, M. M. Yarnell, and R. D. Andrews, Br. J. Cancer, 18, No. 2, 354 (1964).
5. F. Formelli, R. Supino. L. Cleris, and M. Mariani, Br. J. Cancer, 57, No. 4, 343 (1988).
6. J. K. Horton, P. J. Houghton, and J. A. Houghton, Cancer Res., 47, No. 23, 6288 (1987).
7. M. Inaba and E. Maruyama, Cancer Res., 48, No. 8, 2064 (1988).
8. M. Pannelerselvam. R. Bredehorst, and C. W. Vogel, Cancer Res., 47, No. 17, 4601 (1987).
9. T. Tsuro, Jpn. J. Cancer Res. (Gann), 79, No. 3, 285 (1988).