ONCOLOGY

Transport of Nitrullin, a New Nitrosoalkylurea Derivative, Into Tumor and Normal Cells

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The interaction between the antitumor drug nitrullin and the system providing the transport of L-lysine into P388 leukemic cells and murine enterocytes is studied. Two types of lysine carriers with low and high affinity for the substrate are identified. Nitrullin competitively inhibits the transport of ^{3}H-lysine and shows the same affinity for both carriers. It is similar to that of lysine for the low-affinity carrier and is 80-fold lower than lysine affinity for the high-affinity carrier. Kinetic characteristics of the low-affinity transport of ^{3}H-lysine and K_i of nitrullin are similar to those obtained at a reciprocal substrate-inhibitor ratio. Nitrullin does not inhibit active transport of ^{3}H-lysine into enterocytes against the background of considerable (70%) passive diffusion.

Key Words: *nitrullin; lysine; cell transport; competitive inhibition; leukemic cells; enterocytes*

The influx of antitumor alkylating agents (for example, alkylating metabolites) into cells is provided by the transport systems for naturally occurring carriers [2,6,10]. Alkylating derivatives of nitrosourea enter the cell by passive diffusion [5]. This is true for the antitumor agent chlorosotocin, a glucose derivative of nitrosourea [9]. In this study we explored the possibility of active transport of nitrullin (NR), a homocitullin derivative of nitrosourea, into leukemic cells (LC) and normal murine enterocytes via the carrier for the naturally occurring homolog L-lysine.

MATERIALS AND METHODS

Male BDF/1 mice weighing 18-20 g were used.

Nitrullin was synthesized at the Institute of Chemistry, Ural Scientific Center, Russian Academy of Sciences. ^{14}C-NR (specific radioactivity 2.5 GBq/mg) was prepared by Dr. A. S. Singin at the Oncology Research Center, Russian Academy of Medical Sciences.

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Cells from the intestinal mucosa of intact mice were isolated by the method [7] with modifications [4]. P388 cells were inoculated in a dose of 10^6 cells/mouse. Leukemic cells were isolated on day 6 after inoculation, washed 3 times in Earle's balanced salt solution with 8 mM glucose, pH 7.4), and suspended in the same medium.

The rate of the transport of L-4,5-[^{3}H]lysine (specific radioactivity 8.9 GBq/mmol, Chemopol) into cells (final concentration $5\text{-}10\times10^6$ cells/ml) was measured during a 5-min period at 37°C and 0-2°C in incubation medium containing varied amounts of lysine (10^{-6}-3×10^{-3} M). The reaction was terminated by adding an excess of the incubation medium with a simultaneous cooling. The cells were washed twice with the same medium and lyzed in 0.3 N KOH for 18 h at 37°C. Radioactivity was measured in a Rack-beta-1219 counter using a ZhS-8 scintillator cocktail (Russia). The rate of active lysine transport (nmol/min$\times10^9$ cells) was calculated as the difference between lysine accumulation at 37°C and 0-2°C (passive diffusion).

The mechanism of NR transport into tumor cells was studied by the inhibition analysis [1]. Kinetic

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TABLE 1. Kinetic Parameters of ^{3}H-Lysine and Nitrullin (NR) Transport Into P388 Cells (*M±m*)

Transport system	V_{max} ^{3}H-lysine, nmol/min×10^9 cells	K_m ^{3}H-lysine, mM	K_i NR, mM
High-affinity	10.6±1.6	0.026±0.004	2.0±0.1
Low-affinity	356±40	1.5±0.07	2.5±0.6

characteristics (K_m, K_i, V_{max}) were calculated from the Michaelis—Menten equation using Lineweaver—Burke double reciprocal plots [1,3]. The transport of NR into tumor cells was studied using two protocols: 1) ^{3}H-lysine is the substrate and unlabeled NR is the inhibitor; 2) ^{14}C-NR is the substrate and unlabeled L-lysine is the inhibitor. The results of 5-6 determinations were analyzed by Student's *t* test.

RESULTS

Effect of NR on the transport of ^{3}H-lysine in P388 cells. In the concentration ranges 10^{-6}-2×10^{-5} M and 10^{-4}-3×10^{-3} M, the transport of ^{3}H-lysine into LC obeys the kinetics of enzyme reactions for high- and low-affinity transport systems (Table 1, Fig. 1). For the high-affinity system, K_m is 60-fold and V_{max} is 30-fold lower, than for the low-affinity system. Consequently, lysine enters the cell predominantly via the low-affinity system. Generally, the cell contains only one system of lysine transport [8]. Our findings agrees with the observation that lysin is transported into human renal medulla cells by two carriers with K_{m1}=0.02-0.07 mM and K_{m2}=3.4-4 mM [11]. K_m for lysine transport into P388 cells is similar to K_m for the arginine transport into vesicles formed by membranes prepared from rat intestinal mucosa cells [8], which corroborates the hypothesis that diamine acids are transported by the same system.

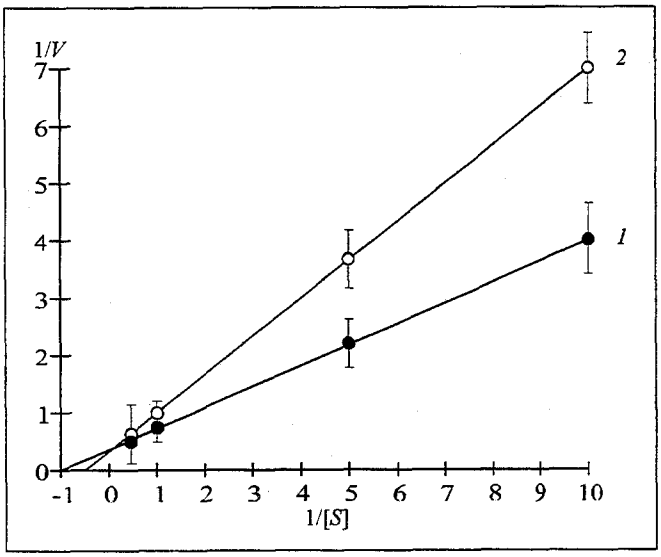

Fig. 1. Inhibition of ^{3}H-lysine transport into P388 cells by nitrullin. *1*) control; *2*) transport of ^{3}H-lysine in the presence of 10^{-3} M nitrullin. Here and in Figs. 2 and 3: *V*: transport rate, nmol/min×10^9 cells; [*S*] concentration of substrate, mM.

Passive diffusion of ^{3}H-lysine is low: 14-16% of the total influx (Fig. 2).

Nitrullin competitively inhibits ^{3}H-lysine transport into LC. Both lysine carriers have the same K_i: 2.0-2.5 mM; however, the affinity of NR for the high-affinity carrier is 80-fold lower than that of the natural substrate (lysine), while for the low-affinity carrier the values are similar. It can be suggested that NR effectively competes with lysine for the low-affinity carrier, which serves as the main means of NR transport into a tumor cell.

Effect of L-lysine on ^{14}C-NR transport into P388 cells. The interaction between NR and lysine carriers was studied at a reciprocal substrate-inhibitor ratio. ^{14}C-NR served as a substrate and L-lysine as an inhibitor. The influx of ^{14}C-NR into cells was studied in the concentration range of 5×10^{-4}-10^{-3} M, which is optimal for the low-affinity L-lysine transport system.

The maximum rate of ^{14}C-NR transport and affinity (V_{max}=366±9 nmol/min×10^9 cells, K_m=0.64 mM) were similar to those for ^{3}H-lysine (Table 1, the low-affinity carrier). Lysine competitively inhibited the transport of ^{14}C-NR into the cells (Fig. 3). The inhibition constants for lysine (2.4±0.04 mM) and NR are similar (Table 1). Thus, similar results were obtained in experiments with two radiolabeled substrates and their inhibitors. This confirms a strong competition between NR with lysine for the low-affinity carrier.

The effect of NR on ^{3}H-lysine transport into normal enterocytes of BDF/1 mice. Active transport of lysine into enterocytes is provided by two systems (Table 2). In the enterocytes, the affinity of a high-affinity carrier for the naturally occurring substrate (lysine)

TABLE 2. Kinetic Parameters of ^{3}H-Lysine Transport Into Enterocytes of BDF/1 Mice (*M±m*)

Transport system	V_{max}, nmol/min×10^9 cells	K_m, mM
High-affinity	0.29±0.05	0.006±0.001
Low-affinity	36±5	1.4±0.06

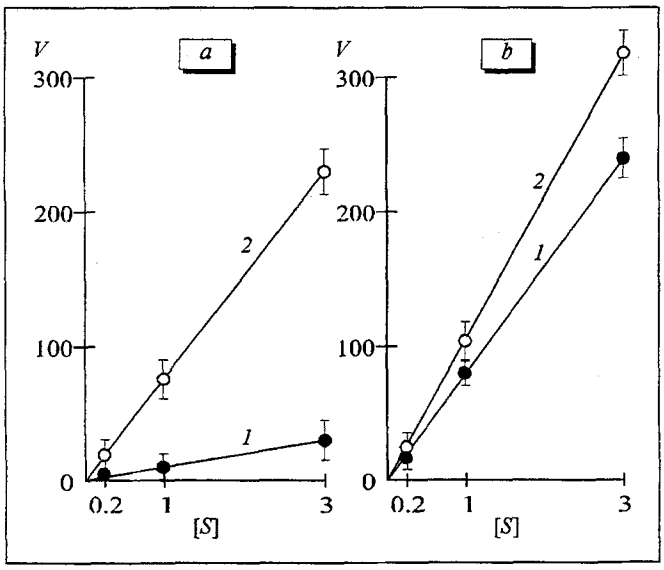

Fig. 2. Transport of ^{3}H-lysine in P388 cells (*a*) and BDF/1 mouse enterocytes (*b*). *1*) passive diffusion (0-2°C); *2*) total transport (37°C).

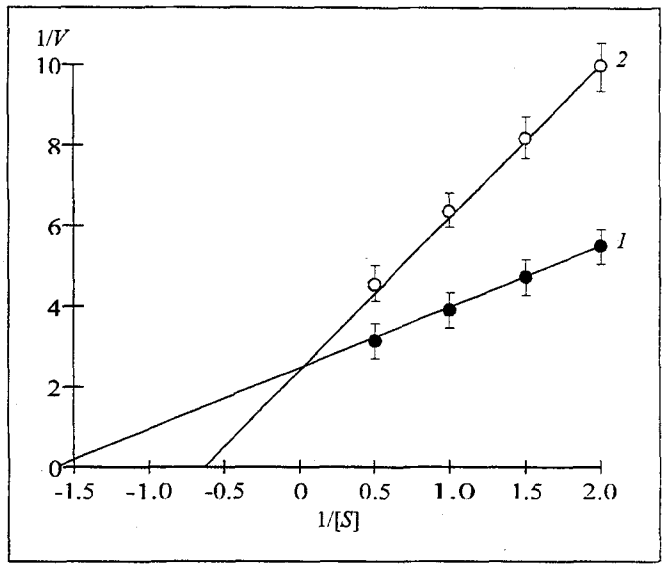

Fig. 3. Inhibition of ^{14}C-nitrullin transport into P388 cells by L-lysine. *1*) control; *2*) transport of ^{14}C-nitrullin in the presence of L-lysine (10^{-3} M).

is higher than that in LC, while the affinities of low-affinity carriers are similar (Tables 1 and 2).

For both carriers, V_{max} of active ^{3}H-lysine transport into enterocytes is 10 times as high as that of lysine transport into LC (Table 1). The total rates of ^{3}H-lysine transport into enterocytes and LC are similar; however, the rate of passive lysine diffusion into enterocytes is 5-fold higher than that into tumor cells, accounting to 70±5% of the total lysine influx (Fig. 3).

Inhibition of ^{3}H-lysine transport into enterocytes by NR (10^{-3} M) was not observed against the background of essentially strong diffusion which may shunt changes in the low rates of the active lysine transport (0.29-36 nmol/min×10^9 cells). An increase in the concentration of NR (2×10^{-3} M) stimulated lysine transport by 10-20% probably due to disruption of the enterocyte plasma membrane. From this finding it can be concluded that NR enters enterocytes by passive diffusion which is the major means of L-lysine transport into these cells.

REFERENCES

1. A. K. Belousova, *Biochemical Approaches to the Chemotherapy of Tumors* [in Russian], Leningrad (1965).
2. G. A. Gorneva, T. S. Shulepova, S. G. Zakhariev, *et al.*, *Eksp. Onkol.*, **13**, No. 4, 41-44 (1991).
3. M. Dixon and E. Webb, *Enzymes* [Russian translation from English], Moscow (1966).
4. A. G. Melik'yants, *Advances in Science and Technology. Series Biophysics* [in Russian], Vol. 16, Moscow (1985).
5. A. Begleiter, H. G. P. Lam, and G. I. Goldenberg, *Cancer Res.*, **37**, No. 4, 1022-1027 (1977).
6. A. K. Belousova, T. I. Solntseva, and T. S. Shulepova, *Sov. Med. Rev. Sect. F. Oncology Rev.*, **4**, Pt. 3 (1992).
7. Y. H. Carter, J. Carter, J. Nussbaum, and A. Eichholz, *J. Cell. Physiol.*, **111**, No. 1, 55-67 (1982).
8. J. W. Foreman, S.-M. Hwang, M. M. Medou, and S. Segal, *Biochim. Biophys. Acta*, **862**, 127-133 (1986).
9. G. I. Goldenberg and A. A. Begleiter, *Antitumor Drug Resistance*, B. W. Fox and M. Fox (Eds.), Vol. 72, Berlin - Haidelberg - New York - Tokyo (1984), pp. 241-298.
10. W. R. Redwood and M. Colvin, *Cancer Res.*, **40**, No. 4, 1144-1149 (1980).
11. L. E. Rosenberg, J. Albrecht, and S. Segal, *Science*, **155**, 1426-1428 (1978).