Possibility of Contribution of Nucleoside Transport Systems to Pirarubicin Uptake by HL60 Cells but Not Mononuclear Cells

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Previously, we reported that pirarubicin (THP), an anthracycline, was taken up, at least in part, by both human leukemic HL60 cells and mononuclear cells (MNCs) via a carrier-mediated system. In this study, the possibility of a contribution of nucleoside transport systems to the uptake of THP by HL60 cells and MNCs was investigated. The experiments were performed after both types of cells had been pretreated with a metabolic inhibitor, 2, 4-dinitrophenol, to deplete cellular ATP. In HL60 cells, THP uptake was increased and decreased significantly by treatment with equilibrative nucleoside transport inhibitors, nitrobenzylthioinosine (NBMPR), nitrobenzylthio-guanosine and dilazep, in the presence and absence, respectively, of an inwardly directed Na^+-gradient. THP uptake by HL60 cells showed an overshoot in the presence of the gradient, and was decreased by treatment of the cells with monensin, indicating that the uptake partially depended on the Na^+-gradient. In HL60 cells in which equilibrative nucleoside transport was inhibited by NBMPR, THP uptake in the presence of the gradient was inhibited by Na^+-dependent concentrative nucleoside transport inhibitors, but no inhibition was observed in the absence of the gradient. In MNCs, conversely, there was no effect of any equilibrative nucleoside transport inhibitor or the Na^+-gradient on THP uptake. These results suggested that THP was taken up, at least in part, via both equilibrative and concentrative nucleoside transport systems in HL60 cells, but not in MNCs.

Key words: Pirarubicin — Uptake mechanism — Nucleoside transport system — Human leukemia HL60 cell — Human mononuclear cell

We have been studying the transport mechanisms for anthracyclines in human leukemia HL60 cells and human mononuclear cells (MNCs), as models of tumor and normal cells, respectively, in order to establish a strategy for selectively delivering anthracyclines to leukemia cells based upon the differences in their transport mechanisms. In our previous studies, it was indicated that pirarubicin (THP) was taken up by HL60 cells and MNCs via a carrier-mediated transport system(s), although what kinds of transporters are involved, and whether or not the system is common to the two types of cells, remained unclear.[1–3]

There have been many reports on the nucleoside transport systems in various animal cells.[4–6] It appears that the nucleoside transport systems comprise both equilibrative (facilitated diffusion) transport systems and concentrative Na^+-cotransport ones. The former can be classified into two subtypes, nitrobenzylthioinosine (NBMPR)-sensitive (*es*) (NBMPR $K_i < 1$ n*M*) and -insensitive (*ei*) (NBMPR $K_i > 1$ μM) systems. At least five classes of concentrative nucleoside transporters have been identified: N1/*cif* transporters are concentrative, NBMPR-insensitive and selective for purines (formycin B), N2/*cit* transporters are concentrative, NBMPR-insensitive and selective for pyri-midines (thymidine), N2/*cit*-like (N4) transporters recognize guanosine as a permeant, N3/*cib* transporters are concentrative, NBMPR-insensitive and exhibit a broad selectivity toward purines and pyrimidines, and *cs* transporters are concentrative and sensitive to inhibition by low nanomolar concentrations of NBMPR.[4–6] The expression of these transporters is not uniform among cell types.[4, 7–9] Lee and co-workers indicated that both equilibrative and Na^+-dependent nucleoside transport systems are expressed in undifferentiated HL60 cells, but only low levels of Na^+-dependent uridine transport exist in the cells.[10–12] On the other hand, human monocytes/macrophages have been reported to possess equilibrative nucleoside transporters.[4, 5] However, the expression of Na^+-dependent nucleoside transport systems in such cells is controversial. One report indicated that adenosine was transported by the Na^+-dependent nucleoside transport systems,[13] whereas other workers, using formycin B as a permeant, showed that the transport was independent of the presence of extracellular sodium.[6, 14]

Recently, it was reported that that dipyridamole, a representative inhibitor of equilibrative nucleoside transport, increases cellular doxorubicin accumulation, and shows potentiated antitumor activity in HeLa, Sarcoma 180 and human ovarian carcinoma 2008 cells.[15, 16] Furthermore, Su

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et al.[17] reported that C3368-A, a nucleoside transport inhibitor, like dipyridamole, inhibits the efflux of antitumor agents, including anthracyclines, and increases the cellular accumulation of drugs and their antitumor effects. These findings suggest the possibility that anthracyclines might be substrates for the nucleoside transport system(s).

In this study, therefore, we investigated the possibility of a contribution of the nucleoside transport system(s) to THP uptake by HL60 cells and MNCs, using various inhibitors, with or without an inwardly directed Na⁺-gradient.

MATERIALS AND METHODS

Chemicals Pure THP and pirarubicinol (THP-OH) were provided by Meiji Seika Kaisha, Ltd. (Tokyo). NBMPR, nitrobenzylthioguanosine (NBTGR), dilazep, formycin B and FCCP (carbonylcyanide *p*-trifluoromethoxyphenylhydrazone) were purchased from Sigma Chemical Co. (St. Louis, MO), and 2, 4-dinitrophenol (DNP), adenosine, uridine, thymidine, cytosine arabinoside (Ara-C), azidothymidine (AZT) and monensin were from Wako Pure Chemical Ind. (Osaka). All other reagents were obtained commercially or were of analytical grade requiring no further purification. Deionized double-distilled water was used throughout the experiments.

Cell line and culture conditions HL60 cells were provided by Dr. R. C. Gallo (NIH, Bethesda, MD). This line was maintained in RPMI1640 medium (GIBCO Laboratories, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum, 10 μg/ml kanamycin sulfate and 25 mM N-2-hydroxyethylpiperazine-2-ethanesulfonic acid (HEPES) under an atmosphere containing 5% CO_2. P-Glycoprotein was not detectable in these cells at either the protein or RNA level.[1] Exponentially growing cells, of which the viability was 98% or more as determined by means of the trypan blue exclusion test, were used in the experiments. We did not synchronize the cell cycle because it has been shown to have little effect on the uptake or release of anthracyclines.[18] As for the incubation media in the uptake experiments, glucose-free Hanks' balanced salt solution (HBSS) (pH 7.4) was used as the Na buffer, and choline-replaced, glucose-free HBSS as the choline buffer.

Preparation of MNCs The isolation and purification of MNCs from human peripheral blood were performed by the method described previously.[3, 19] With this method, the recovered cells accounted for about 27% of the whole leukocytes in the blood, and consisted of about 90% MNCs, 10% polymorphonuclear leukocytes and a negligible amount of platelets, as determined by differential counting and observation under a light microscope, respectively.[3, 19] Their viability was 98% or more, as determined by means of the trypan blue exclusion test.

For the experiments involving MNCs, glucose-free 0.1% gelatin-containing HBSS (pH 7.4, Na buffer) or choline-replaced, glucose-free 0.1% gelatin-containing HBSS (pH 7.4, choline buffer) was used as the incubation medium, as indicated.

Uptake experiments Uptake experiments were performed by the methods previously reported.[1–3, 19] HL60 cells (density, 5×10^6 cells/ml) were incubated with 4 mM DNP in choline buffer for 20 min at 37°C to deplete cellular ATP, and then centrifuged at 3000 rpm for 10 min at room temperature. After obtaining the cell pellet, the reaction was initiated by the addition of an appropriate volume of an incubation medium (Na or choline buffer) containing 0.3 μM THP and 4 mM DNP, with or without an inhibitor, warmed at 37°C. Each nucleoside transport inhibitor was used at the concentration reported to afford approximately the maximum inhibitory effect.[4–6] In the experiments involving MNCs, the cells (density, 4×10^6 cells/ml) were preincubated with 4 mM DNP in a volume of choline buffer for 20 min, and then the reaction was initiated by adding the same volume of Na or choline buffer containing 1 μM THP and 4 mM DNP, with or without an inhibitor, warmed at 37°C (final cell density, 2×10^6 cells/ml). Because of the decrease in viability and the low level of THP uptake in MNCs, the sampling procedures were different for HL60 cells and MNCs. Since the substrate concentrations used in this study were much lower than the Michaelis constants for the THP uptake, which were 108 and 42.8 μM in HL60 cells and MNCs, respectively,[3] we think comparison of transport mechanisms between the two cell types is valid. For both types of cells, the reaction was terminated by the addition of ice-cold choline buffer (in the case of MNCs, Ca^{2+}, Mg^{2+}-free, 0.5 mM ethylenediaminetetraacetic acid (EDTA)-containing choline buffer). After centrifugation, the cell pellet was washed with ice-cold choline buffer and then resuspended in 1 ml of water. Samples were frozen at −80°C until assayed. In the case of pretreatment with the inhibitor and ionophore, they were preincubated in choline and Na buffer, respectively, with 4 mM DNP for 20 min at 37°C, and then the reaction was initiated as described above.

Assay procedure The THP concentrations in cells were determined by high-performance liquid chromatography (HPLC) as reported previously.[1, 3] Briefly, to a 1 ml sample, 3 ml of 0.1 M ammonia-ammonium chloride buffer (pH 9.5), 50 or 100 μl of a 0.16 μM THP-OH solution in methanol as an internal standard, and 13.5 ml of chloroform-methanol (2:1, v/v) mixture were added, followed by vigorous shaking for 1 min. After centrifugation at 3000g for 10 min, the organic layer was collected and evaporated to dryness at 30°C under a stream of nitrogen gas. The residue was dissolved in 0.15 or 0.2 ml of HPLC mobile phase. The HPLC system consisted of an LC-6A

system (Shimadzu, Kyoto) equipped with a fluorescence detector (RF-535; Shimadzu). The analytical conditions were as follows: column, STR-ODS II (5 μm, 250 mm×4 mm i.d.; Shimadzu); mobile phase, acetonitrile/0.2 M acetic acid-ammonium formate buffer, pH 4.0 (30:70, v/v); and flow rate, 0.7 ml/min. Fluorescence was measured at 470 nm (excitation) and 550 nm (emission). No interference peaks due to endogenous substances were observed.

Statistical analysis   For HL60 cells, the data are expressed as means±SE. For MNCs, each experiment was carried out in triplicate with MNCs obtained from a male donor, and the data were averaged. Then, the same experiments as described above were performed with MNCs obtained from three or more separate donors, and the data were expressed as means±SEM. Comparisons between two groups and among three or more groups were performed by means of Student's unpaired or paired t test and one-way analysis of variance followed by Scheffe's S test or the Bonferroni-Dunn (Control) test, respectively, a difference with a P value of 0.05 or less being considered statistically significant.

RESULTS

Effects of equilibrative nucleoside transport inhibitors
Fig. 1(A) shows the effects of inhibitors of equilibrative

nucleoside transport on THP uptake by HL60 cells. As shown in Fig. 1(A), the THP uptake in the presence of an inwardly directed Na$^+$-gradient at 1 min was significantly increased by the simultaneous addition of 10 μM NBMPR, 10 μM NBTGR or 100 μM dilazep. However, in the absence of the gradient they all, except NBTGR, decreased the THP uptake significantly (Fig. 1A).

The effects of equilibrative nucleoside transport inhibitors on THP uptake by MNCs are shown in Fig. 1(B). In the absence of an inwardly directed Na$^+$-gradient, the uptake of THP by MNCs was not affected by any inhibitor, in contrast with the case of HL60 cells (Fig. 1B).

Effect of an inwardly directed Na$^+$-gradient   The time course of THP uptake by HL60 cells with or without an inwardly directed Na$^+$-gradient is depicted in Fig. 2(A). The uptake of THP by HL60 cells in the presence of the Na$^+$-gradient was transiently stimulated ("overshoot"). As shown in Table I, the THP uptake by HL60 cells pretreated with 10 μg/ml monensin was significantly ($P<$ 0.001) lower than those in the cases of non- and carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP)-treated cells.

As shown in Fig. 2(B), the THP uptakes by MNCs in the presence and absence of an inwardly directed Na$^+$-gradient were approximately equal at any time point. Table I shows the effects of ionophores on the uptake by MNCs.
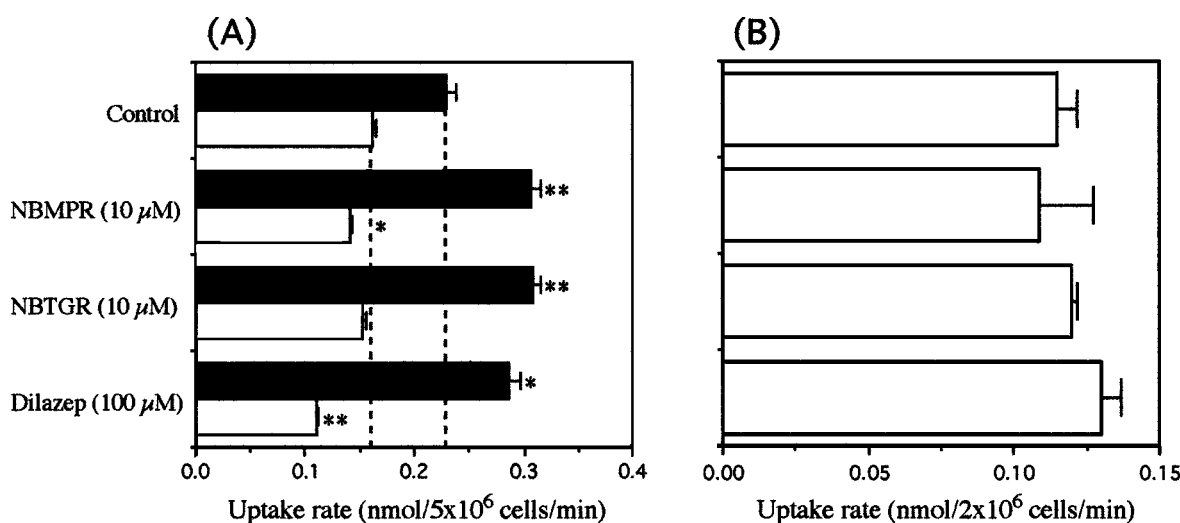

Fig. 1. Effects of equilibrative nucleoside transport inhibitors on THP uptake by HL60 cells (A) and MNCs (B) in the presence and absence of an inwardly directed Na$^+$-gradient. (A) After HL60 cells had been pretreated with 4 mM DNP for 20 min in choline buffer, they were incubated with 0.3 μM THP and the indicated concentration of an inhibitor in Na (closed bar) or choline (open bar) buffer containing 4 mM DNP for 1 min at 37°C. (B) MNCs were preincubated with 4 mM DNP in a volume of choline buffer for 20 min, and then the reaction was initiated by adding the same volume of choline buffer containing 1 μM THP, the indicated concentration of an inhibitor and 4 mM DNP, as the final concentration, and continued for 1 min at 37°C. Each bar represents the mean±SE or SEM for three experiments with HL60 cells or experiments with MNCs obtained from three separate donors, respectively. * and **: significantly different from each control group, $P<0.01$ and 0.001, respectively.
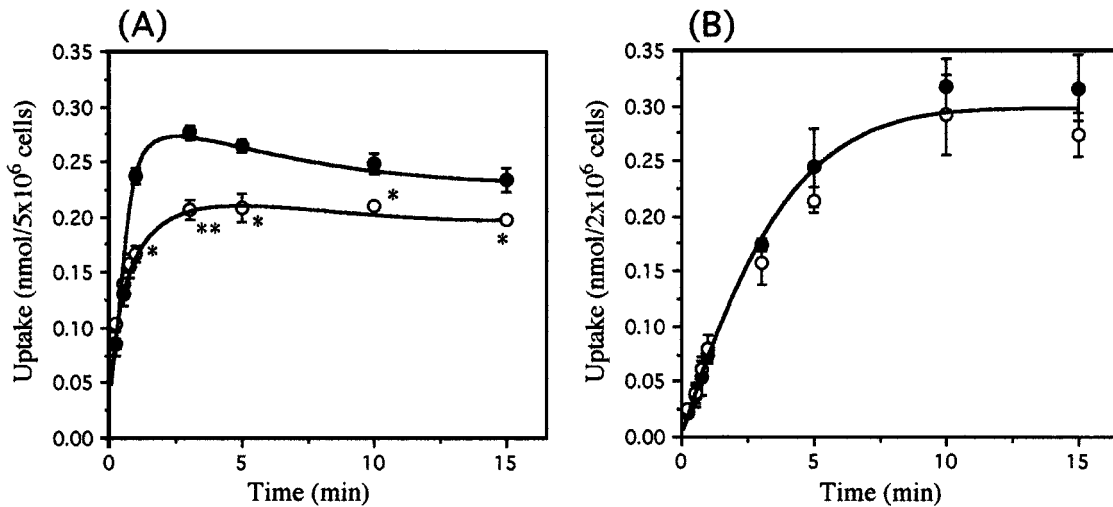
Fig. 2. Time courses of THP uptake by HL60 cells (A) and MNCs (B) in the presence and absence of an inwardly directed Na$^+$-gradient. (A) After HL60 cells had been pretreated with 4 mM DNP for 20 min in choline buffer, they were incubated with 0.3 μM THP in Na (closed circles) or choline (open circles) buffer containing 4 mM DNP for the indicated times at 37°C. (B) MNCs were preincubated with 4 mM DNP in a volume of choline buffer for 20 min, and then the reaction was initiated by adding the same volume of Na (closed circles) or choline (open circles) buffer containing 1 μM THP and 4 mM DNP, as the final concentration, for the indicated times at 37°C. Each point represents the mean±SE or SEM for three experiments. * and **: significantly different from each value for the Na buffer group at the corresponding time point, $P<0.05$ and 0.01, respectively.

Table I. Effects of Ionophores on THP Uptake by HL60 Cells and MNCs

Treatment	Uptake	
	HL60 cells (nmol/5×10^6 cells/min)	MNCs (nmol/2×10^6 cells/min)
Control	0.254±0.018	0.108±0.005
Monensin (10 μg/ml)	0.167±0.009**	0.126±0.011*
FCCP (10 μg/ml)	0.237±0.011	0.119±0.010

Cells were pretreated with 4 mM DNP and 10 μg/ml ionophore for 20 min, and then incubated with 0.3 (HL60 cells) or 1 (MNCs) μM THP for 1 min in Na buffer at 37°C. Each value represents the mean±SE or SEM for three experiments with HL60 cells or experiments with MNCs obtained from three separate donors, respectively. * and **: significantly different from each control, $P<0.05$ and 0.001, respectively.

The THP uptake was significantly increased by pretreatment with monensin, but n ot FCCP.

Effects of Na$^+$/nucleoside cotransport inhibitors Fig. 3 shows the effects of inhibitors of Na$^+$/nucleoside cotransport on THP uptake by HL60 cells in the presence of an inwardly directed Na$^+$-gradient. One hundred micromolar formycin B and guanosine, and 500 μM thymidine, uridine, adenosine and Ara-C significantly decreased the
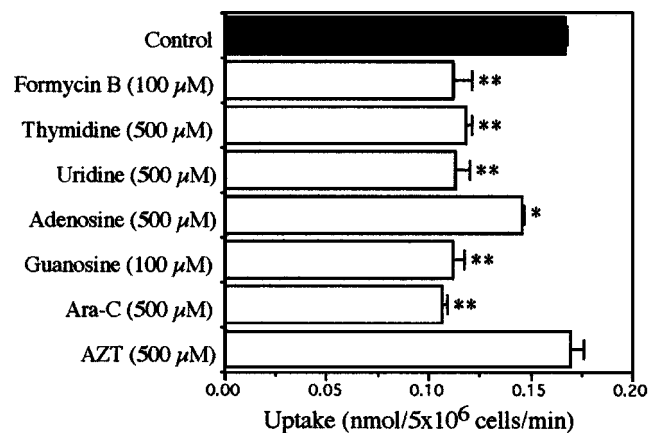

Fig. 3. Effects of Na$^+$/nucleoside cotransport inhibitors on THP uptake by HL60 cells in the presence of an inwardly directed Na$^+$-gradient. After cells had been pretreated with 4 mM DNP for 20 min in choline buffer, they were incubated with 0.3 μM THP and the indicated concentration of an inhibitor in Na buffer containing 4 mM DNP for 1 min at 37°C. Each value represents the mean±SE for three experiments. * and **: significantly different from each control, $P<0.001$ and 0.0001, respectively.

THP uptake, although 500 μM AZT had no effect. As found in similar experiments involving NBMPR-pretreated HL60 cells (Fig. 4), the THP uptake was signifi-
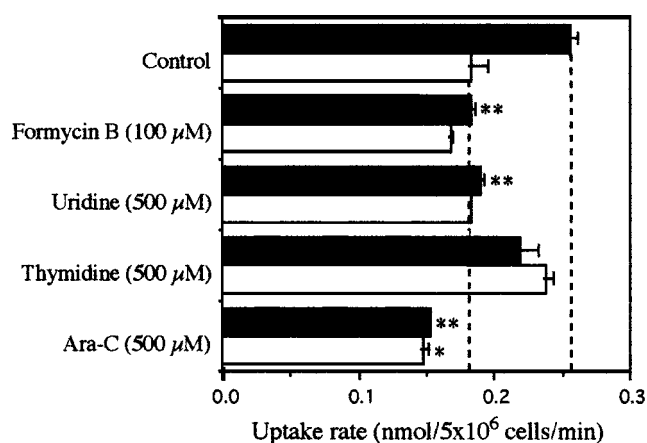
Fig. 4. Effects of Na$^+$/nucleoside cotransport inhibitors on THP uptake by NBMPR-pretreated HL60 cells in the presence and absence of an inwardly directed Na$^+$-gradient. After cells had been pretreated with 4 mM DNP and 10 μM NBMPR for 20 min in choline buffer, they were incubated with 0.3 μM THP and the indicated concentration of an inhibitor in Na (closed bar) or choline (open bar) buffer containing 4 mM DNP and 10 μM NBMPR for 1 min at 37°C. Each bar represents the mean±SE for three to five experiments. ∗ and ∗∗: significantly different from each control group, $P<0.01$ and 0.001, respectively.

cantly decreased by the addition of 100 μM formycin B, 500 μM uridine or 500 μM Ara-C, but not 500 μM thymidine. On the other hand, in the absence of the Na$^+$-gradient, formycin B, uridine and thymidine did not affect the THP uptake by NBMPR-pretreated HL60 cells (Fig. 4). Furthermore, the THP uptake decrease on addition of the inhibitors with the Na$^+$-gradient was equivalent to that without the gradient (Fig. 4). In addition, Ara-C decreased the uptake significantly in the absence of the Na$^+$-gradient.

DISCUSSION

As shown in Fig. 1(A), the THP uptake apparently increased on the addition of representative equilibrative nucleoside transport inhibitors, NBMPR, NBTGR and dilazep, in the presence of a Na$^+$-gradient, implying that THP may be a substrate for the nucleoside transport system in HL60 cells. When these inhibitors were used in the absence of the Na$^+$-gradient, they apparently inhibited the uptake (Fig. 1A). Crawford et al.[8] and Cai and Lee[20] reported that under physiological conditions the equilibrative transporters which show directional symmetry act mainly as efflux systems, and the Na$^+$-cotransporters act as influx systems. So, the contrary results in Fig. 1(A) could be accounted for as follows. The increased uptake in the presence of the Na$^+$-gradient could be due to inhibi-

tion of the efflux (via equilibrative transport) of THP transported into the cells via the Na$^+$-cotransport system and other systems, although the precise mechanisms are not clear, and the decreased uptake in the absence of the Na$^+$-gradient could be due to the inhibition of influx via the equilibrative transport system. Therefore, we speculate that THP might be transported via the equilibrative nucleoside transport system.

Next, in order to clarify the possible contribution of the Na$^+$/nucleoside cotransport system to the THP uptake by HL60 cells, we investigated the effects of Na$^+$-cotransport inhibitors on THP uptake. THP uptake was apparently stimulated in the presence of the gradient and showed an overshoot (Fig. 2A). Thus, it appeared that the THP uptake by HL60 cells partially depended on an inwardly directed Na$^+$-gradient, and this was supported by the finding that a Na$^+$-selective ionophore, monensin, but not a protonophore, FCCP, significantly decreased the uptake (Table I). The overshoot observed in the THP uptake, as shown in Fig. 2(A), was lower in comparison with that in the uptake of nucleosides and their analogues by other cell types isolated from intestine, kidney, liver, blood (erythrocytes and leukemia cell lines), choroid plexus, etc.[4–6, 21] This might be explained by the finding that the expression of the Na$^+$/nucleoside cotransport system is much lower than that of the equilibrative transport system in HL60 cells.[10–12]

Sodium/nucleoside cotransport inhibitors, formycin B, thymidine, uridine, adenosine, guanosine and Ara-C significantly inhibited the THP uptake in the presence of a Na$^+$-gradient (Fig. 3). Barcelo et al.[22] reported that anthracyclines (5.5–7.5 μM), and high concentrations of purine (>2–6 mM) or pyrimidine (>85–100 mM) nucleoside derivatives formed complexes in aqueous solution. This might result in a decrease in the THP uptake. However, we used THP and nucleoside derivatives at much lower concentrations than those reported by them. Moreover, we found that there was no change in the ultraviolet spectrum when each compound was mixed in the transport buffer (data not shown). Thus, the results were thought to imply the inhibition of the transport system by these inhibitors.

In 10 μM NBMPR-pretreated HL60 cells, there appeared to be significant inhibition of THP uptake by formycin B and uridine in the presence of a Na$^+$-gradient (Fig. 4). Furthermore, the THP uptake in the formycin B and uridine groups with the gradient was found to be approximately equal to that in the control group without the gradient. It is known that 10 μM NBMPR almost completely inhibits both es and ei equilibrative nucleoside transporters.[4–6] Thus, although cis-inhibition and trans-stimulation experiments could not be performed due to the formation of complexes of THP and nucleosides as mentioned above, we considered that THP might be taken

up, at least in part, into HL60 cells via the Na$^+$/nucleoside cotransport system, and that THP uptake depending on a Na$^+$-gradient might be accounted for by the uptake via the Na$^+$/nucleoside cotransport system. We cannot clearly explain the results for thymidine in this study, although it was reported that thymidine might have an inhibitory effect on equilibrative nucleoside transport.[4–6] Ara-C caused significant inhibition of the THP uptake under all the experimental conditions examined (Figs. 3 and 4). So, we speculate that Ara-C might inhibit other system(s) of THP transport in addition to the equilibrative and concentrative nucleoside transport systems. In clinical leukemia chemotherapy, especially for acute myeloid leukemia, it is known that the combination of anthracycline and Ara-C exhibits a synergistic antitumor activity.[23–26] So, there seems to be a discrepancy between the clinical efficacy and our basic results. However, we only examined the uptake of THP, and did not assess the uptake of Ara-C or their pharmacological interaction. Thus, we are now examining the interaction between THP and Ara-C in more detail in order to explain this discrepancy.

On the basis of these findings, the nucleoside transport systems might contribute, at least in part, to the THP uptake by HL60 cells. Additional studies using gene expression systems, etc. will be necessary to confirm this hypothesis.

We also examined the possibility that nucleoside transport systems contributed to the THP uptake by MNCs, as in the case of HL60 cells. As shown in Fig. 1(B), equilibrative nucleoside transport inhibitors had no effect on the THP uptake by MNCs in the absence of an inwardly directed Na$^+$-gradient. Moreover, the THP uptake by MNCs appeared to be independent of the Na$^+$-gradient, because THP uptake with the gradient was equal to that without it (Fig. 2B). However, the Na$^+$-selective ionophore, monensin, significantly increased the THP uptake (Table I). It was reported that human peripheral monocytes express functional P-glycoprotein,[17, 27–29] and that monensin had an inhibitory effect on P-glycoprotein.[30, 31] Considering these findings, the increase in THP uptake caused by monensin was speculated to be due, at least in part, to the inhibition of THP efflux via P-glycoprotein, although further studies would be needed to confirm this speculation. Therefore, we think that the THP uptake by MNCs does not depend on an inwardly directed Na$^+$-gradient, and that THP is not taken up by MNCs via both equilibrative and concentrative nucleoside transport systems, in contrast to the case of HL60 cells.

Goh et al. reported that the total amounts of NBMPR binding sites in various human tumor tissues were 1.5- to 5-fold higher than those in the respective normal tissues of the same patient, in spite of the fact that the NBMPR binding affinities of both tumor and normal tissues were statistically insignificant.[32] In addition, nucleoside transporters are suggested to be heterogeneous and functionally dissimilar among tissues.[33–35] These findings might explain the differences between the results for HL60 cells and MNCs in this study, although the details of the mechanisms involved are unclear. Further investigations using other anthracyclines, such as doxorubicin, daunorubicin, etc., are in progress in our laboratory to elucidate the substrate specificity of these transporters.

In conclusion, we suggest that THP is, at least in part, taken up via both equilibrative and concentrative nucleoside transport systems in HL60 cells, but not in MNCs.

REFERENCES

1) Nagasawa, K., Natazuka, T., Chihara, K., Kitazawa, F., Tsumura, A., Takara, K., Nomiyama, M., Ohnishi, N. and Yokoyama, T. Transport mechanism of anthracycline derivatives in human leukemia cell lines: uptake and efflux of pirarubicin in HL60 and pirarubicin-resistant HL60 cells. *Cancer Chemother. Pharmacol.*, **37**, 297–304 (1996).

2) Nagasawa, K., Natazuka, T., Nomiyama, M., Ohnishi, N. and Yokoyama, T. Transport mechanism of anthracycline derivatives in human leukemia cell lines: uptake and efflux of daunorubicin and doxorubicin in HL60 and its resistant cells and comparison with those of pirarubicin. *Biol. Pharm. Bull.*, **19**, 100–105 (1996).

3) Nagasawa, K., Tsumura, A., Nomiyama, M., Ohnishi, N. and Yokoyama, T. Transport mechanism of pirarubicin in human mononuclear cells. *Biol. Pharm. Bull.*, **19**, 1203–1209 (1996).

4) Plagemann, P. G. W. and Wohlhueter, R. M. Permeation of nucleosides, nucleic acid bases, and nucleotides in animal cells. *Curr. Top. Membr. Transp.*, **14**, 225–329 (1980).

5) Plagemann, P. G. W., Wohlhueter, R. M. and Woffendin, C. Nucleoside and nucleobase transport in animal cells. *Biochim. Biophys. Acta*, **947**, 405–443 (1988).

6) Griffith, D. A. and Jarvis, S. M. Nucleoside and nucleobase transport systems of mammalian cells. *Biochim. Biophys. Acta*, **1286**, 153–181 (1996).

7) Plagemann, P. G. W. and Woffendin, C. Species differences in sensitivity of nucleoside transport in erythrocytes and cultured cells to inhibition by nitrobenzylthioinosine, dipyridamole, dilazep and lidoflazine. *Biochim. Biophys. Acta*, **969**, 1–8 (1988).

8) Crawford, C. R., Ng, C. Y. C., Noel, D. and Belt, J. A.

Nucleoside transport in L1210 murine leukemia cells. *J. Biol. Chem.*, **265**, 9732–9736 (1990).

9) Deckert, J., Hennemann, A., Bereznai, B., Fritze, J., Vock, R., Marangos, P. J. and Riederer, P. [³H]Nitrobenzylthioinosine binding sites at the human parietal cortex and erythrocyte adenosine transporter: a comparison. *Life Sci.*, **55**, 1675–1682 (1994).

10) Lee, C. W., Sokoloski, J. A., Sartorelli, A. C. and Handschumacher, R. E. Induction of the differentiation of HL-60 cells by phorbol 12-myristate 13-acetate activates a Na⁺-dependent uridine-transport system. Involvement of protein kinase C. *Biochem. J.*, **274**, 85–90 (1991).

11) Sokoloski, J. A., Sartorelli, A. C., Handschumacher, R. E. and Lee, C. W. Inhibition by pertussis toxin of the activation of Na⁺-dependent uridine transport in dimethyl-sulphoxide-induced HL-60 leukemia cells. *Biochem. J.*, **280**, 515–519 (1993).

12) Goh, L.-B., Sokoloski, J. A., Sartorelli, A. C. and Lee, C.-W. Enhancement of pertussis-toxin-sensitive Na⁺-dependent uridine transporter activity in HL-60 granulocytes by N-formylmethionyl-leucyl-phenylalanine. *Biochem. J.*, **294**, 693–697 (1993).

13) Plagemann, P. G. W. and Aran, J. M. Na⁺-dependent, active nucleoside transport in mouse spleen lymphocytes, leukemia cells, fibroblasts and macrophages, but not in equivalent human or pig cells; dipyridamole enhances nucleoside salvage by cells with both active and facilitated transport. *Biochim. Biophys. Acta*, **1025**, 32–42 (1990).

14) Plagemann, P. G. W. Na⁺-dependent, concentrative nucleoside transport in rat macrophages. Specificity for natural nucleosides and nucleoside analogues, including dideoxynucleosides, and comparison of nucleoside transport in rat, mouse and human macrophages. *Biochem. Pharmacol.*, **42**, 247–252 (1991).

15) Kusumoto, H., Maehara, Y., Anai, H., Kusumoto, T. and Sugimachi, K. Potentiation of adriamycin cytotoxicity by dipyridamole against HeLa cells *in vitro* and Sarcoma 180 cells *in vivo*. *Cancer Res.*, **48**, 1208–1212 (1988).

16) Howell, S. B., Hom, D., Sanga, R., Vick, J. S. and Abramson, I. S. Comparison of the synergistic potentiation of etoposide, doxorubicin, and vinblastine cytotoxicity by dipyridamole. *Cancer Res.*, **49**, 3178–3183 (1989).

17) Su, J., Zhen, Y.-S., Qi, C.-Q. and Hu, J.-L. Antibiotic C3368-A, a fungus-derived nucleoside transport inhibitor, potentiates the activity of antitumor drugs. *Cancer Chemother. Pharmacol.*, **36**, 149–154 (1995).

18) Tarasiuk, J., Foucrier, J. and Garnier-Suillerot, A. Cell cycle dependent uptake and release of anthracycline by drug-resistant and drug-sensitive human leukemic K562 cells. *Biochem. Pharmacol.*, **45**, 1801–1808 (1993).

19) Nagasawa, K., Ohnishi, N. and Yokoyama, T. Transport mechanisms of idarubicin, an anthracycline derivative, in human leukemia HL60 cells and mononuclear cells, and comparison with those of its analogs. *Jpn. J. Cancer Res.*, **88**, 750–759 (1997).

20) Cai, J. and Lee, C. W. Tamoxifen inhibits nitrobenzylthio-

inosine-sensitive equilibrative uridine transport in human MCF-7 breast cancer cells. *Biochem. J.*, **320**, 991–995 (1996).

21) Suzuki, H., Sawada, Y., Sugiyama, Y., Iga, T. and Hanano, M. Anion exchanger mediates benzylpenicillin transport in rat choroid plexus. *J. Pharmacol. Exp. Ther.*, **243**, 1147–1152 (1987).

22) Barcelo, F., Barcelo, I., Ferragut, F. A., Yanovich, S. and Gonzalez-Ros, J. M. Interaction of anthracyclines with nucleotides and related compounds studied by spectroscopy. *Biochim. Biophys. Acta*, **884**, 172–181 (1986).

23) Drach, D., Zhao, S., Drach, J., Mahadevia, R., Gattringer, C., Huber, H. and Andreeff, M. Subpopulations of normal peripheral blood and bone marrow cells express a functional multidrug resistant phenotype. *Blood*, **80**, 2729–2734 (1992).

24) Chaudhary, P. M., Mechetner, E. B. and Roninson, I. B. Expression and activity of the multidrug resistance P-glycoprotein in human peripheral blood lymphocytes. *Blood*, **80**, 2735–2739 (1992).

25) Klimecki, W. T., Futscher, B. W., Grogan, T. M. and Dalton, W. S. P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood*, **83**, 2451–2458 (1994).

26) Naito, M., Hoshino, T., Matsushita, Y., Hirai, R. and Tsuruo, T. Two types of interaction between P-glycoprotein and ionophore antibiotics. *J. Cell Pharmacol.*, **2**, 263–267 (1991).

27) Dutcher, J. P., Wiernik, P. H., Markus, S., Weinberg, V., Schiffer, C. A. and Harwood, K. V. Intensive maintenance therapy improves survival in adult acute nonlymphocytic leukemia: an eight-year follow-up. *Leukemia*, **7**, 413–419 (1988).

28) Yates, J. W., Glidewell, O., Wiernik, P. H., Cooper, M. R., Steinberg, D., Dosik, H., Levy, R., Hoagland, C., Henry, P., Gottleib, A., Cornell, C., Berenberg, J., Hutchison, J. L., Raich, P., Nissen, N., Ellison, R. R., Frelick, R., James, G. W., Falkson, G., Silver, R. T., Haurani, F., Green, M., Henderson, E., Leone, L. and Holland, J. F. Cytosine arabinoside with daunorubicin or adriamycin for therapy of acute myelocytic leukemia: a CALGB study. *Blood*, **60**, 454–462 (1982).

29) Van Sloten, K., Wiernik, P. H., Schiffer, C. A. and Schimpff, S. C. Evaluation of levamisole as an adjuvant to chemotherapy for treatment of ANLL. *Cancer*, **51**, 1576–1580 (1983).

30) Yates, J. W., Wallace, H. R., Ellison, R. R. and Holland, J. F. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother. Rep.*, **57**, 485–488 (1973).

31) Klopman, G., Srivastava, S., Kolossvary, I., Epand, R. F., Ahmed, N. and Epand, R. M. Structure-activity study and design of multidrug-resistant reversal compounds by a computer automated structure evaluation methodology. *Cancer Res.*, **52**, 4121–4129 (1992).

32) Goh, L.-B., Mack, P. and Lee, C.-W. Nitrobenzylthioinosine-binding protein overexpression in human breast,

liver, stomach and colorectal tumour tissues. *Anticancer Res.*, **15**, 2575–2580 (1995).

33) Deckert, J., Morgan, P. F. and Marangos, P. J. Adenosine uptake site heterogeneity in the mammalian CNS? Uptake inhibitors as probes and potential neuropharmaceuticals. *Life Sci.*, **42**, 1331–1345 (1988).

34) Jones, K. W. and Hammond, J. R. Heterogeneity of [³H]dipyridamole binding to CNS membranes: correlation with [³H]nitrobenzylthioinosine binding and [³H]uridine influx studies. *J. Neurochem.*, **59**, 1363–1371 (1992).

35) Williams, E. F. Chronic dipyridamole administration downregulates [³H]nitrobenzylthioinosine binding site affinity in guinea pig kidney but not heart and brain. *Life Sci.*, **59**, 2051–2057 (1996).