Jpn. J. Cancer Res. 88, 750–759, August 1997

Transport Mechanisms of Idarubicin, an Anthracycline Derivative, in Human Leukemia HL60 Cells and Mononuclear Cells, and Comparison with Those of Its Analogs

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Transport mechanisms of idarubicin (IDA) in HL60 cells, as leukemia cells, and human mononuclear cells (MNCs), as normal cells, were investigated, and compared with those of its analogs. The uptake of IDA by both cell types was temperature- and concentration-dependent, was inhibited competitively by daunorubicin (DNR) and noncompetitively by adriamycin (ADR), and was stimulated by preloading of the cells with DNR and ADR, indicating the partial involvement of a carrier-mediated mechanism. On pretreatment of the cells with 2,4-dinitrophenol, IDA uptake by HL60 cells increased, but that by MNCs decreased, suggesting that IDA was partially taken up into HL60 cells *via* an energy-independent carrier system, and into MNCs *via* an energy-dependent one. We speculated that in HL60 cells the carrier concerned with IDA uptake was common to DNR and ADR, and that the binding site of IDA on the carrier was the same as that for DNR, but not that for ADR, while in MNCs the carrier system consisted of, at least in part, a carrier for DNR uptake and one for ADR uptake, and the binding site of IDA was identical to that for DNR in the former, but different from that for ADR in the latter. It appeared that the uptake of IDA was greater than those of pirarubicin, DNR and ADR in both HL60 cells and MNCs, and that IDA was incorporated into MNCs more efficiently than into HL60 cells because of the higher uptake efficacy of the carrier(s).

Key words: Idarubicin — HL60 cell — Human mononuclear cell — Transport mechanism

Previously, we investigated the transport mechanisms of several anthracycline derivatives, pirarubicin (THP), daunorubicin (DNR), and adriamycin (ADR) (Fig. 1), in human leukemia HL60 cells, as leukemia cells, and human mononuclear cells (MNCs), as normal cells, in order to establish a strategy for selectively delivering anthracyclines to leukemia cells based upon the differences in their transport mechanisms.[1–3] A carrier-mediated system was found to be involved in their uptake by both cell types, but there are apparent differences in the characteristics of their uptake between HL60 cells and MNCs.[3]

Idarubicin (IDA), newly synthesized by Arcamone *et al.*, has a hydrogen atom at position 14 like DNR, whereas THP and ADR have a hydroxyl group (Fig. 1).[4] It was reported that IDA shows an antitumor effect similar or superior to those of other analogs *in vitro*[5–10] and *in vivo*,[11–15] and also exhibits efficacy against tumor cells showing multidrug resistance,[8, 16–21] but it has serious side effects, particularly leukopenia, as compared with other anthracyclines, and thus its clinical usefulness is limited.[12–15] It would be interesting to determine whether or not IDA is taken up into tumor and normal cells *via* the carrier(s) reported previously, and whether or not the high antitumor efficacy and side effects of IDA are due to its uptake characteristics.

The aims of this study were (1) to elucidate the transport mechanisms of IDA in HL60 cells and MNCs, (2) to compare them with those of its analogs, and (3) to obtain more insight into the substrate specificity of the carrier in both types of cells.

MATERIALS AND METHODS

Chemicals Pure IDA (idarubicin hydrochloride) was provided by Pharmacia K. K. (Tokyo). Tetrahydropyranyldoxorubicinol (the internal standard), DNR (daunorubicin hydrochloride; Meiji Seika Kaisha, Ltd., Tokyo), and ADR (adriamycin hydrochloride; Kyowa Hakko Kogyo Co., Tokyo) were also used. 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). We confirmed that there was no expression of P-glycoprotein in HL60 cells by means of northern and western blot analyses.[1] The mean cell volume of HL60 cells was about 1300 μm^3, as reported previously,[1] and the IC_{50} values for IDA, THP, DNR and ADR in HL60 cells, evaluated by tetrazolium dye (MTT) assay,[1] were 1, 86, 28 and 146 ng/ml, respectively. This line was maintained in RPMI1640 medium (Gibco BRL, Life Technologies,

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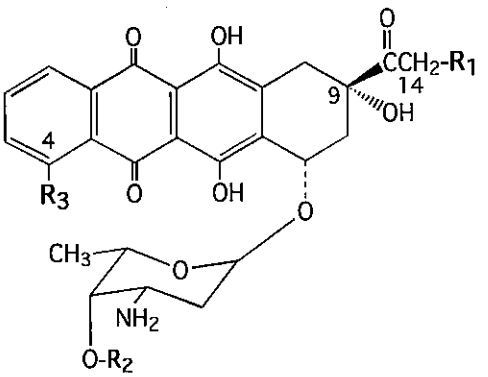

	R_1	R_2	R_3	PC
ADR	OH	H	OCH_3	1.4
DNR	H	H	OCH_3	8.0
IDA	**H**	**H**	**H**	**32.1**
THP	OH		OCH_3	52.0

Fig. 1. Chemical structures of anthracycline derivatives and their octanol/PBS partition coefficients (PC).

Inc., Grand Island, NY) containing 10% heat-inactivated fetal bovine serum, 10 μg/ml kanamycin sulfate and 25 m*M* HEPES, under an atmosphere containing 5% CO_2. Exponentially growing cells, of which the viability was 98% or more, as determined by 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.[22] For the uptake experiments, Hanks' balanced salt solution (HBSS, pH 7.4, buffer A) or glucose-free HBSS (pH 7.4, buffer B) and phosphate-buffered saline (PBS, pH 7.5) were used as the incubation medium and washing medium, respectively.

Preparation of MNCs The isolation and purification of MNCs from human peripheral blood were performed by the methods described previously.[3] With these methods, 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] Their viability was 98% or more, as determined by the trypan blue exclusion test, and the mean cell volume of MNCs was about 195 μm^3.[3] For the experiments involving MNCs, 0.1% gelatin-containing HBSS (pH 7.4, buffer C), Ca^{2+}, Mg^{2+}-free, 0.5 m*M* EDTA-containing buffer C (pH 7.4, buffer D) or glucose-free buffer C (pH 7.4, buffer E) was used as the incubation or washing medium, as indicated.

Uptake experiments Uptake experiments were performed with the same procedure as previously reported.[1,3] Briefly, the reaction suspension of HL60 cells or MNCs was preincubated for 10 min with shaking (160 strokes/min) at a designated temperature (0 or 37°C) in a sterilized plastic tube, except in the experiments involving 2,4-dinitrophenol (DNP) (it was always used in glucose-free medium), in which the reaction suspension was preincubated without and then with DNP for 10 and 20 min, respectively. The reaction was initiated by the addition of an IDA solution to the incubation medium and the final concentration in the medium was 0.3 μM unless otherwise stated. At appropriate time intervals, the reaction was terminated by the addition of ice-cold PBS, and then the suspension was centrifuged. The cell pellet was first washed with ice-cold PBS and then resuspended in 1 ml of water. Samples were frozen at −80°C until assayed.

Assay procedure The IDA concentrations in HL60 cells and MNCs were determined by high-performance liquid chromatography (HPLC) under the same conditions as for the assaying of THP, DNR and ADR.[1,23,24] No interference peaks due to endogenous substances were observed for either HL60 cells or MNCs, but the retention times of IDA and THP were approximately equal (21.5 and 21.3 min, respectively). Hence, we could not examine the effect of THP on IDA uptake, and so its effect was estimated from the results for DNR and ADR, and our previous findings.[1-3] The ratios of the peak height of IDA to that of the internal standard were plotted against known concentrations of a drug, and standard curves were generated by least-squares linear regression analysis. The standard curves obtained for IDA were linear over the concentration range of 0.003–0.3 μM ($r^2 > 0.999$).

Measurement of the partition coefficient The octanol/PBS (pH 7.5) partition coefficient (PC) of IDA was determined, as previously reported,[3] to be 32.1 ± 1.0 ($n = 3$).

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 or female donor, and the data were averaged. Then, the same experiments as described above were performed using MNCs obtained from three or more separate donors. The data are 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 or two-way analysis of variance, respectively. A difference with a P value of 0.05 or less was considered statistically significant.

RESULTS

Uptake of IDA by HL60 cells

Kinetics and temperature dependence: The time course of uptake and the apparent cell/medium concentration (C/M) ratio, which was calculated using the cell volume (1266 μm^3), of IDA in HL60 cells at 37°C are shown in Fig. 2, A and B, respectively. The uptake was rapid and reached equilibrium in 5–10 min. To calculate the initial amount of IDA adsorbed on the cell membrane surface, the Y section (Y value) was obtained by extrapolation of the regression line to time zero (inset in Fig. 2A). Consequently, the adsorbed amount in HL60 cells was 0.00125 nmol/2×10^6 cells, this value being 0.856% of the equilibrium value (0.146 nmol/2×10^6 cells). The uptake of IDA at 0°C was also time-dependent, and the steady-state level (0.0292 nmol/2×10^6 cells) was greater than the Y value described above. However, the IDA uptake at 0°C was significantly lower than that at 37°C, indicating a clear temperature dependency of the uptake by HL60 cells.

Effect of a metabolic inhibitor: Table I shows the effect of a metabolic inhibitor, DNP, on the initial rate of IDA uptake by HL60 cells. The IDA uptake by HL60 cells treated with 4 mM DNP in glucose-free medium for 20 min was significantly greater than that by the control cells ($P<0.001$). Therefore, the following experiments involving HL60 cells were performed using cells preincubated with 4 mM DNP in buffer B for 20 min.

Concentration dependence: Fig. 3A shows the relationship between the drug concentration and the initial uptake rate in HL60 cells. The IDA uptake by the cells was a saturable concentration-dependent process, and with a concentration of 100 μM or more it increased linearly with increasing concentrations of IDA. The kinetic constants for this uptake estimated using the MULTI program[25] are summarized in Table II. The apparent Michaelis constants (K_m), maximum velocity (V_{max}), per-

Table I. Effects of a Metabolic Inhibitor on IDA Uptake by HL60 Cells and MNCs

Treatment	Uptake rate (% of control)	
	HL60 cells	MNCs
Control	100.1±4.4	99.9±0.03
4 mM DNP	115.7±4.3[a)]	63.3±7.9[a)]

HL60 cells and MNCs were preincubated in buffer A and buffer C, respectively, or with 4 mM DNP in buffer B and beffer E, respectively, for 20 min at 37°C, and then incubated with 0.3 μM IDA for 15 s. Each value represents the mean± SE or SEM for three experiments with HL60 cells or experiments with MNCs obtained from three separate donors.
a) $P<0.001$, significantly different from each control.

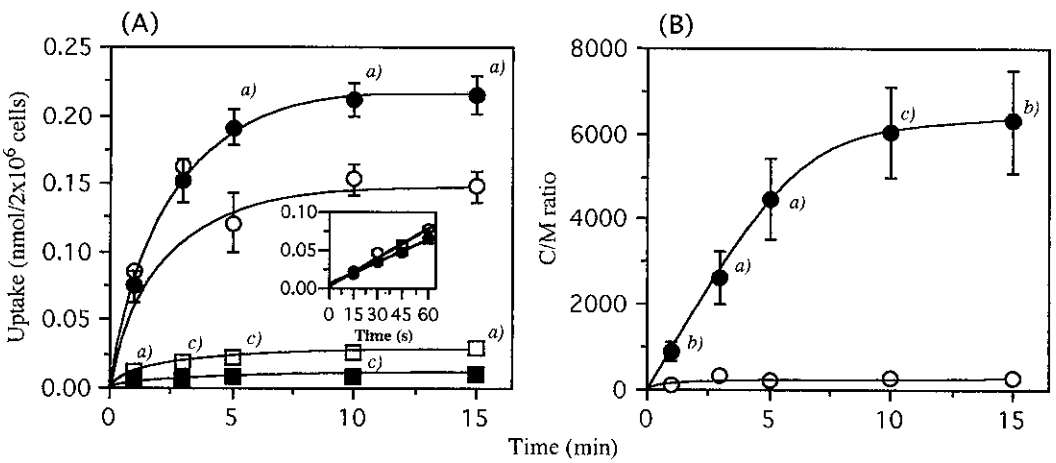

Fig. 2. Time courses of uptake amount (A) and C/M ratio (B) of IDA in HL60 cells and MNCs at 0 and 37°C. HL60 cells and MNCs were incubated with 0.3 μM IDA in buffers A and C, respectively, for the indicated times at 0 or 37°C. Panel (A) represents the uptake amounts of IDA in HL60 cells (open symbols) and MNCs (closed symbols) at 0 (squares) or 37 (circles) °C. The inset shows the linear regression of the initial four time points (up to 60 s) at 37°C. Panel (B) represents the C/M ratios of IDA in HL60 cells (open symbols) and MNCs (closed symbols) at 37°C, which were calculated using the data in panel (A) and each mean cell volume (taken from ref. 1). Each point represents the mean±SE or SEM for three experiments with HL60 cells or experiments with MNCs obtained from three to four separate donors, respectively. *a*), *b*) and *c*) $P<0.05$, 0.01 and 0.001, significantly different from the value of HL60 cells at the corresponding time point, respectively.

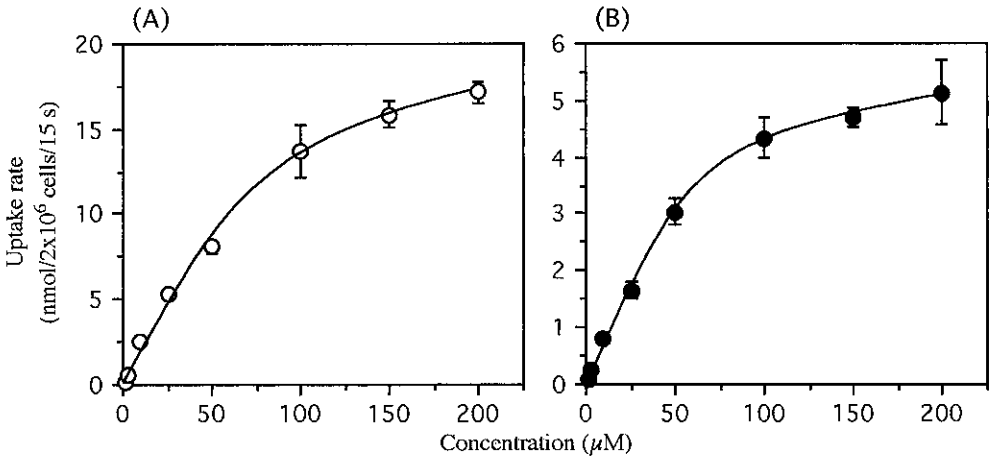

Fig. 3. Concentration dependence of IDA uptake by HL60 cells (A) and MNCs (B). HL60 cells, after pretreatment with 4 m*M* DNP for 20 min in buffer B, and MNCs were incubated with the indicated concentrations of IDA in buffers B and C, respectively, for 15 s at 37°C. Each point represents the mean±SE or SEM for three experiments with HL60 cells or experiments with MNCs obtained from three separate donors.

Table II. Apparent Kinetic Constants for IDA Uptake by HL60 Cells and MNCs

Type of cell	HL60 cells	MNCs
Cell volume (μm^3)[a]	1266	195
DNA concentration (mg/2×10^6 cells)[a]	0.07±0.001	0.02±0.002
C/M ratio (Initial phase)	25.0±1.5	152.1±7.3[d]
(Steady state)	297.9±46.5	6044.5±1063.7[c]
K_m (μM)	146.2±10.9	67.0±2.4[c]
V_{max} (nmol/2×10^6 cells/15 s)	22.1±4.9	5.3±0.3[b]
(nmol/mm^3 cell volume/15 s)	8.7±2.0	13.6±0.9
(nmol/mm^3 cell volume/mg DNA/15 s)	125.2±28.0	72.1±46.6[c]
V_{max}/K_m (ml/2×10^6 cells/15 s)	0.15±0.02	0.08±0.07[b]
(ml/mm^3 cell volume/15 s)	0.06±0.01	0.20±0.02[c]
(ml/mm^3 cell volume/mg DNA/15 s)	0.8±0.1	10.8±0.9[c]
k_d (ml/2×10^6 cells/15 s)	0.031±0.008	0.008±0.003[b]

Kinetic constants were estimated by an iterative nonlinear least-squares method using the MULTI program.[25] Each value represents the mean±SE or SEM for three experiments with HL60 cells or experiments with MNCs obtained from three separate donors.
a) These data were taken from reference Nos. 1 and 3.
b), *c*) and *d*) $P<0.05$, 0.01 and 0.001, significantly different from the value of HL60 cells, respectively.

meation clearance (V_{max}/K_m), and nonspecific diffusion constant (k_d) were found to be 146.2 μM, 22.1 nmol/2×10^6 cells per 15 s, 0.15 ml/2×10^6 cells per 15 s, and 0.03 ml/2×10^6 cells per 15 s, respectively. These parameters, corrected using the cell volume and/or the DNA content,[3] are also shown in Table II.

Effect of DNR or ADR: To examine the *cis*-inhibitory effect of DNR or ADR on IDA uptake, the cellular uptake of IDA as a substrate was observed after IDA and either DNR or ADR as an inhibitor had been introduced into HL60 cell culture simultaneously. In a Dixon plot, the reciprocals of the IDA uptake rate increased with increasing doses of DNR or ADR, with either 1 or 5 μM IDA as the substrate. In addition, the Y coordinate at the intersection of the two extrapolated regression lines for DNR was positive and that for ADR was negative (Fig. 4). The apparent inhibition constants (K_is) of DNR and ADR were 72.7 and 320.5 μM, respectively.

Fig. 5 shows the results of *trans*-stimulation experiments on HL60 cells. IDA uptake by the cells was sig-

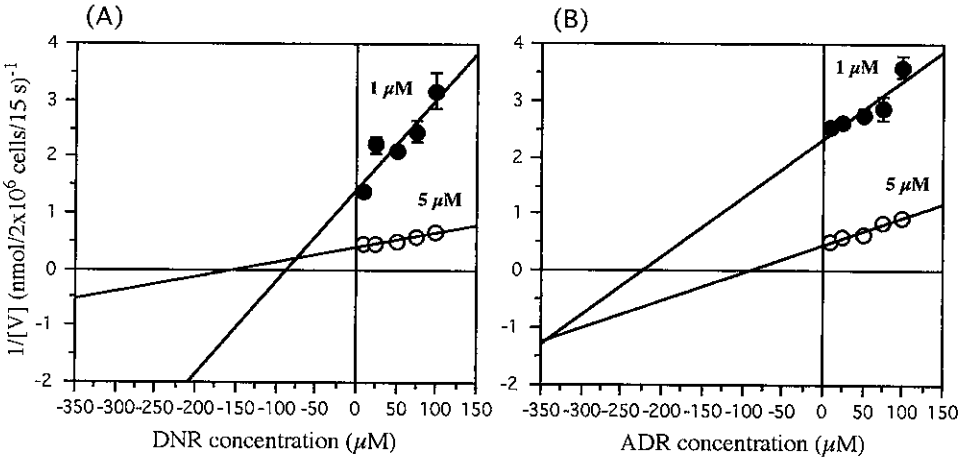

Fig. 4. Dixon plots for IDA uptake showing inhibition by DNR (A) and ADR (B) in HL60 cells. Cells were preincubated with 4 m*M* DNP for 20 min, and then incubated with 1 or 5 μM substrate (IDA) and the indicated concentrations of an inhibitor (DNR or ADR) in buffer B for 15 s at 37°C. Each point represents the mean±SE for three experiments.

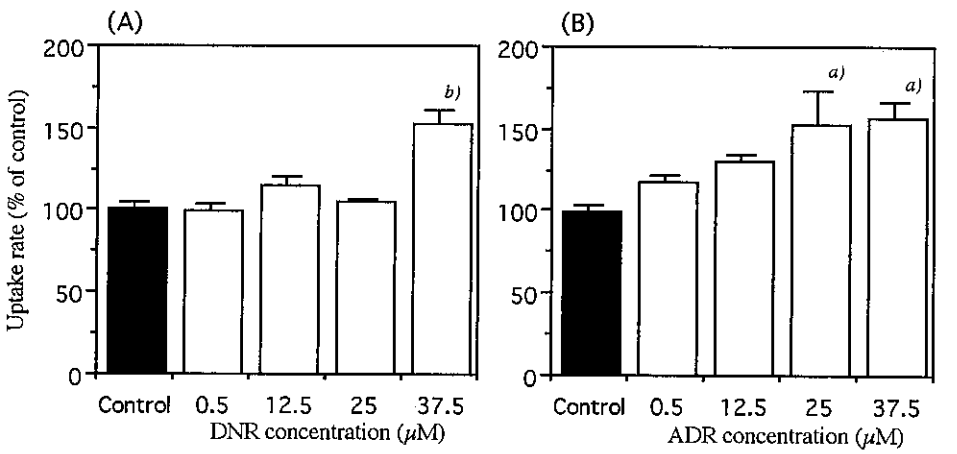

Fig. 5. Effects of preloaded DNR (A) and ADR (B) on IDA uptake by HL60 cells. Cells were preincubated with the indicated concentrations of DNR or ADR in buffer B containing 4 m*M* DNP for 15 min at 37°C, and then washed once with ice-cold buffer B. Thereafter, the cells were suspended in buffer B containing 0.3 μM IDA and 4 m*M* DNP warmed at 37°C, and then incubated for 15 s at 37°C. Each bar represents the mean±SE for three experiments. *a*) and *b*) $P<0.01$ and 0.001, significantly different from each control, respectively.

nificantly elevated by increasing the preloaded amount of DNR or ADR. Thus, it appeared that DNR and ADR had *trans*-stimulatory effects on IDA uptake by HL60 cells.

Uptake of IDA by MNCs

Kinetics and temperature dependence: The uptake and C/M ratio of IDA in MNCs at 37°C are depicted as a function of time in Fig. 2, A and B, respectively. The uptake was rapid and reached equilibrium in 5–10 min. The linear regression of the initial four time points (up to 60 s) indicated that the rate of uptake was linear ($P<0.01$) in this portion of the curve, as in the case of HL60 cells (inset in Fig. 2A). At 0°C, IDA was taken up by MNCs time-dependently, and the amount at the steady state was two-fold greater than the *Y* value (0.0056 nmol/2×10^6 cells). At 0°C, the IDA uptake by MNCs was significantly less than that by HL60 cells at each time point (Fig. 2A).

The C/M ratios, calculated using the cell volume (195 μm^3)[3], in MNCs were 152.1 and 6044.5 in the initial uptake phase and steady state, respectively, and thus were remarkably greater than those in HL60 cells at all time points (Fig. 2B and Table II).

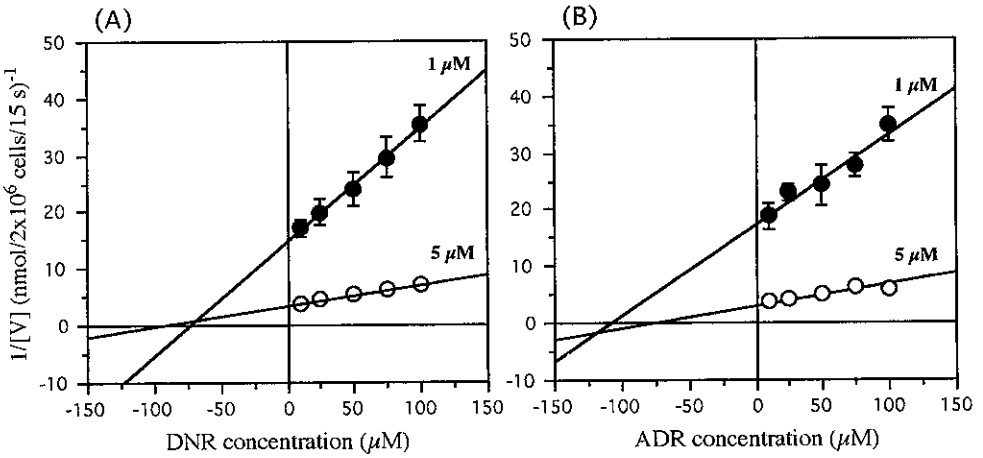

Fig. 6. Dixon plots for IDA uptake showing inhibition by DNR (A) and ADR (B) in MNCs. Cells were incubated with 1 or 5 μM substrate (IDA) and the indicated concentrations of an inhibitor (DNR or ADR) in buffer C for 15 s at 37°C. Each point represents the mean±SEM for experiments with MNCs obtained from three separate donors.

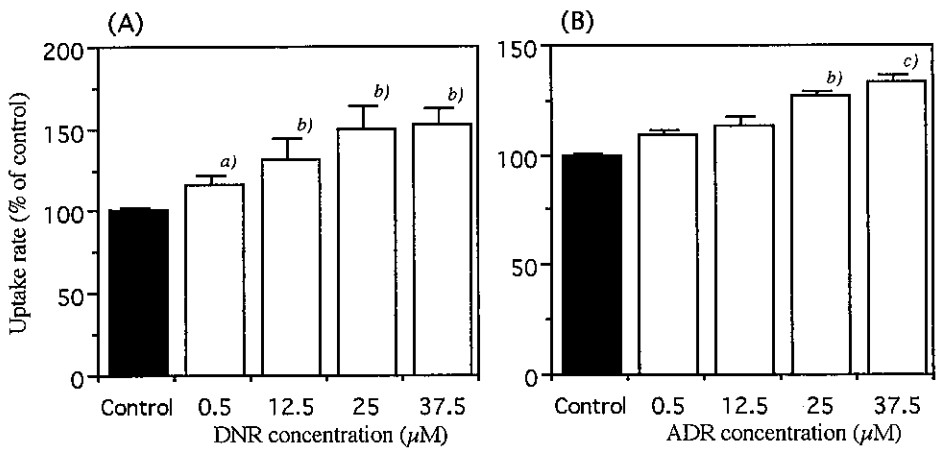

Fig. 7. Effects of preloaded DNR (A) and ADR (B) on IDA uptake by MNCs. Cells were preincubated with the indicated concentrations of DNR or ADR in buffer C for 15 min at 37°C, and then washed once with ice-cold buffer D. Thereafter, the cells were suspended in buffer C containing 0.3 μM IDA warmed at 37°C, and then incubated for 15 s at 37°C. Each bar represents the mean±SEM for experiments with MNCs obtained from three separate donors. *a*), *b*) and *c*) $P<0.01$, 0.001 and 0.0001, significantly different from each control, respectively.

Effect of a metabolic inhibitor: MNCs were pretreated with 4 mM DNP for 20 min in glucose-free medium (buffer E) before the reaction was started. As shown in Table I, a metabolic poison, DNP, partially inhibited the IDA uptake ($P<0.001$).

Concentration dependence: The uptake by MNCs appeared to consist of both saturable concentration-dependent and nonsaturable processes (k_d was calculated to be 0.008 ml/2×10^6 cells per 15 s), the k_d value being approximately one-fourth smaller than that in HL60 cells (Table II). The apparent kinetic constants for IDA uptake were calculated using an equation that assumes two uptake processes. K_m, V_{max}, and V_{max}/K_m in MNCs were 67.0 μM, 5.28 nmol/2×10^6 cells per 15 s, and 0.08 ml/2×10^6 cells per 15 s, respectively, and thus were significantly smaller than those in HL60 cells (Table II). However, after correction of these parameters for cell volume and/or DNA content, the V_{max}s and V_{max}/K_ms in MNCs were greater than those in HL60 cells (Table II).

Effect of DNR or ADR: As in the case of HL60 cells, the

IDA uptake by MNCs was inhibited dose-dependently by DNR and ADR, the inhibition being competitive and noncompetitive, respectively, and the K_is were estimated to be 65.5 μM and 116.8 μM, respectively (Fig. 6).

As shown in Fig. 7, DNR and ADR had *trans*-stimulatory effects on the uptake of IDA by MNCs.

DISCUSSION

Since the uptake of IDA by HL60 cells was found to be time-, temperature- and concentration-dependent, and to occur against a concentration gradient (Figs. 2 and 3), and since its analogs had *cis*-inhibitory and *trans*-stimulatory effects (Figs. 4 and 5), it appears that IDA is taken up into HL60 cells *via* a carrier-mediated system. As a result of concentration-dependence experiments, it was found that a nonspecific diffusion mechanism was also involved, in part, in its uptake (Fig. 3). The contribution of the carrier-mediated uptake to the total uptake $(V_{max}/K_m)/(V_{max}/K_m+k_d)$ was estimated to be approximately 83%. The involvement of nonspecific diffusion may explain the disagreement of the Y value with the amount taken up in the steady state at 0°C (Fig. 2A).

As judged from an examination of the effect of a metabolic inhibitor, DNP, on IDA uptake by HL60 cells, the rate of initial uptake was significantly increased by pretreatment with DNP, as shown in Table I. It was thought that this increase in the uptake was due to the inhibition of an energy-dependent efflux system, suggesting that an energy-independent uptake process partially contributes to the IDA uptake, although we could not rule out the possibility that an energy-dependent process might also be involved. This agrees with the results of our previous experiments on THP, DNR and ADR uptake.[1,2] Thus, as previously reported, for evaluation of the influx mechanism of IDA in HL60 cells, DNP-pretreated cells were used.[1,2,26]

The IDA uptake by HL60 cells was inhibited competitively by DNR and noncompetitively by ADR, and was stimulated by the preloading of cells with both analogs (Figs. 4 and 5). These findings indicate that IDA might be partially taken up into HL60 cells *via* a carrier-mediated system common to DNR and ADR. Under the HPLC conditions used in this study, IDA and THP could not be quantified simultaneously, as mentioned under "Materials and Methods," and thus we could not evaluate the mutual effects of THP and IDA on their own uptake.

We showed previously that in HL60 cells, THP, DNR and ADR were, at least in part, taken up by a common carrier, and that the binding sites for THP and ADR on the carrier were the same, while that for DNR was different.[1,2] As regards the substrate specificity of the carrier for anthracyclines in HL60 cells, in the *cis*-inhibition experiments, IDA was shown to bind to the site of the carrier common to DNR, but not to ADR (Fig. 4) and THP.[1,2] The findings that the K_i value of DNR for IDA uptake was comparable to the K_m value for IDA uptake, but the K_i value of ADR for IDA uptake was incompatible with the K_m value for IDA uptake support this consideration (Table II).

As shown in Fig. 1, R_1 at position 14 of IDA is a hydrogen atom, as in the case of DNR, but different from the cases of THP and ADR. Previously we speculated that the difference in the structure at position 14 might be related to the recognition of the drug at the binding site of the carrier.[2] The findings in this study support our hypothesis. We reported that nonspecific diffusion was observed in the uptake of DNR, but not that of THP and ADR in HL60 cells.[1,2] In addition, nonspecific uptake of IDA by diffusion was found to occur in this study. These results suggest that the role of this diffusion in the uptake process might be affected by the moiety at position 14.

Next, we compared the kinetic constants for IDA uptake with those for THP, DNR and ADR in HL60 cells. The K_m value of IDA (146.2 μM) was almost the same as those of THP (108.2 μM) and ADR (230.0 μM), but was six-fold greater than that of DNR (25.9 μM).[1-3] Hence, it was indicated that the affinities for the carrier of IDA, THP and ADR were equal but lower than that of DNR, and were not affected by lipophilicity (Fig. 1). On the other hand, the V_{max} and V_{max}/K_m values for IDA uptake (22.1 nmol/2$\times 10^6$ cells per 15 s and 0.15 ml/2$\times 10^6$ cells per 15 s, respectively) are two-fold greater than those for THP uptake (10.1 nmol/2$\times 10^6$ cells per 15 s and 0.09 ml/2$\times 10^6$ cells per 15 s, respectively). Thus, it appeared that the uptake capacity and efficacy in the case of IDA were higher than those of THP, implying that the potent antitumor activity of IDA as compared with THP, DNR and ADR might be due, in part, to these factors. We previously found that the uptake efficacy of THP, DNR and ADR in HL60 cells might be correlated with their PC values.[2] As shown in Fig. 1, the PC value of IDA is 32.1 and that of THP is 52.0 (Fig. 1), so the results obtained in this study are not in agreement with the above idea. Recently, however, Rivory *et al.* reported that the hepatocellular uptake of anthracyclines was correlated with the logarithm of the PC value.[27] We can not explain this apparent contradiction at present, and further investigation is needed.

On the other hand, it appeared that the uptake of IDA by MNCs was mediated by a carrier-mediated system and nonspecific diffusion, as in the case of HL60 cells, and the contribution of the former to the total uptake was estimated to be approximately 90% (Figs. 2–5 and Table II). In contrast to the uptake mechanism in HL60 cells, the IDA uptake by MNCs partially required adenosine-5′-triphosphate as a driving force (Table I), in

agreement with the finding for THP reported previously.[3] An examination of the substrate specificity of the carrier indicated that the IDA uptake was inhibited competitively by DNR and noncompetitively by ADR, and was stimulated by preloading of the cells with both compounds (Figs. 6 and 7). Although these results are similar to those for HL60 cells, there are marked differences in the mechanisms between HL60 cells and MNCs. We previously reported that THP and DNR bound to the same binding site on a carrier and then were taken up into MNCs, and ADR was incorporated into the cells *via* a system different from that for THP and DNR.[3] In the light of these findings, at least two different systems for DNR (and THP) uptake and ADR uptake were suggested to be involved in the IDA uptake by MNCs. It was thought that, in addition, IDA bound to the DNR (and THP) binding site in the carrier, but bound to a different site from that for ADR in the ADR uptake carrier, and this speculation is supported by the findings that the K_m value for the IDA uptake was comparable to the K_i value of DNR for the IDA uptake, but not the K_i value of ADR for the IDA uptake (Table II). We are currently examining the substrate specificity of the carriers in more detail.

As judged from a comparison of the kinetic constants for IDA uptake with those for THP, DNR and ADR,[3] the K_m value of IDA (67.0 μM) was comparable with those of THP (42.8 μM) and DNR (54.0 μM), and significantly greater than that of ADR (10.0 μM), indicating that there was no difference in the affinity for the carrier among IDA, THP and DNR, whereas ADR showed five-fold higher affinity. In addition, the affinity of these drugs was not unrelated to their lipophilicity, as previously reported.[3] The V_{max} and V_{max}/K_m values of IDA (5.3 nmol/2×10^6 cells per 15 s and 0.08 ml/2×10^6 cells per 15 s, respectively) were greater than those of THP (1.5 nmol/2×10^6 cells per 15 s and 0.04 ml/2×10^6 cells per 15 s, respectively), DNR (0.3 nmol/2×10^6 cells per 15 s and 0.006 ml/2×10^6 cells per 15 s, respectively), and ADR (0.04 nmol/2×10^6 cells per 15 s and 0.004 ml/2×10^6 cells per 15 s, respectively).[3] Therefore, the uptake capacity and efficacy of the carrier for IDA were superior to those for the other drugs.

Finally, we compared the uptake of IDA in HL60 cells and MNCs. As shown in Fig. 2A, the profiles of IDA uptake in the two cell types were similar, but the amount taken up in the steady state at 37°C was significantly less in HL60 cells than that in MNCs, and this difference was remarkable in terms of the C/M ratio (Fig. 2B and Table II). These results indicated that IDA was taken up by MNCs very efficiently as compared to the uptake by HL60 cells. In order to determine whether or not the uptake kinetics of the carrier could account for the difference in cellular incorporation between HL60 cells and MNCs, we examined the kinetic constants for IDA uptake in both types of cell. Further, the kinetic constants, V_{max} and V_{max}/K_m, were compared after correction for cell volume in the case of initial uptake, and for cell volume and DNA content in the case of the steady state, because the cell volume and DNA content appear to have marked effects on evaluation of anthracycline uptake.[3,28–30] The K_m value of IDA for the carrier in HL60 cells was two-fold higher than that in MNCs, and the affinity order corresponded with that of the C/M ratios. The ratios of the V_{max}/K_m values of IDA in MNCs to those in HL60 cells in the initial phase (corrected for cell volume) and steady state (corrected for cell volume and DNA content), which were calculated to be 3 and 13, respectively, were approximately comparable with the ratios of the C/M ratios in MNCs to those in HL60 cells, which were 6 and 20, respectively (Table II). These results suggested that the greater incorporation of IDA into MNCs than HL60 cells was, at least in part, due to the higher uptake efficacy of the carrier. It is, however, unclear whether or not IDA is taken up by these cells against an extremely steep concentration gradient in the steady state, because the intracellular free anthracycline concentration is low, and most of the anthracycline incorporated into cells binds to lysosomes and the nucleus.[31–35] Although the IDA uptake by HL60 cells was greater than that by MNCs at 0°C, this was thought to be due to the difference in the contribution of nonspecific diffusion to the total uptake between these cells (Fig. 2 and Table II).

Considering the data presented here, and our previous findings, we conclude that (1) IDA is concentrated in HL60 cells *via* an energy-independent (in part) carrier-mediated mechanism and nonspecific diffusion, (2) the carrier is common to THP, DNR and ADR, and the binding site of IDA on the carrier is the same as that for THP and DNR, but not that for ADR, (3) IDA is concentrated in MNCs *via* an energy-dependent (in part) carrier system and nonspecific diffusion, (4) the system consists of a carrier for THP and DNR uptake, and a carrier for ADR uptake, and (5) the IDA binding site in the former is identical to that for THP and DNR, but in the latter is different from that for ADR. It appeared that the uptake of IDA was greater than those of THP, DNR and ADR in both HL60 cells and MNCs, and that IDA was incorporated into MNCs more efficiently than into HL60 cells, because of the higher uptake efficacy for IDA of the carrier(s).

ACKNOWLEDGMENT

The authors are grateful to Pharmacia K. K. for the gift of idarubicin hydrochloride.

(Received April 28, 1997/Accepted June 11, 1997)

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