Jpn. J. Cancer Res. **89**, 1067–1073, October 1998

Cytotoxicity of Amrubicin, a Novel 9-Aminoanthracycline, and Its Active Metabolite Amrubicinol on Human Tumor Cells

Takashi Yamaoka,[1] Mituharu Hanada,[1] Shinji Ichii,[1] Shinya Morisada,[1] Toshihiro Noguchi[1, 3] and Yoshikazu Yanagi[2]

[1]Research Center, Sumitomo Pharmaceuticals Co., Ltd., 1-98 Kasugadenaka 3-chome, Konohana-ku, Osaka 554-0022 and [2]Clinical R & D Division, Sumitomo Pharmaceuticals Co., Ltd., 2-8 Doshomachi 2-chome, Chuo-ku, Osaka 541-8510

Amrubicin, a completely synthetic 9-aminoanthracycline derivative, was previously shown to have potent antitumor activities against various human tumor xenografts. In this study, the *in vitro* activities of amrubicin and its major metabolite, amrubicinol, were examined using 17 human tumor cell lines. Amrubicinol was 5 to 54 times more potent than amrubicin, and as potent as doxorubicin, in inhibiting the growth of the cells following 3-day continuous drug exposure. Amrubicinol closely resembled doxorubicin in its profile of activities on the 17 human tumor cell lines. Cells were incubated with the drugs for 1 h, and the intracellular drug concentration and cell growth inhibition after 3 days were determined. Amrubicinol attained similar intracellular concentrations at lower medium concentrations compared to amrubicin, and the intracellular concentration of amrubicinol necessary to produce 50% cell growth inhibition was 3 to 8 times lower than that of amrubicin in 4 cell lines tested. Amrubicinol has a higher activity level inside the cells than does amrubicin. When cells were incubated with amrubicin for 5 h, a substantial amount of amrubicinol, more than 9% of that of amrubicin, was found in cells in 4 of the 8 cell lines tested. Amrubicinol may contribute to the *in vitro* growth-inhibitory effect of amrubicin on these cells. The results suggest that amrubicinol plays an important role in the *in vivo* antitumor effect of amrubicin as an active metabolite.

Key words: Anthracycline — Amrubicin — SM-5887 — Metabolism

Amrubicin hydrochloride, (+)-(7*S*,9*S*)-acetyl-9-amino-7-[(2-deoxy-β-D-*erythro*-pentopyranosyl)oxy]-7,8,9,10-tetrahydro-6,11-dihydroxy-5,12-naphthacenedione hydrochloride (SM-5887), is a completely synthetic 9-aminoanthracycline derivative.[1)] It has potent antitumor activities against various human tumor xenografts, being more potent than doxorubicin.[2)] In contrast to its potent *in vivo* antitumor activities, the *in vitro* growth-inhibitory activities of amrubicin were more than 5 times lower than those of doxorubicin in several human tumor cell lines.[3)] The dissociation between the *in vitro* and *in vivo* activities suggests that amrubicin is converted *in vivo* to metabolites which are more effective cell growth inhibitors than the parent compound. To test this possibility, the growth-inhibitory activities of the metabolites of amrubicin were examined using various human tumor cell lines.

A major pathway of anthracycline metabolism is known to be the reduction of the C-13 carbonyl group to a hydroxyl group by cytoplasmic carbonyl reductase, and another involves the reductive cleavage of the glycosidic bond between the amino sugar and the chromophore by microsomal glycosidases.[4)] The C-13 hydroxy derivatives and aglycones of doxorubicin, epirubicin, daunorubicin and idarubicin were found in the plasma of cancer patients,[5–8)] as well as in the plasma and tissues of experimental animals treated with these drugs.[9–11)] These metabolites were also formed *in vitro* in human hepatocytes and human tumor cells.[12–16)] The conversion of anthracycline derivatives to their 13-hydroxy metabolites is generally regarded as an inactivation pathway for elimination, and the 13-hydroxy derivatives of doxorubicin, epirubicin and daunorubicin are less potent than the respective parent compounds.[16–19)] In contrast, the 13-hydroxy derivative of idarubicin is essentially equipotent to idarubicin in cell growth-inhibitory activity.[16, 17, 20)] The aglycones of anthracyclines are also far less potent than the respective parent compounds.[12, 17, 18)]

Several metabolites of amrubicin were found in amrubicin-injected mice[21)] and rats (unpublished results), i.e., amrubicinol, 7-deoxyamrubicin aglycone, amrubicinol aglycone, 7-deoxyamrubicinol aglycone and 9-deaminoamrubicin. Amrubicinol, the 13-hydroxy derivative of amrubicin, is a major metabolite of amrubicin. In the present study, amrubicin and amrubicinol were examined for growth-inhibitory activities *in vitro*. The profiles of cellular uptake and metabolism in cultured cells were also examined to elucidate the contribution of the metabolite to the *in vivo* antitumor effect of amrubicin. The cellular

[3] To whom correspondence should be addressed.

incorporation of amrubicin and amrubicinol and the metabolism of amrubicin in cells were studied using human tumor cell lines.

MATERIALS AND METHODS

Chemicals Amrubicin hydrochloride, amrubicinol hydrochloride (diastereoisomeric mixture), 7-deoxyamrubicin aglycone, amrubicinol aglycone, 7-deoxyamrubicinol aglycone and 9-deaminoamrubicin were prepared by Sumitomo Pharmaceuticals Co. (Osaka).[1)] Doxorubicin was purchased from Kyowa Hakko Co. (Tokyo). The chemical structures of amrubicin, amrubicinol, and doxorubicin are shown in Ref. 21.

Cells Eighteen human tumor cell lines were used: Calu-1, A549, QG-56, PC-8 (lung cancer), MG-63, Saos-2 (osteosarcoma), T24, RT4 (bladder cancer), KU-2, G-401 (kidney cancer), COLO 205, WiDr (colon cancer), K-562, CCRF-CEM, CCRF-HSB-2, U-937, MOLT-4 and P3HR-1 (hematopoietic cancer). QG-56, K-562 and PC-8 were provided by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo. KU-2 was a gift from Dr. H. Tazaki, Keio University. P3HR-1 was a gift from Dr. Y. Hinuma, Kyoto University. The other 13 tumor cell lines were provided by the American Type Culture Collection (Rockville, MD). Calu-1, G-401, RT4, T24 and Saos-2 were grown in McCoy's 5A medium supplemented with 10% fetal calf serum (FCS). A549 was grown in Dulbecco's modified Eagle's medium supplemented with 10% FCS, MG-63, WiDr and KU-2 were grown in minimum essential medium supplemented with 10% FCS. The other 9 cell lines were grown in RPMI1640 supplemented with 10% FCS. Cells were grown at 37°C in humidified 5% CO_2 in air.

Cell growth inhibition test Cell growth inhibition was examined by means of a 1-h drug exposure test and a 3-day continuous drug exposure test. For monolayer cell cultures, cells were seeded at 2–8×10^4 cells/ml in 24-well plates, and were grown for a day before the drug treatment. In the 1-h drug exposure test, cells were incubated with different concentrations of the drugs at 37°C for 1 h. After that, the drugs were removed and the cells were washed twice with growth medium. The resulting cells were grown in fresh growth medium for 3 days. In the 3-day continuous drug exposure test, medium containing different concentrations of the drugs was added to cells, and the cells were grown for 3 days. Cells were harvested with trypsin solution (phosphate-buffered saline (PBS(–)) containing 0.125% trypsin and 0.05% EDTA) at the time when the cell number was counted. The suspension cell cultures were tested as follows. In the 1-h drug exposure test, cells at 2×10^4 to 4×10^5 cells/ml were incubated with different concentrations of drugs at 37°C for 1 h. After that, the cells were collected by centrifugation at 1,200 rpm for 5 min and washed with growth medium. The resulting cells were resuspended in fresh growth medium at 2–5×10^4 cell/ml, and were grown for 3 days. In the 3-day continuous drug exposure test, cells were suspended in the growth medium containing different concentrations of drugs at 2–5×10^4 cells/ml, and then grown for 3 days. All assays were carried out in triplicate. Cell growth inhibition was assessed by counting the cell number with a Coulter Counter (Coulter Electronics, Harkenden, England). The treated/control (T/C(%)) ratio was calculated by use of the following formula: T/C(%)=[(mean cell number of drug-treated cells at day 3)−(mean cell number of drug-treated cells at day 0)]/[(mean cell number of control cells at day 3)−(mean cell number of control cells at day 0)]. The concentration necessary to inhibit the cell growth by 50% (IC_{50}) was calculated from a plot of the T/C(%) values versus drug concentrations. Each experiment was performed at least two to three times.

Determination of intracellular drug concentration Cells were incubated with different concentrations of amrubicin or amrubicinol at 37°C in triplicate. At 0, 1, 2 or 5 h, the drugs were removed and the cells were washed twice with ice-cold PBS(−). The cell number was counted, then 10^5 to 5×10^6 cells were harvested in test tubes and frozen at −20°C. The intracellular concentrations of amrubicin, amrubicinol and aglycones were determined by means of high-performance liquid chromatography (HPLC) using a modification of the method described by Matsushita *et al*.[22)] The cells were resuspended in 0.1 ml of 16 m*M* citric acid-16 m*M* Na_2HPO_4-0.9% NaCl solution and 0.8–1.0 ml of 0.1 *M* NH_3-HCl (pH 9.0)-9% NaCl-5% bovine serum albumin (BSA) solution. The samples were then extracted with 7–8 ml of chloroform:MeOH (2:1) with shaking for 30 min at room temperature. After centrifugation, the organic layer was evaporated at 35°C under N_2 gas flow. The dried samples were dissolved in 200 μl of MeOH and 200 μl of 50 m*M* NaH_2PO_4 (pH 3.0), 2% $(CH_3)_4NCl$:CH_3CN (73:27). One hundred microliters of the solution was injected into a liquid chromatography system (Shimadzu, Kyoto) with a Sumipax ODS A-212 column (Sumika Chemical Analysis Service, Osaka). The mobile phase consisted of 4 m*M* sodium 1-heptanesulfonate, 2.3 m*M* acetic acid:tetrahydrofuran:dioxane (15:2:6) pumped at a flow rate of 1 ml/min. The eluate was monitored with a fluorescence detector set at an excitation wavelength of 465 nm and a detection wavelength of 560 nm. The intracellular drug concentrations were calculated with the aid of a standard curve and related to 10^6 cells.

RESULTS

Cell growth inhibition Amrubicin, amrubicinol, three aglycones and 9-deaminoamrubicin were examined for

growth-inhibitory activities by means of a 3-day continuous drug exposure test on 2 hematopoietic (CCRF-CEM, U-937) and 2 lung (PC-8, A549) cancer cell lines, and their activities were compared to those of doxorubicin. As shown in Table I, the concentration of amrubicin necessary to produce 50% cell growth inhibition, i.e. its IC_{50} value, ranged from 0.062 to 0.58 μM, but amrubicin was 11 to 51 times less potent than doxorubicin. 7-Deoxyamrubicin aglycone, amrubicinol aglycone, 7-deoxyamrubicinol aglycone, and 9-deaminoamrubicin were less potent than the parent compound. Amrubicinol, in contrast, showed 8 to 68 times higher activity than amrubicin, and it was as potent as doxorubicin. Amrubicin, amrubicinol, and doxorubicin were further tested on 17 human tumor cell lines comprised of 11 solid and 6 hematopoietic cancer cell lines. Fig. 1 illustrates the IC_{50} values of each drug. The IC_{50} values of amrubicin, amrubicinol, and doxorubicin ranged from 0.06–0.7 μM, 0.005–0.04 μM, and 0.003–0.03 μM, respectively. Amrubicin was 3 to 130 times less potent than doxorubicin. Amrubicinol showed 5 to 54 times higher activity than the parent compound, and it was as potent as doxorubicin in all of the cell lines tested. The finding indicates that amrubicinol is similar to doxorubicin in the profile of growth-inhibitory activities on these 17 tumor cell lines. Amrubicin, amrubicinol and doxorubicin were examined by means of a 1-h drug exposure test using the same cell lines. Fig. 2 illustrates the mean IC_{50} values of each drug in this test. The IC_{50} values of amrubicin, amrubicinol and doxorubicin were in the ranges of 0.6–15 μM, 0.01–0.3 μM, and 0.03–0.9 μM, respectively. Amrubicin was 2 to 73 times less potent than doxorubicin. Amrubicinol showed 18 to 220 times higher activity than did amrubicin, and it showed higher activity than did doxorubicin in most of the cell lines; in particular, amrubicinol was more than 5 times as potent as doxorubicin towards RT4, KU-2, CCRF-CEM and P3HR-1 cells.

Cellular incorporation and growth-inhibitory activity level inside the cells Cellular incorporation and the potency of the incorporated drug were elucidated using 2 solid (QG-56 and G-401) and 2 hematopoietic (U-937 and CCRF-CEM) cancer cell lines. Cells were incubated with medium containing different concentrations of amrubicin or amrubicinol for 1 h, and then the intracellular drug concentrations were determined by HPLC. Fig.3 illustrates the plots of intracellular concentration versus medium concentration. Amrubicinol and amrubicinol aglycone were also detected in the amrubicin-treated cells in some cases, but their concentrations were less than 10% of the respective amrubicin concentrations. Linear relationships were observed between the intracellular and medium drug concentrations. In all 4 of the cell lines tested, the intracellular concentrations of amrubicinol were equal to those of amrubicin when the medium concentrations of amrubicinol were 5 to 10 times lower than those of amrubicin, indicating that amrubicinol is incorporated in the cells to a greater extent than is amrubicin. Aliquots of 1-h drug-treated cells were grown in drug-free medium for 3 days, and the cell growth inhibition was assessed. Table II shows the IC_{50} values of amrubicin and amrubicinol calculated based on both the medium and intracellular drug concentrations. The intracellular concentration of amrubicinol after 1-h exposure to amrubicin was negligible in all cell lines used (data not shown). Amrubicinol showed 27 to 67 times higher activity than did amrubicin when assessed in terms of the medium concentration. Amrubicinol also showed 3 to 8 times higher activity than amrubicin when assessed in terms of the intracellular concentration, indicating that amrubicinol has a higher activity than amrubicin at the same intracellular concentration.

Metabolism of amrubicin Metabolites of amrubicin in cells were analyzed using 2 lung (A549 and Calu-1), 2 colon (COLO 205 and WiDr), 2 kidney (G-401 and KU-

Table I. Growth Inhibition of Human Tumor Cells Following 3-Day Continuous Exposure to Amrubicin, Its Metabolites, and Doxorubicin[a)]

Drug	IC_{50} (μM)[b)]			
	CCRF-CEM	U-937	PC-8	A549
Amrubicin	0.58 ±0.03	0.48 ±0.06	0.26 ±0.16	0.062 ±0.008
Amrubicinol	0.017±0.008	0.0071±0.0011	0.021±0.015	0.0079±0.0022
7-Deoxyamrubicin aglycone	1.1 ±0.1	13 ±0	1.3 ±0.4	0.80 ±0.21
Amrubicinol aglycone	0.79 ±0.04	0.76 ±0.08	0.76 ±0.27	0.45 ±0.25
7-Deoxyamrubicinol aglycone	0.73 ±0.02	0.93 ±0.00	0.92 ±0.25	0.77 ±0.16
9-Deaminoamrubicin	1.2 ±0.3	2.3 ±0.2	9.2 ±5.4	0.70 ±0.08
Doxorubicin	0.034±0.001	0.010 ±0.001	0.010±0.004	0.0057±0.0000

a) Cells were grown in medium containing various concentrations of the drugs for 3 days.
b) The data are the mean IC_{50} value (μM) ± standard deviation of two experiments. CCRF-CEM and U-937 are hematopoietic cell lines; A549 and PC-8 are lung cancer cell lines.

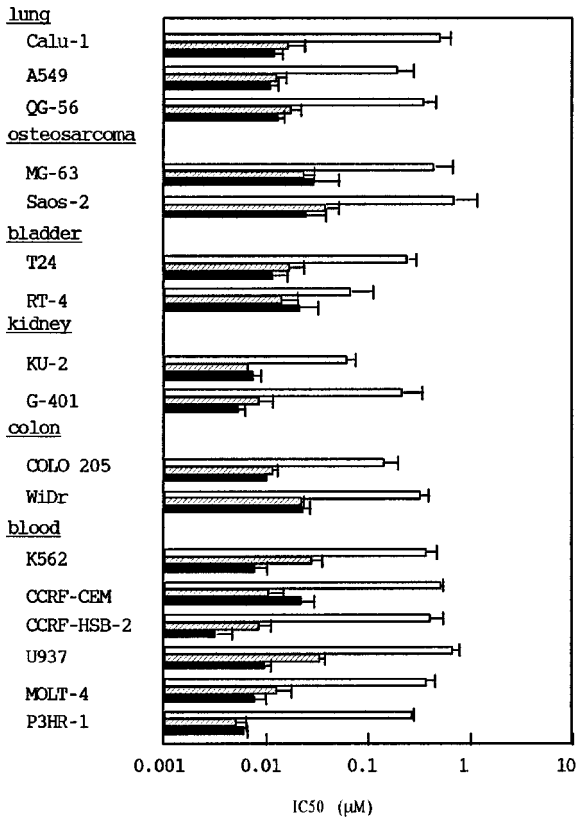

Fig. 1. Growth-inhibitory activities of amrubicin (open bars), amrubicinol (hatched bars) and doxorubicin (closed bars) on human tumor cells with a 3-day continuous drug exposure. Cells were grown in medium containing various concentrations of the drugs for 3 days. The data are the mean IC_{50} value ± standard deviation of two or three experiments.

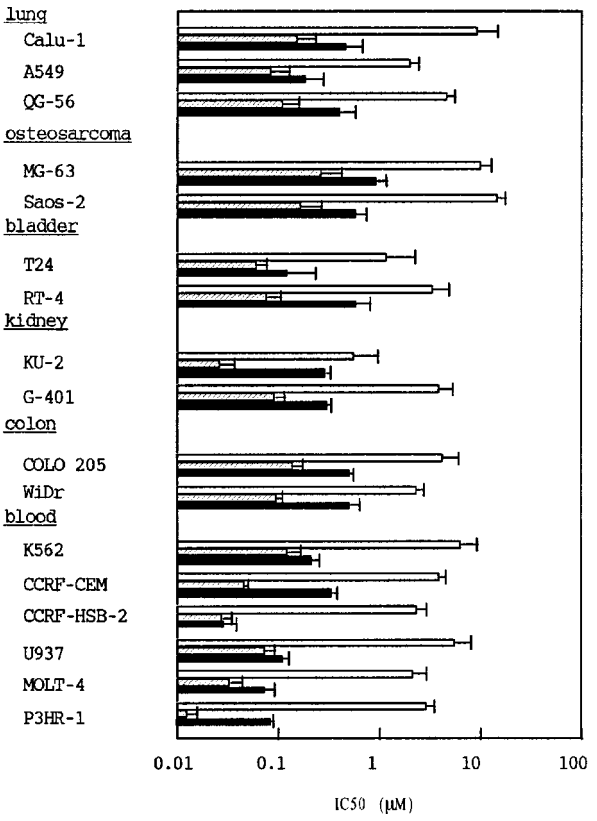

Fig. 2. Growth-inhibitory activities of amrubicin (open bars), amrubicinol (hatched bars) and doxorubicin (closed bars) on human tumor cells with a 1-h drug exposure. Cells were incubated for 1 h with various concentrations of the drugs, and were grown in drug-free medium for 3 days. The data are the mean IC_{50} value ± standard deviation of two or three experiments.

2) and 2 hematopoietic (U-937 and CCRF-CEM) cancer cell lines which show different responsiveness to amrubicin. Cells were incubated with 19 μM amrubicin (10 μg/ml of amrubicin hydrochloride) for 0, 2 or 5 h, and the intracellular concentrations of amrubicin, amrubicinol and aglycones were determined by HPLC. Fig. 4 illustrates the intracellular concentrations of amrubicin and amrubicinol. The intracellular concentration of amrubicin was saturated within 2 h in all cell lines tested, and the saturating levels were different among the cell lines. For example, amrubicin reached a 5-times-higher level in the A549 cells compared to that in the U-937 cells. Amrubicinol was detected in the A549, COLO 205, WiDr, KU-2 and CCRF-CEM cells, and the intracellular concentrations of amrubicinol were more than 9% of those of amrubicin after a 5-h incubation with A549, COLO 205, WiDr and KU-2 cells. In contrast, amrubicinol was under the detection limit in the Calu-1, G-401 and U-937 cells during the incubation period (the intracellular concentrations of amrubicinol were <0.1, <0.1 and <0.01 ng/10^6 cells for Calu-1, G-401 and U-937, respectively). The intracellular concentration of amrubicinol was saturated within 2 h during incubation of COLO 205, WiDr, KU-2 and CCRF-CEM cells with amrubicin. In the A549 cells, the amrubicinol concentration in the cells increased up to 5 h. The intracellular concentrations of amrubicinol after 2-h incubation were markedly different among the cell lines; the amrubicinol concentration in the A549 cells was 23 times higher than that in the CCRF-CEM cells. The ratios of amrubicin to amrubicinol varied from 0.04 to 0.26, indicating that the intracellular amrubicin-metabolizing activities differ markedly, depending on the cell lines.

DISCUSSION

The growth-inhibitory activities of amrubicin, its metabolites, and doxorubicin were compared using 4 human tumor cell lines in a 3-day continuous drug expo-

sure test. Among the metabolites of amrubicin, the three aglycones and the 9-deamino derivative were less potent than amrubicin, whereas amrubicinol showed much higher activity than the parent compound, and was as potent as doxorubicin. The C-13 hydroxy metabolites of anthracycline derivatives are generally less potent than the parent compound in cell growth-inhibitory activity.[12, 16–20] Amrubicin is unique among the anthracycline derivatives in that the growth-inhibitory activity of its C-13 hydroxy derivative is much higher than that of the parent compound. Amrubicin, amrubicinol, and doxorubicin showed similar activity profiles towards 17 human tumor cell lines in both the 1-h and 3-day drug exposure tests. Amrubicinol was as potent as doxorubicin in the 3-day continuous drug exposure test, whereas the former was more potent than the latter in the 1-h drug exposure test. We have observed

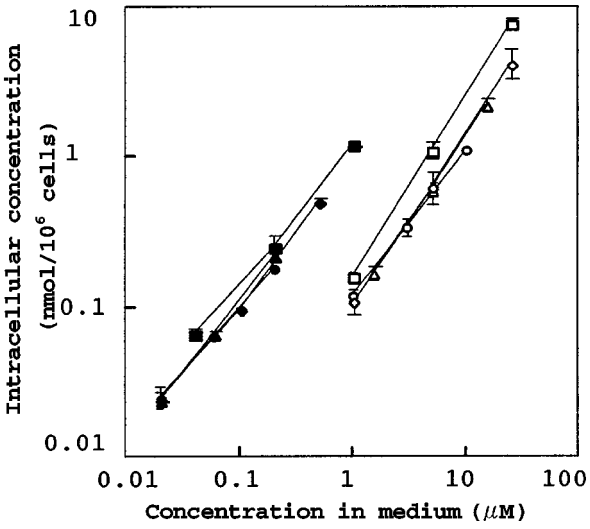

Fig. 3. Cellular incorporation of amrubicin and amrubicinol. CCRF-CEM (●, ○), U-937 (▲, △), QG-56 (◆, ◇) or G-401 cells (■, □) were incubated in medium containing various concentrations of amrubicin (open symbols) or amrubicinol (closed symbols) in triplicate for 1 h, and the intracellular concentrations were measured by HPLC as described in "Materials and Methods." Each point represents the mean value±standard deviation of triplicate wells. This figure shows the result in one of the two experiments. The values were reproduced well in the other experiment.

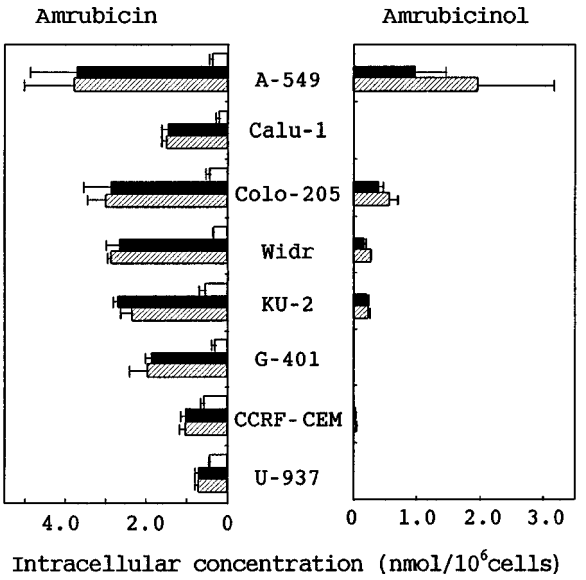

Fig. 4. Metabolism of amrubicin to amrubicinol in human tumor cells. Cells were incubated with 10 μg/ml of amrubicin hydrochloride for 0 h (open bars), 2 h (closed bars) or 5 h (hatched bars) in triplicate and the intracellular concentrations of amrubicin and amrubicinol were measured by HPLC as described in "Materials and Methods." The data are the mean value±standard deviation of three experiments.

Table II. Relationship between Growth-inhibitory Activities and Intracellular Drug Concentrations in Human Tumor Cells[a)]

Cell line	IC_{50} (μM)[b)]			IC_{50} (nmol/10^6 cells)[c)]		
	Amrubicin	Amrubicinol	Ratio	Amrubicin	Amrubicinol	Ratio
CCRF-CEM	2.8±0.0	0.042±0.001	67	0.30±0.02	0.042±0.008	7.0
U-937	6.0±0.1	0.093±0.007	64	0.55±0.24	0.12 ±0.01	4.6
QG-56	9.4±3.7	0.24 ±0.08	39	3.1 ±2.1	0.41 ±0.25	7.5
G-401	1.8±0.4	0.068±0.036	27	0.23±0.01	0.077±0.021	3.0

a) Cells were incubated with various concentrations of the drugs for 1 h, and were grown in drug-free medium for 3 days. Intracellular drug concentrations were measured after a 1-h drug incubation. The data are the mean IC_{50} value±standard deviation of two experiments.
b) IC_{50} assessed using the drug concentration in the medium.
c) IC_{50} assessed using the intracellular drug concentration after the 1-h incubation.

that amrubicinol was incorporated into cells faster than was doxorubicin during 1-h drug exposure (data not shown). The ratio of the intracellular concentration of amrubicinol to that of doxorubicin after the 1-h drug exposure may be higher than that during the 3-day drug exposure, and this may explain why amrubicinol has a higher activity than doxorubicin in the case of 1-h drug exposure.

It is thought that the growth-inhibitory activity of the C-13 hydroxy metabolite of an anthracycline derivative is associated with the degree of cellular incorporation.[18, 23] The intracellular concentrations of doxorubicinol, daunorubicinol and epirubicinol were found to be much lower than those of the respective parent compound at the same extracellular drug concentrations, suggesting that the reduced cytotoxicity of the C-13 hydroxy derivative resulted from low intracellular drug concentrations. The intracellular concentrations of idarubicinol were in the same range as those of idarubicin, and idarubicin was equipotent to idarubicinol in cell growth inhibition.[16, 17, 20] In contrast to the C-13 hydroxy metabolites of other anthracycline derivatives, amrubicinol was shown here to be incorporated to a higher extent than was the parent compound. Furthermore, the intracellular concentration of amrubicinol necessary to produce 50% cell growth inhibition was 3 to 8 times lower than that of amrubicin. This finding, which has not been observed for any other anthracycline derivative, suggests that amrubicinol has a higher activity level inside the cells than does amrubicin. Further studies are necessary to explore this possibility. The present results suggest that amrubicinol showed a higher growth-inhibitory activity than amrubicin due to increased cellular incorporation and a higher activity level inside the cells. Table II indicates that QG-56 is the most resistant cell line to not only extracellular, but also intracellular amrubicinol. It appears that this resistance could not be explained by lower uptake of amrubicinol. We found a low level of cleavable complex formation after treatment of QG-56 cells with amrubicin or amrubicinol [unpublished results]. It was reported that VM-26 resistant cell line, which had a low level of topoisomerase II, produced a low level of cleavable complex. Therefore, the expression of topoisomerase II in QG-56 cells may be low, and this may be the reason why QG-56 is relatively resistant to amrubicin or amrubicinol.

The metabolism of anthracycline derivatives by cultured cells of human origin has been studied by several investigators.[12, 14, 16] Kuffel *et al.* demonstrated that 5–20% of idarubicin and daunorubicin were converted to the C-13 hydroxy metabolites during 3-day incubations with human glioblastoma and leukemia cells.[16] Chevillard *et al.* reported that doxorubicinol in the organotypic culture of A549 cells, and the intracellular concentration of doxorubicinol was about 3% of that of the parent compound.[14] It is known that carbonyl reductase catalyzes the reduction of an anthracycline derivative to its C-13 hydroxy derivative, and the conversion is primarily regarded as an inactivation pathway for elimination.[24] On the other hand, it appears that the conversion of amrubicin to amrubicinol increases the cytotoxicity of amrubicin, because amrubicinol in the cells has higher growth-inhibitory activity than amrubicin, as described above. The present study showed that amrubicin is metabolized to amrubicinol by human tumor cells. The metabolizing activity varied depending upon the cell lines, and a substantial amount of amrubicinol, more than 9% of amrubicin, was found in the cells after a 5-h drug incubation in 4 of the 8 cell lines tested. Moreover, we have found that the concentration of amrubicinol was higher than that of the parent compound in tumor tissues of nude mice treated with amrubicin.[21] These findings show that not only does amrubicin itself exert growth-inhibitory activities on the cell lines that do not metabolize amrubicin to amrubinol (see Figs. 1 and 2), but also amrubicinol contributes to the growth-inhibitory activities of amrubicin in the cell lines that metabolize amrubicin to amrubicinol. In order to clarify the significance of amrubicinol in the growth-inhibitory activity of amrubicin, we intend to examine whether the expression of carbonyl reductase cDNA increases the growth-inhibitory activity of amrubicin in cells which do not metabolize amrubicin to amrubicinol well.

Although the *in vitro* growth-inhibitory effects of amrubicin on various human tumor cells were lower than those of doxorubicin, its antitumor effects in nude mice-human tumor xenografts are superior to those of doxorubicin.[2] Amrubicinol, which was found in plasma, normal tissues, and tumor tissues of mice treated with amrubicin, was shown to be much more effective than amrubicin on various human tumor cells *in vitro*. Moreover, amrubicin was metabolized to amrubicinol in some of the human tumor cell lines, and amrubicinol had a higher activity level inside the cells compared to amrubicin. These findings suggest that amrubicinol plays an important role in the *in vivo* antitumor effect of amrubicin as an active metabolite.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Y. Tsunashima, Ms. Y. Takenaka, and Ms. Y. Matsumura for their expert technical assistance.

(Received May 6, 1998/Revised July 27, 1998/Accepted August 3, 1998)

REFERENCES

1) Ishizumi, K., Ohashi, N. and Tanno, N. Stereospecific total synthesis of 9-aminoanthracyclines: (+)-9-amino-9-deoxydaunomycin and related compound. *J. Org. Chem.*, **52**, 4477–4485 (1987).
2) Morisada, S., Yanagi, Y., Noguchi, T., Kashiwazaki, Y. and Fukui, M. Antitumor activities of a novel 9-aminoanthracycline SM-5887 against mouse experimental tumors and human tumor xenografts. *Jpn. J. Cancer Res.*, **80**, 69–76 (1989).
3) Takigawa, N., Ohnoshi, T., Ueoka, H., Kiura, K. and Kimura, I. Comparison of antitumor activity of new anthracycline analogues, ME2303, KRN8602, and SM5887 using human lung cancer cell lines. *Acta Med. Okayama*, **46**, 249–256 (1992).
4) Bachur, N. R. Anthracycline antibiotic pharmacology and metabolism. *Cancer Treat. Rep.*, **63**, 817–820 (1979).
5) Robert, J., Bui, N. B. and Vrignaud, P. Pharmacokinetics of doxorubicin in sarcoma patients. *Eur. J. Clin. Pharmacol.*, **31**, 695–700 (1987).
6) Mross, K., Maessen, P., Van Der Vijgh, W. J., Gall, F. H., Boven, E. and Pinedo, H. M. Pharmacokinetics and metabolism of epidoxorubicin and doxorubicin in humans. *J. Clin. Oncol.*, **6**, 517–526 (1988).
7) Zanette, L., Zucchetti, M., Freshi, A., Erranti, D., Tirrelli, U. and D'Incalci, M. Pharmacokinetics of 4-demethoxydaunorubicin in cancer patients. *Cancer Chemother. Pharmacol.*, **25**, 445–448 (1990).
8) Reid, J. M., Pendergrass, T. W., Krailo, M. D., Hammond, G. D. and Ames, M. M. Plasma pharmacokinetics and cerebrospinal fluid concentrations of idarubicin and idarubicinol in pediatric leukemia patients. A Children's Cancer Study Group report. *Cancer Res.*, **50**, 6525–6528 (1990).
9) Broggini, M., Sommacampagna, B., Paolini, A., Dolfini, E. and Donelli, M. G. Comparative metabolism of daunorubicin and 4-demethoxydaunorubicin in mice and rabbits. *Cancer Treat. Rep.*, **70**, 697–702 (1986).
10) Cusack, B. J., Young, S. P., Driskell, J. and Olson, R. D. Doxorubicin and doxorubicinol pharmacokinetics and tissue concentrations following bolus injection and continuous infusion of doxorubicin in the rat. *Cancer Chemother. Pharmacol.*, **32**, 53–58 (1993).
11) Cusack, B. J., Young, S. P. and Olson, R. D. Daunorubicin and daunorubicinol pharmacokinetics in plasma and tissues in the rat. *Cancer Chemother. Pharmacol.*, **35**, 213–218 (1995).
12) Le Bot, M. A., Begue, J. M., Kernaleguen, D., Robert, J., Ratanasavanh, D., Airiau, J., Riche, C. and Guillouzo, A. Different cytotoxicity and metabolism of doxorubicin, daunorubicin, epirubicin, esorubicin, and idarubicin in cultured human and rat hepatocytes. *Biochem. Pharmacol.*, **37**, 3877–3888 (1988).
13) Geetha, V. and Ahmed, N. K. Uptake and metabolism of daunorubicin by human myelocytic cells. *Cancer Chemother. Pharmacol.*, **15**, 35–39 (1985).
14) Chevillard, S., Vielh, P., Bastian, G. and Coppey, J. Adriamycin uptake and metabolism in organotypic culture of A549 human adenocarcinoma cells according to the exposure time. *J. Cancer Res. Clin. Oncol.*, **116**, 633–638 (1990).
15) Le Bot, M. A., Glaise, D., Kernaleguen, D., Ratanasavanh, D., Carlhant, D., Riche, C. and Guillouzo, A. Metabolism of doxorubicin, daunorubicin and epirubicin in human and rat hepatoma cells. *Pharmacol. Res.*, **24**, 243–252 (1991).
16) Kuffel, M. J., Reid, J. M. and Ames, M. M. Anthracyclines and their C-13 alcohol metabolites: growth inhibition and DNA damage following incubation with human tumor cells in culture. *Cancer Chemother. Pharmacol.*, **30**, 51–57 (1992).
17) Ferrazzi, E., Woynarowski, J. M., Arakali, A., Brenner, D. E. and Beerman, T. A. DNA damage and cytotoxicity induced by metabolites of anthracycline antibiotics doxorubicin and idarubicin. *Cancer Commun.*, **3**, 173–180 (1991).
18) Dessypris, E. N., Brenner, D. E., Baer, M. R. and Hande, K. R. Uptake and intracellular distribution of doxorubicin metabolites in B-lymphocytes of chronic lymphocytic leukemia. *Cancer Res.*, **48**, 503–506 (1988).
19) Ozols, R. F., Willson, J. K. V., Weltz, M. D., Grotzinger, K. R., Myers, C. E. and Young, R. C. Inhibition of human ovarian cancer colony formation by adriamycin and its major metabolites. *Cancer Res.*, **40**, 4109–4112 (1980).
20) Limonta, M., Biondi, A., Giudici, G., Specchia, G., Catapano, C., Masera, G., Barbui, T. and D'Incalci, M. Cytotoxicity and DNA damage caused by 4-demethoxydaunorubicin and its metabolite 4-demethoxy-13-hydroxydaunorubicin in human acute myeloid leukemia cells. *Cancer Chemother. Pharmacol.*, **26**, 340–342 (1990).
21) Noguchi, T., Ichii, S., Morisada, S., Yamaoka, T. and Yanagi, Y. *In vivo* efficacy and tumor-selective metabolism of amrubicin to its active metabolite. *Jpn. J. Cancer Res.*, **89**, 1055–1060 (1998).
22) Matsushita, Y., Iguchi, H., Kiyosaki, T., Tone, H., Ishikura, T., Takeuchi, T. and Umezawa, H. A high performance liquid chromatographic method of analysis of 4′-O tetrahydropyranyladriamycin and its metabolites in biological samples. *J. Antibiot.*, **36**, 880–886 (1983).
23) Schott, S. and Robert, J. Comparative cytotoxicity, DNA synthesis inhibition and drug incorporation of eight anthracyclines in a model of doxorubicin-sensitive and -resistant rat glioblastoma cells. *Biochem. Pharmacol.*, **38**, 167–172 (1989).
24) Gonzalez, B., Akman, S., Doroshow, J., Rivera, H., Kaplan, W. D. and Forrest, G. L. Protection against daunorubicin cytotoxicity by expression of a cloned human carbonyl reductase cDNA in K562 leukemia cells. *Cancer Res.*, **55**, 4646–4650 (1995).