Biochemical Pharmacology, Vol. 38, No. 22, pp. 4069–4074, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
Pergamon Press plc

COMPARATIVE ACTIVITY OF ANTHRACYCLINE 13-DIHYDROMETABOLITES AGAINST RAT GLIOBLASTOMA CELLS IN CULTURE

BRIGITTE SCHOTT and JACQUES ROBERT*

Fondation Bergonié and Université de Bordeaux II, 180, rue de Saint-Genès, 33076 Bordeaux-Cedex, France

(*Received* 20 *February* 1989; *accepted* 13 *June* 1989)

Abstract—We have studied the growth inhibition, DNA synthesis inhibition and cell incorporation of five 13-dihydrometabolites of anthracyclines in a model of doxorubicin-sensitive and -resistant rat C6 glioblastoma cells. These compounds were major metabolites for doxorubicin, epirubicin, daunorubicin, idarubicin and the new anthracycline 4′-deoxy-4′-iododoxorubicin and are known to be present in appreciable amounts in the plasma of patients treated with these drugs. We have shown that *in vitro* growth inhibition in sensitive cells was either much lower than that of the parent drug (doxorubicinol, epirubicinol, daunorubicinol), or similar to it (idarubicinol, 4′-iodoxorubicinol). In resistant cells, growth inhibition was about 100 times lower than in wild cells, and was always lower than that of the parent anthracycline. DNA synthesis inhibition occurred in sensitive cells for doses about 100 times higher than those required for growth inhibition, but in resistant cells, similar doses provided growth inhibition and DNA synthesis inhibition. Metabolite incorporation was always lower than that of the corresponding parent anthracycline; it was greatly reduced in resistant cells as compared to sensitive ones. The calculated intracellular concentrations obtained for the same growth inhibition are higher in resistant cells than in sensitive cells; in contrast, the calculated intracellular concentrations obtained for the same DNA synthesis inhibition are similar in resistant and sensitive cells, and similar for all the metabolites studied. These results suggest that the amount of drug incorporated is primarily responsible for DNA synthesis inhibition, which is directly correlated to growth inhibition in resistant cells, but not in sensitive cells.

Most anthracyclines undergo in humans and experimental animals a reductive biotransformation of carbon 13, replacing thus a carbonyle by a hydroxyle (see Fig. 1). These metabolites have been named after the name of the anthracycline by adding the suffix -ol. The aldoketoreductase responsible for this reduction is a ubiquitous cytoplasmic enzyme present in most organs [1] and having a higher activity for anthracyclines of the daunorubicin series than for those of the doxorubicin series. As a result of this common metabolic pathway, 13-dihydroderivatives can be found in the plasma, urine and bile of patients treated with anthracycline [2]. The circulating levels of these metabolites are sometimes relatively low, barely reaching those of the unchanged drug in the case of doxorubicin and epirubicin, and sometimes much higher than those of the unchanged drug in the case of daunorubicin and idarubicin [3–6].

These 13-dihydrometabolites are generally considered as "active" in contrast to the inactive aglycones or glucuronides; however, very few data are available on the activity of these compounds, in terms of cytotoxicity, DNA synthesis inhibition or cell incorporation. Concerning doxorubicinol, an activity ten times lower *in vitro* than that of doxorubicin against human ovarian cancer cells [7] and bone marrow progenitors *in vitro* [8] has been reported. Daunorubicinol cytotoxicity was also evaluated on *in vitro* systems [9–12] and this compound entered thereafter a clinical trial several years ago [13]. It has been also shown that idarubicinol had a cytotoxic activity against HeLa cells similar to that of idarubicin [14].

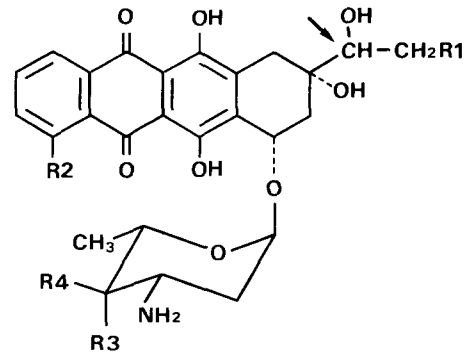

Fig. 1. Structural formulas of the anthracycline metabolites used in this study. Carbon 13 is indicated by an arrow.

R1	R2	R3	R4	Name
OH	OCH_3	OH	H	Doxorubicinol.
OH	OCH_3	H	OH	Epirubicinol.
H	OCH_3	OH	H	Daunorubicinol.
H	H	OH	H	Idarubicinol.
OH	OCH_3	I	H	4′-Iododoxorubicinol.

We have investigated the activities of several 13-dihydrometabolites of anthracyclines on an *in vitro* model we had already developed. Growth inhibition, DNA synthesis inhibition, as well as drug intracellular incorporation, have been evaluated and compared to the data previously obtained on the same

* To whom all correspondence should be addressed.

model with eight anthracyclines [15]. The cellular model is a rat glioblastoma and its multidrug-resistant variant selected with doxorubicin [16]. The multidrug-resistant variant presented an overexpression of the gene responsible for the synthesis of P-glycoprotein, as tested with a cDNA probe originating from colchicine-resistant Chinese Hamster ovary cells [17]. The anthracycline metabolites studied were doxorubicinol (adriamycinol), epirubicinol, daunorubicinol, idarubicinol; all of them are derivatives of clinically used anthracyclines. To this panel was added the 13-dihydroderivative of a new anthracycline presently in development, 4′-iodo-4′-deoxydoxorubicin.

MATERIAL AND METHODS

Material. The following metabolites were obtained from Farmitalia-Carlo Erba (Milan, Italy): epirubicinol, idarubicinol and 4′-iodo-4′-deoxydoxorubicinol; and from Rhône-Poulenc-Santé (Vitry-sur-Seine, France): doxorubicinol and daunorubicinol. They were dissolved in distilled water at a concentration of 1–10 mg/ml and stored at −20°.

Media and sera for cell cultures originated from Seromed, and Petri dishes from Nunc. [^{3}H-methyl]thymidine was purchased from Amersham France (Les Ulis, France).

Cell cultures. The C6 clone originated from a rat brain tumor induced by *N*-nitrosomethylurea [18] and its doxorubicin-resistant counterpart was obtained in our laboratory by continuous exposure to stepwise increasing amounts of doxorubicin [16]. It was able to grow permanently in the presence of 0.5 μg/ml of doxorubicin (clone C6 0.5 E). The cells were routinely cultivated as monolayers in Petri dishes with Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and 1% antibiotic mixture, at 37°, in a humidified atmosphere containing 5% CO_2. The cultures were replicated each week and the medium was changed one or two times in between. Growth curves of both cell lines have been described [16] and were used for the evaluation of growth inhibition.

Evaluation of growth inhibition. Adequate numbers of cells were seeded in 10 or 20 cm^2 Petri dishes in order to obtain 5×10^5 cells three days later. Incubations of the monolayers were then performed for 2 h in the presence of various amounts of the metabolite considered (generally 0.01–1 μg/ml for sensitive cells and 1–100 μg/ml for resistant cells). After rinsing the monolayers, the cells were further incubated for 2.5 complete cell cycles as evaluated from growth curves. At this time, cells were harvested in fresh medium with the help of trypsin, and counted in an automatic hemocytometer (Royco Cell Crit 920 A).

All measurements were performed in triplicate, and two or three independent evaluations were performed. We expressed the cytotoxicity as GIC_{50}, i.e. the concentration causing 50% growth inhibition as compared to controls grown and incubated simultaneously without drug.

Evaluation of drug incorporation and DNA synthesis inhibition. Appropriate numbers of cells were seeded in 10 or 20 cm^2 Petri dishes, in order to obtain 2×10^6 cells in an exponential phase of growth 4 days later. Incubation of the monolayers were then performed for 2 hr in the presence of various amounts of the metabolite considered (generally 0.1–10 μg/ml for sensitive cells and 1–100 μg/ml for resistant cells); during the last hour of incubation, 1 μCi of [^{3}H-methyl]thymidine was added in each dish. The cells were then harvested in 0.15 M NaCl after two rinses, and pelleted at 3000 rpm for 5 min; 0.5 ml of water and 0.5 ml of 40% trichloroacetic acid were successively added, and the samples were kept overnight at 4°, then centrifuged for 30 min at 3000 rpm. The acid-soluble part was used to evaluate the intracellular concentration of the drug by fluorometry (Jobin-Yvon NE 1 spectrofluorometer) with excitation and emission wavelengths set at the maximum fluorescence of each drug in the trichloroacetic solution (*ca.* 470 nm for excitation and 550 nm for emission). The acid-insoluble pellet was solubilized in 1 M NaOH and used to evaluate both the protein content [19] and the [^{3}H] radioactivity in a Beckman LS 1207 liquid scintillation spectrometer.

All incubations were performed in triplicate and two or three independent experiments were performed. In all cases, the incorporation of [^{3}H-methyl]thymidine was referred to controls realized in the same conditions and incubated without drug. It was possible to define a TIC_{50} value, i.e. the concentration of drug providing a 50% decrease of [^{3}H-methyl]thymidine incorporation.

RESULTS

Growth inhibition of wild and doxorubicin-resistant cells in the presence of the 13-dihydroderivatives studied is presented in Table 1. By comparison, the GIC_{50} of the parent anthracyclines measured in the same conditions [15] have been indicated. It appears in this table that the cytotoxic activity of the metabolite in wild cells may vary in important proportions; idarubicinol and 4′-iodo-4′-deoxydoxorubicinol are as active as idarubicin and 4′-iodo-4′-deoxydeoxydoxorubicin in sensitive cells, whereas doxorubicinol, epirubicinol and daunorubicinol are 10–30 times less cytotoxic than the corresponding parent drug.

The doses required for 50% growth inhibition of doxorubicin-resistant cells are 100–200 times higher than for wild cells, and the differences observed from one metabolite to the other in wild cells are also found in resistant cells, with markedly lower GIC_{50} for idarubicinol and 4′-iododoxorubicinol than for doxorubicinol, epirubicinol and daunorubicinol. In the resistant line, metabolites are always less active than the original drugs in approximately constant proportions (2–3 times).

The inhibition of DNA synthesis was evaluated by inhibition of tritiated thymidine incorporation (Table 2). In sensitive cells, TIC_{50} was 60–80 times higher than GIC_{50}, whereas the two parameters were much closer in resistant cells.

Incorporation of the metabolites in the cells were evaluated after 2 hr exposures to doses ranging over 2 or 3 logs. A good linearity was always observed between exposure dose and intracellular concen-

Table 1. Growth inhibition of C6 and C6 0.5 E cells by anthracycline metabolites (compared with that obtained by the corresponding parent drug)

	GIC_{50} (μg/ml)	
	C6 cells	C6 0.5 E cells
Doxorubicinol	2.47 ± 1.10 (0.076)	>100 (30.3)
Epirubicinol	1.05 ± 0.07 (0.123)	>100 (>100)
Daunorubicinol	0.32 ± 0.10 (0.028)	51.3 ± 6.7 (21.7)
Idarubicinol	0.011 ± 0.001 (0.010)	2.43 ± 0.98 (0.78)
4'-Iododoxorubicinol	0.014 ± 0.005 (0.012)	1.30 ± 0.28 (0.81)

GIC_{50} values are evaluated as the drug extracellular concentration providing a decrease of 50% of cell survival after 2-hr exposure to the compound and 2.5 cell cycles further growth of the cells. Results are means ± SE of two to three experiments performed in triplicate. The values obtained for original anthracyclines are indicated in brackets.

Table 2. DNA synthesis inhibition of C6 and C6 0.5 E cells by anthracycline metabolites (compared with that obtained by the corresponding parent drug)

	TIC_{50} (μg/ml)	
	C6 cells	C6 0.5 E cells
Doxorubicinol	>100 (1.15)	>100 (33.5)
Epirubicinol	62.7 ± 14.6 (1.37)	>100 (>100)
Daunorubicinol	2.25 ± 0.65 (0.53)	38.5 ± 1.5 (12.3)
Idarubicinol	0.76 ± 0.20 (0.33)	10.5 ± 0.5 (1.43)
4'-Iododoxorubicinol	1.11 ± 0.39 (0.93)	3.7 ± 0.2 (1.90)

TIC_{50} values are evaluated as the drug extracellular concentration providing a decrease of 50% of [^{3}H-methyl]thymidine incorporation after 2-hr exposure to the compound and 1-hr exposure to thymidine. Results are mean ± SE of two to three experiments performed in triplicate. The values obtained for the original anthracyclines are indicated in brackets.

Table 3. Incorporation of anthracycline metabolites in C6 and C6 0.5 E cells for an exposure dose of 1 μg/ml (compared to that obtained with the corresponding parent drug)

	Cell incorporation (μg/mg prot)	
	C6 cells	C6 0.5 E cells
Doxorubicinol	0.020 ± 0.006 (1.64)	0 (0.15)
Epirubicinol	0.073 ± 0.013 (2.19)	0.020 ± 0.010 (0.20)
Daunorubicinol	1.47 ± 0.52 (4.08)	0.187 ± 0.021 (0.24)
Idarubicinol	3.73 ± 0.08 (5.00)	0.329 ± 0.025 (3.05)
4'-Iododoxorubicinol	1.78 ± 0.08 (3.85)	0.143 ± 0.038 (3.07)

Cell incorporation was evaluated by spectrofluorometry after 2-hr exposure to the compounds. Values are means ± SE of two to three experiments performed in triplicate. The values obtained for original anthracyclines are indicated in brackets.

tration. We have selected for presentation (Table 3) the levels obtained for exposure doses of 1 μg/ml. In all cases, the incorporation was lower than that of the original drug, the difference being especially important in sensitive cells for doxorubicinol and epirubicinol as compared to doxorubicin and epirubicin. A reduction of drug incorporation by resistant cells as compared to sensitive cells was exhibited for all metabolites. We have evaluated what would be the incorporation of drugs in cells exposed to similarly cytotoxic concentrations, i.e. to concentrations equal to GIC_{50} and TIC_{50} as defined before (Table 4). For a similar growth inhibition, resistant cells incorporated much higher levels of compound than sensitive cells; in contrast, for a similar DNA synthesis inhibition, resistant and sensitive cells incorporated similar amounts of compound.

DISCUSSION

We have shown in this paper that the activity of anthracycline 13-dihydrometabolites cannot be predicted from the structure of their parent drug; doxorubicinol and epirubicinol appear as completely

Table 4. Incorporation of anthracycline metabolites in C6 and C6 0.5 E cells for exposure doses corresponding to GIC_{50} or TIC_{50}

	Cell incorporation (μg/mg prot) Exposure to GIC_{50}		Exposure to TIC_{50}	
	C6 cells	C6 0.5 E	C6 cells	C6 0.5 E
Doxorubicinol	0.115	>0.70	>2	>2
Epirubicinol	0.092	>0.63	2.69	>2
Daunorubicinol	0.054	4.74	3.15	3.65
Idarubicinol	0.035	1.11	2.98	5.42
4′-Iododoxorubicinol	0.036	0.30	2.59	1.36

Cell incorporation at GIC_{50} or TIC_{50} was evaluated by interpolation between two actual drug concentrations to which the incorporations of drugs by the cells were determined. Linearity of intracellular concentration as a function of exposure dose was assessed to validate this procedure.

inactive at the concentrations they can reach *in vivo*; idarubicinol and 4′-iododoxorubicinol are in contrast as active as the parent compounds; and daunorubicinol appears as intermediate. Neither the structure of the side chain, nor the modifications at the 4′ position can be used as a rational guide to predict the cytotoxic activity of the metabolites. It had already been shown *in vitro* that doxorubicinol was about 10 times less active than doxorubicin against human ovarian cancers [7] and bone marrow progenitors [8]. Daunorubicinol also displays a cytotoxic activity, which was shown to be much lower than that of daunorubicin, both on normal CFU cells [9] and on tumoral cells in culture [10–12]. It was shown in a preliminary communication that idarubicinol was as cytotoxic as idarubicin against HeLa cells *in vitro* [14].

It must be kept in mind that doxorubicinol and epirubicinol reach relatively low concentrations in humans treated with doxorubicin or epirubicin: the ratios of the total AUC (0–∞) metabolite/parent compound are respectively about 0.3 for doxorubicin [3] and 0.25 for epirubicin [4]. In contrast, the three other 13-dihydroderivatives are major metabolites of the corresponding anthracycline, with AUC ratios of 2–5 for daunorubicin [5], idarubicin [6] and 4′-iododoxorubicin (personal unpublished results). The very high *in vitro* cytotoxic activity of idarubicinol and 4′-iododoxorubicinol may be an important factor in the clinical efficacy of these drugs; the relatively lower activity of daunorubicinol (10 times less than its parent drug) might explain in contrast why daunorubicin displays only a marginal activity in most solid tumors.

No 13-dehydroderivative of the series presented a significant activity against doxorubicin-resistant cells. Whereas idarubicinol and 4′-iododoxorubicinol were as active against sensitive cells as idarubicin and 4′-iododoxorubicin, they appeared two to three times less active than their parent drugs against doxorubicin-resistant cells. However, the most active compounds against sensitive cells remain the most active against resistant cells (idarubicinol and 4′-iododoxorubicinol), but the cytotoxic concentrations are still high and are barely reached after an injection of the parent drug in humans.

Drug incorporation for a similar drug exposure is correlated with growth and DNA synthesis inhibition, both in sensitive and resistant cells. This is generally the case for anthracyclines, as previously discussed [15]. When the cells are exposed to the same extracellular dose, the incorporation of all metabolites is reduced by a factor of 2–80 when compared to the parent drug, both for sensitive and resistant cells. Moreover, the incorporation of metabolites is reduced by a factor 5–10 in doxorubicin-resistant cells when compared to the wild line; this reduction had been observed for doxorubicin, epirubicin and daunorubicin, but not for idarubicin and 4′-iododoxorubicin [15]; this can probably explain why the metabolites of these two drugs have a lower activity than parent drugs against resistant cells.

Evaluation of drug incorporation could be done for drug exposures corresponding to GIC_{50}. The intracellular concentration providing 50% growth inhibition in sensitive cells are relatively close to each other (maximal ratio = 3.3) for exposure doses varying with a maximal ratio of 220. This had been observed in the parent anthracyclines [15] and suggests that cytotoxicity occurs for each drug at similar intracellular doses. In sensitive cells, these incorporations at GIC_{50} are slightly lower for each metabolite than for its parent anthracycline, whereas they are much lower in resistant cells. This would suggest that metabolites are at least as effective as the parent drug once they have entered the cell.

Similarly, evaluation of drug incorporation could be done for drug exposures corresponding to TIC_{50}. These incorporations are very close to each other both in sensitive and resistant cells, and they are also very close to the values obtained for parent anthracyclines [15]. This suggests that the level of drug incorporated is responsible for DNA synthesis inhibition, and that this effect is similar for all compounds and both lines.

There is a relationship between GIC_{50} and TIC_{50}, both in sensitive and resistant cells; however, the ratio TIC_{50}/GIC_{50} is high in sensitive cells (8–80) but remains close to 1 in resistant cells. This had been observed for the parent anthracyclines [15] and suggests that DNA synthesis inhibition might be responsible for growth inhibition in resistant cells, but not in sensitive ones. Siegfried *et al.* [20] had also emphasized the lack of direct relationship between inhibition of nucleic acid synthesis and cytotoxicity

of doxorubicin. In addition, the incorporations obtained at TIC_{50} in sensitive cells are very close to those obtained at TIC_{50} and GIC_{50} in resistant cells, whereas the incorporations obtained at GIC_{50} in sensitive cells remain much lower.

As for parent anthracyclines, it appears that resistant cells can still proliferate with intracellular concentrations of metabolites which are lethal to sensitive cells. This raises the question of the mechanism of doxorubicin resistance. The widely accepted theory of drug extrusion through a glycoprotein pump as primary mechanism for resistance cannot explain this observation, which has been made by other authors [21–24]. Since the resistant cells studied do overexpress the mdr gene (personal unpublished results), it must be admitted that other supplementary mechanisms may be developed during selection of cellular variants resistant to doxorubicin.

It is generally admitted that anthracycline cytotoxicity occurs via the interaction of the intercalated drug with DNA topoisomerase II [25]. Resistance could be then due to an inhibition of this enzyme activity, rendering the DNA of the cells more resistant to protein associated strand breaks, as shown by Capranico *et al.* [22] in P388 cells. If this mechanism of resistance occurred in our cells, it would be expected in resistant cells higher intracellular drug levels than in sensitive cells for a similar DNA synthesis inhibition, which we never observed. Tritton and Yee [26] have proposed a completely different mechanism for doxorubicin cytotoxicity, showing that this drug can be cytotoxic without entering the cells. The link we observed between drug incorporation and cytotoxicity is not in favour of such a mechanism. Another hypothesis, developed in 1987 by Beck [27] has related the resistance to a modification of the subcellular distribution of the drug, allowing only a small percentage of the drug to reach its targets. In our model, the high incorporation observed in resistant cells at GIC_{50} agrees with this hypothesis; however, the similarity of the drug incorporations at TIC_{50} in sensitive and resistant cells remains unexplained. A selective effect of anthracycline drugs on enzymes involved in thymidine uptake, phosphorylation and utilization cannot be excluded and could explain the apparent change of target between sensitive and resistant cells. Other explanations of our observations can also be made; an increase in the DNA repair activity occurring between drug exposure and cell counting could explain why resistant cells can proliferate with intracellular drug concentrations which are lethal to sensitive cells; modifications of detoxication systems or of drug targets could also be involved in our resistant cells.

In addition to the involvement of P-glycoprotein overexpression, several mechanisms responsible for resistance probably occur in our cellular model of glioblastoma cells. In this respect, the 13-dihydrometabolites behave similarly to the parent anthracyclines. The study of other resistant cell models by the same approaches is now necessary.

Acknowledgements—We are grateful to Farmitalia-Carlo Erba for the generous gift of epirubicinol, idarubicinol and 4′-iododoxorubicinol, and to Rhône-Poulenc-Santé for the generous gift of doxorubicinol and daunorubicinol. This work was supported by grants from INSERM, and the Ligue Nationale Française contre le Cancer. We are grateful to Mrs C. Ries for excellent technical assistance and to Miss M. Sanchez for preparation of the manuscript.

REFERENCES

1. Loveless H, Arena A, Felsted RL and Bachur NR, Comparative mammalian metabolism of adriamycin and daunorubicin. *Cancer Res* **38**: 593–598, 1978.
2. Robert J, Pharmacocinétique des nouvelles anthracyclines. *Bull Cancer* **75**: 167–174, 1988.
3. Robert J, Bui NB and Vrignaud P, Pharmacokinetics of doxorubicin in sarcoma patients. *Eur J Clin Pharmacol* **31**: 695–699, 1987.
4. Vrignaud P, Eghbali H, Hoerni B, Iliadi A and Robert J, Pharmacokinetics and metabolism of epirubicin during repetitive courses of administration in Hodgkin's patients. *Eur J Cancer Clin Oncol* **21**: 1307–1313, 1985.
5. Rahman A, Goodman A, Foo W, Harvey J, Smith FP and Schein PS, Clinical pharmacology of daunorubicin in phase I patients with solid tumors: development of an analytical methodology for daunorubicin and its metabolites. *Semin Oncol* **11**(suppl. 3): 36–44, 1984.
6. Robert J, Rigal-Huguet F, Harousseau JL, Pris J, Huet S, Reiffers J, Hurteloup P and Tamassia V, Pharmacokinetics of idarubicin after daily intravenous administration in leukemic patients. *Leuk Res* **11**: 961–964, 1987.
7. Ozols RF, Willson JKV, Weltz MD, Grotzinger KR, Myers CE and Young RC. Inhibition of human ovarian cancer colony formation by adriamycin and its major metabolites. *Cancer Res* **40**: 4109–4117, 1980.
8. Dessypris EN, Brenner DE and Hande KR, Toxicity of doxorubicin metabolites to human marrow erythroid and myeloid progenitors *in vitro*. *Cancer Treat Rep* **70**: 487–490, 1986.
9. Beran M, Andersson B, Eksborg S and Ehrsson H, Comparative studies on the *in vitro* killing of human normal and leukemic clonogenic cell (CFUC) by daunorubicin, daunorubicinol and daunorubicin-DNA complex. *Cancer Chemother Pharmacol* **2**: 19–24, 1979.
10. Yesair M, Thayer PS, McNitt S and Teague K, Comparative uptake, metabolism and retention of anthracyclines by tumors growing *in vitro*. *Ger J Cancer* **16**: 901–907, 1980.
11. Yanovich S, Gewirtz D and Weinert C, Cellular pharmacokinetics of daunorubicinol. *Proc Am Assoc Cancer Res* **26**: 223 (Abstract No. 877), 1985.
12. Bachur NR, Steele M, Meriweather WD and Hildebrand RC, Cellular pharmacodynamics of several anthracycline antibiotics. *J Med Chem* **19**: 651–654, 1976.
13. Chauvergne J, Carton M, Berlie J, Brulé G, Clavel B, Gary-Bobo J, Guerrin J, Klein T and Pommatau E, Essai de chimiothérapie anticancéreuse par la duborimycine. Analyse de 151 observations. *Bull Cancer* **63**: 41–58, 1976.
14. Casazza AM, Barbieri B, Fumagalli A and Geroni MC, Biologic activity of 4-demethoxy-13-dihydrodaunorubicin. *Proc Am Assoc Cancer Res* **24**: 251 (Abstract No. 991), 1983.
15. Schott B 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.
16. Vrignaud P, Londos-Gagliardi D and Robert J, Cellular pharmacology of doxorubicin in sensitive and resistant rat glioblastoma cells in culture. *Oncology* **43**: 60–66, 1986.

17. Van der Bliek A, Van der Velde-Koerts T, Ling V and Borst P, Overexpression and amplification of five genes in a multidrug resistant chinese hamster ovary cell line. *Mol Cell Biol* **6**: 1671–1676, 1986.
18. Benda P, Lightbody J, Sato G, Levine L and Sweet W, Differentiated rat glial cell strain in tissue culture. *Science* **161**: 370–371, 1968.
19. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
20. Siegfried JM, Sartorelli AC and Tritton TR, Evidence for the lack of relationship between inhibition of nucleic acid synthesis and cytotoxicity of adriamycin, *Cancer Biochem Biophys* **6**: 137–142, 1983.
21. Kessel D and Wilberding C, Anthracycline resistance in P 388 murine leukemia and its circumvention by calcium antagonists. *Cancer Res* **45**: 1687–1691, 1985.
22. Capranico G, Dasdia T and Zunino F, Comparison of doxorubicin-induced DNA damage in doxorubicin-sensitive and resistant P 388 murine leukemia cells. *Int J Cancer* **37**: 227–232, 1986.
23. Tsuruo T, Iida-Saito H, Kawabata H, Oh-hara T, Hamada H and Utakoji T, Characteristics of resistance to adriamycin in human myelogenous leukemia K 562 resistant to adriamycin and in isolated clones. *Jap J Cancer Res* **77**: 682–692, 1986.
24. Munger C, Ellis A, Woods K, Randolph J, Yanovich S and Gewirtz D, Evidence for inhibition of growth related to compromised DNA synthesis in the interaction of daunorubicin with H-35 rat hepatoma. *Cancer Res* **48**: 2404–2411, 1988.
25. Tewey KM, Chen GL, Nelson EM and Liu LF, Intercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. *J Biol Chem* **259**: 9182–9187, 1984.
26. Tritton TR and Yee G, The anticancer agent adriamycin can be actively cytotoxic without entering cells. *Science* **217**: 248–250, 1982.
27. Beck WT, The cell biology of multiple drug resistance. *Biochem Pharmacol* **36**: 2879–2887, 1987.