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EFFECTS OF VERAPAMIL ON THE CELLULAR ACCUMULATIONS AND TOXICITY OF SEVERAL ANTITUMOR DRUGS IN 9-HYDROXY-ELLIPTICINE-RESISTANT CELLS*

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Abstract—9-OH-Ellipticine (9-OH-E)-resistant cells are not only resistant to the DNA topoisomerase II inhibitors, but also to some other antitumor agents, such as actinomycin D (AD), adriamycin (ADM), daunorubicin and vincristine. It was previously shown that a decreased uptake accounts for the cross-resistance of these cells to AD and ADM which then suggested that the 9-OH-E-resistant cells might display some of the properties usually associated with the multidrug resistance phenotype. In this work, we have examined the effects of verapamil, a drug which is known to overcome the multidrug resistance, on the toxicity and the cellular accumulation of four cytotoxic agents: 9-OH-E, 2*N*-methyl-9-hydroxy-ellipticinium (NMHE), AD and ADM, either on 9-OH-E resistant cells or on a multidrug resistant subline derived from the same sensitive parental cells. Verapamil inhibited the cellular accumulation of the ellipticine derivatives in the sensitive DC-3F cells, and the toxicity of these drugs on these cells was correspondingly decreased. On either one of the resistant cell lines, verapamil had no effect on the toxicity and the cellular accumulation of 9-OH-E. In contrast, in the presence of verapamil, the cellular accumulation of NMHE by the 9-OH-E and the multidrug resistant cells was about 50% and 300% increased, respectively. The increased NMHE cellular concentration in the multidrug resistant cells was associated with an 8-fold increased toxicity. The major structural characteristics which might account for this difference between the sensitivities of both ellipticine derivatives to the effects of verapamil on the multidrug resistant cells is the presence of a positive charge on the nitrogen in position 2 of the 6H-pyridocarbazole molecule. Finally, verapamil circumvented partially the cross-resistance of DC-3F/9-OH-E cells to AD and ADM by increasing the accumulation of these drugs inside the cells.

As previously reported [1], Chinese hamster lung cells resistant to 9-OH-E‡ have been selected. Detailed drug uptake and retention studies did not show any significant difference between the sensitive parental cells and the resistant cells [1, 2]. Further studies [3] revealed that the toxicity of 9-OH-E in the sensitive cells required the presence of a cellular protein which, in the resistant cells, seems to be modified so that it is no longer recognized by the drug. Ellipticines, other DNA intercalating drugs and epipodophyllotoxins, are known to produce protein-associated DNA breaks [4–6]. It has been proposed that these drugs inhibit the breakage–reunion reaction catalysed by DNA topoisomerase II, by stabilizing a reversible enzyme–DNA complex [7, 8]. It was recently shown that the capacity of ellipticines to induce DNA breaks was markedly decreased in the 9-OH-E resistant cells [9], which are also cross-resistant to other DNA topoisomerase II inhibitors [9]. All these data suggested that resistance to ellipticines in these cells is related to an alteration of the DNA topoisomerase II activity, which was recently demonstrated [10].

In addition, the 9-OH-E resistant cells displayed some properties indicating that the 9-OH-E resistance was also associated with cell membrane alterations. These changes are a loss of the oncogenic potential, morphological modifications of the resistant cells which exhibit the usual growth characteristics of normal cells, and a cross-resistance to various antitumor agents, such as AD, ADM, daunorubicin and vincristine [1, 9]. Furthermore, the cross-resistance to AD and ADM was found to be related to a decreased cellular accumulation of these drugs [1, 9 and this paper]. All these properties suggested that, in parallel to the alteration of the DNA topoisomerase II activity, the DC-3F/9-OH-E cells also have acquired some properties usually associated with the multidrug resistance (MDR) phenotype. However, in these cells, these membrane modifications would have no effect on the uptake of some topoisomerase II inhibitors, such as 9-OH-E (unpublished data), NMHE [2], or m-Amsa [9] which accumulate normally.

Since the original demonstration of this phenom-

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‡ The abbreviations used are: 9-OH-E, 9-hydroxy-ellipticine; NMHE, 2*N*-methyl-9-hydroxy-ellipticinium; AD, actinomycin D; ADM, adriamycin; m-Amsa, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide; VPM, verapamil; MEM, Eagle's minimal essential medium modified; FCS, fetal calf serum; ED_{50}, the dose which reduces the cloning efficiency by 50%.

enon by Biedler and Riehm [11], numerous MDR variants have been described. Several biochemical and pharmacological alterations (reviewed in Ref. 12) have been shown to be consistently associated with this phenotype. The most striking is the presence of high molecular weight glycoproteins which have been found on the surfaces of MDR cells of rodent [13, 14] and human origin [15]. Multiple reports [11, 16–19] indicate that a reduced cellular accumulation of the drugs is, at least in part, responsible for the resistance phenotype in these variants. The cDNA sequences of the genes coding for these high molecular weight glycoproteins (MDR genes) revealed that these proteins could behave as energy-dependent pumps which would be responsible for the decreased drug accumulation in the MDR cells [20, 21]. Recently, several studies (reviewed in Ref. 22) demonstrated that MDR can be circumvented by Ca^{2+} channel blockers, like verapamil, or calmodulin inhibitors.

The purpose of this work was to compare the consequences of the membrane alterations in 9-OH-E and multidrug resistant cells on different antitumor agents. We have examined the effects of verapamil on the toxicity and cellular accumulation of two ellipticine derivatives, the 9-OH-E and the NMHE, in three different cell lines: the sensitive parental cells DC-3F, the 9-OH-E resistant variant, and a highly AD resistant cell line [11]. This latter cell line (DC-3F/AD) was also derived from the DC-3F cells and exhibited a well defined MDR phenotype [11, 16, 23]. We also studied the effects of verapamil on the toxicity and cellular accumulation of AD and ADM in the same cell lines.

MATERIALS AND METHODS

Cells and culture medium. The Chinese hamster lung cells DC-3F and the 9-OH-E resistant subline (DC-3F/9-OH-E) have been previously described [1]. The actinomycin D resistant variant DC-3F/AD was originally isolated by Dr J. L. Biedler [11], and was kindly given to us by Dr G. Barski. These three cell lines have been previously cloned. Monolayer cultures were maintained in MEM, supplemented with 7% FCS, streptomycin (50 μg/ml), and penicillin (100 U/ml). 9-OH-E and AD resistant cells were permanently grown in the presence of the selective agent (0.6 μg/ml 9-OH-E and 10 μg/ml AD respectively).

Drugs and chemicals. 9-OH-E and NMHE are 6H-pyridocarbazole derivatives, the structures of which have been previously shown [1]. These drugs were kindly provided by Sanofi, Paris, France, and further purified by Dr G. Muzard. Actinomycin D (Lyovac Cosmegen) was obtained from Merck, Sharp & Dohme, Inc. (Rahway, NY), adriamycin was from Roger Bellon (Neuilly sur Seine, France); verapamil (Isoptine) was from Biosedra (Knoll) (Malakoff, France). All other chemicals were of reagent grade.

Cellular drug accumulation was studied with the following radiolabeled molecules: [1-^{14}C]9-OH-E (58 mCi/mmole) and NMHE carrying a ^{3}H-labeled group at position 2 (29.5 Ci/mmole) were synthesized and kindly provided by Dr Chenu (Sanofi, Toulouse, France); [14-^{14}C]doxorubicin hydrochloride (53.3 mCi/mmole) and [^{3}H]actinomycin D (13.3 Ci/mmole) were from Amersham. Radiochemical purities were 94–97% depending on the drug and batch. With respect to the high specific activities of the drugs, we carried out the experiments within two months after arrival.

Drug cellular uptake and retention. Uptake and efflux kinetics of the different radiolabeled molecules were carried out as previously described in detail [2]. Briefly, the cells were plated the day before in order to have about 1×10^6 cells/dish at the time of the uptake experiment. The number of cells was precisely determined in duplicate using a Coulter Counter ZM: the difference between 2 separate dishes was less than 5%. After incubation with 1 ml of drug containing medium at 37°, the cells were washed with 9‰ NaCl at 4° and harvested. The cells were separated from the surrounding medium by filtration, and the radioactivity of the filter which retains the cells was measured. Efflux kinetics were determined after loading the cells for 3 hr at 37° in the absence of verapamil (except as indicated in Figure 6); after washing, the cells were incubated at 37° in 5 ml of drug-free medium and the amount of the drug which remained associated with the cells was determined at the indicated times (0.25, 0.5, 1, 2 and 3 hr).

Unless otherwise specified in the legends to Figs 5 and 6, these experiments were carried out at a drug concentration close to the ED_{50} value of each drug on the corresponding cell line in absence of verapamil.

Uptakes of the drugs at different external drug concentrations in the three cell lines were also carried out after 3 hr contact.

Cell survival determination. All the experiments were carried out on exponentially growing cells in the conditions previously described [3].

We have shown previously [3] that the 9-OH-E ED_{50} value on the sensitive cells was constant whether the cells were exposed to the drug for 3 hr or cloned in the presence of the drug, which requires an 8–9 days drug exposure. This behavior is likely to be the consequence of the mechanism of action of this drug on the parental sensitive cells [3]. On the resistant cells, which are killed by a different mechanism, the 9-OH-E toxicity was time dependent, with an ED_{50} value at 3 hr about 10 times higher than that obtained when the cells were cloned in the presence of the drug. As a result, the level of cellular resistance to ellipticine derivatives varied with the drug exposure time. In DC-3F/9-OH-E cells, the resistance to 9-OH-E was over 100-fold after a 3 hr drug exposure, as compared to only about 12–15-fold after 8–9 days exposure. In contrast, as usually observed, the resistance levels of these cells to AD and ADM were independent of the experimental conditions. This time-dependent variation of the cellular resistance to ellipticine derivatives complicates any quantitative comparison with the resistance levels to other drugs.

In order to measure the cytotoxicity and the cellular accumulation of the different molecules used in this work under comparable conditions, we chose to determine the drug sensitivity of the three cell lines after a 3 hr exposure time.

Table 1. ED_{50} (μM) of the different drugs on DC-3F, DC-3F/9-OH-E, and DC-3F/AD cells in the absence or in the presence of verapamil

		DC-3F	DC-3F/9-OH-E	DC-3F/AD
9-OH-E	− VPM	0.09 ± 0.04*	14.4 ± 1.3	0.16 ± 0.06
	+ VPM	0.16 ± 0.04	14.4 ± 1.4	0.16 ± 0.06
NMHE	− VPM	1.14 ± 0.2	109 ± 21	8.82 ± 0.5
	+ VPM	2.28 ± 0.4	114 ± 11	1.26 ± 0.2
AD	− VPM	0.02 ± 0.004	0.063 ± 0.001	84.2 ± 11
	+ VPM	0.012 ± 0.002	0.038 ± 0.004	1.07 ± 0.03
ADM	− VPM	0.05 ± 0.002	0.40 ± 0.08	5.3 ± 1.2
	+ VPM	0.03 ± 0.005	0.17 ± 0.02	0.27 ± 0.04

Each cell line was exposed for 3 hr to graded drug concentrations with or without 10 μg/ml (22 μM) verapamil and the cloning efficiency was then determined as described in Materials and Methods.

* Mean ± SD of 3 independent determinations.

Verapamil was used at a concentration of 10 μg/ml (22 μM) which is not cytotoxic on the three cell lines (90–100% survival).

RESULTS

Cross-resistance studies

We first examined the cross-resistance of the DC-3F/9-OH-E and DC-3F/AD cells to the different drugs used in this work. For each drug, the resistance level of a particular resistant line was defined as the ratio of the ED_{50} of the drug (± verapamil) on that cell line over the ED_{50} of the same drug (− verapamil) on the parental DC-3F cells.

Table 1 shows that, in these conditions, the DC-3F/9-OH-E cells were about 110-fold resistant to 9-OH-E, 100-fold to NMHE, 3-fold to AD, and 8-fold to ADM. The DC-3F/AD cells were about 4200-fold resistant to AD, 100-fold to ADM, and 8-fold to NMHE, but did not display any significant cross-resistance to 9-OH-E.

Effect of verapamil on drug toxicity and cellular accumulation in DC-3F cells

Figure 1 shows that the treatment of DC-3F cells by 9-OH-E or NMHE in the presence of verapamil

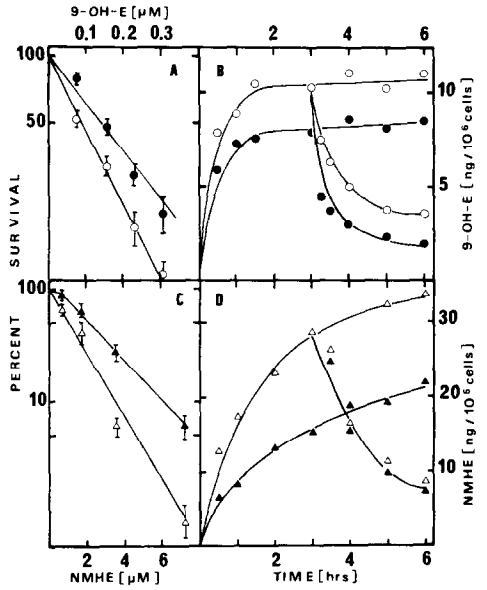

Fig. 1. Effect of verapamil on the toxicity and cellular accumulation of 9-OH-E and NMHE by DC-3F cells. A and C: The cells were incubated with 9-OH-E or NMHE at the indicated concentrations either in the presence (●,▲) or in the absence (○,△) of 10 μg/ml (22 μM) verapamil. The percent survival was then determined as described in Materials and Methods. Each point represents the mean (± SD) of three independent experiments. B and D: The cells were incubated for the indicated times with 0.11 μM (0.03 μg/ml) 9-OH-E or 1.2 μM (0.35 μg/ml) NMHE, either in the presence (●, ▲) or in the absence (○,△) of 10 μg/ml verapamil. Drug cellular concentrations were then determined as described in Materials and Methods. Each point represents the average of two independent experiments (each value is within 10% of the mean) in which each determination was carried out in duplicate (each value is within 5% of the mean).

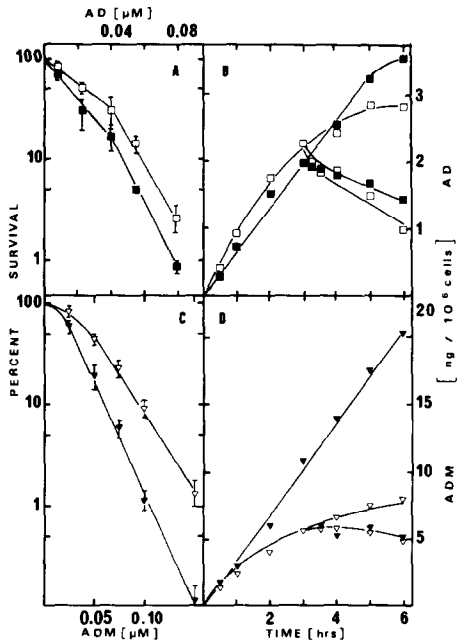

Fig. 2. Effect of verapamil on the toxicity and cellular accumulation of AD and ADM by DC-3F cells. Same legend as Fig. 1. B and D: the cells were incubated with 0.024 μM (0.03 μg/ml) AD or 0.05 μm (0.03 μg/ml) ADM: ■ and ▼, with verapamil; □ and ▽, no verapamil.

resulted in a nearly 50% decrease of the toxicity of either one of these drugs. Uptake kinetics were determined by incubating DC-3F cells either with 9-OH-E at 0.03 μg/ml (0.1 μM) or with NMHE at 0.35 μg/ml (1.2 μM). As shown in Fig. 1, after incubation for 2 hr, the 9-OH-E uptake reaches a steady state level corresponding to a concentration of 10 ng/10^6 cells. Thus, as previously observed with the 2*N*-methyl-ellipticinium [2], the 9-OH-E uptake appears to reach an equilibrium. In the presence of verapamil, the plateau was reached at the same time, but at a concentration about 20% lower (8 ng/10^6 cells). Figure 1 also shows that the 9-OH-E efflux was increased in the presence of verapamil, since only 20% of the drug remained associated with the cells after 3 hr of efflux (the time that will be used throughout for comparison) as compared to about 35% in the control. This increased efflux may then contribute to the decrease of 9-OH-E cellular accumulation in DC-3F cells.

Figure 1 shows that the NMHE cellular concentration continues to increase after 6 hr of incubation with the drug, which is consistent with our previous data [2]. The effect of verapamil on the cellular accumulation of NMHE by DC-3F cells was noticeably different from that on 9-OH-E, since the NMHE uptake was about 40% decreased whereas the efflux rate was not modified. There was also no efflux modification when it was measured in the presence and absence of verapamil after cells were loaded with NMHE in the presence of verapamil (data not shown). Because verapamil contains a tertiary amine, it could directly interfere with NMHE which is a quaternary ammonium compound and hence decrease its accumulation.

Figure 2 shows that verapamil provoked about a 2-fold increase of the AD and ADM toxicity on the DC-3F cells. Studies on the cellular accumulation of these drugs were carried out by incubating the cells with either one of them at a concentration of 0.03 μg/ml. Figure 2 shows that only minor modifications of the AD uptake and efflux kinetics were observed in the presence of verapamil. In contrast, verapamil induced a marked increase of the ADM uptake which, after 3 hr of incubation, was about 2-fold higher than in absence of verapamil. Verapamil had no effect on the ADM kinetics from DC-3F cells.

Effect of verapamil on drug toxicity and cellular accumulation in DC-3F/9-OH-E cells

Figure 3 shows that verapamil has no effect on the toxicity of 9-OH-E and NMHE on the 9-OH-E resistant cells. For uptake and efflux kinetics studies, the DC-3F/9-OH-E cells were then incubated either with 9-OH-E at 3 μg/ml (12 μM) or with NMHE at 24 μg/ml (100 μM). Figure 3 shows that the 9-OH-E uptake and efflux were not modified in the presence of verapamil, whereas the NMHE uptake was about 50% increased. To this increased uptake of NMHE corresponds an increased toxicity which is limited, most likely because, in these resistant cells, large variations in the drug cellular concentration are required to affect the cell viability. For example, as shown in Fig. 3, the cell viability only decreased from 90 to 70% when the external drug concentration was 3-fold increased.

Figure 4 shows that, in the presence of verapamil, the uptake of AD by DC-3F/9-OH-E cells after 3 hr of incubation was about 30% increased; on the other

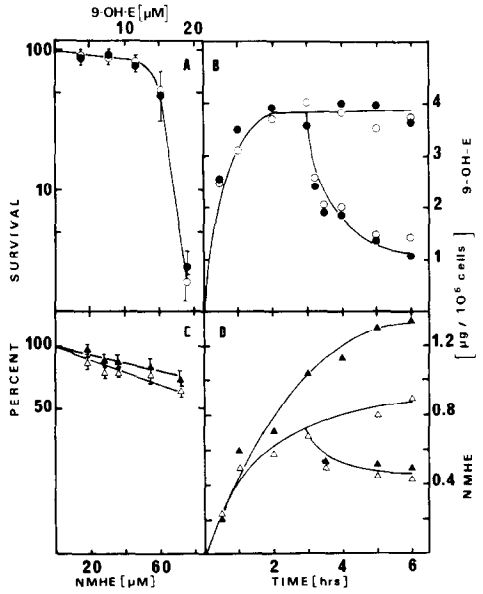

Fig. 3. Effect of verapamil on the toxicity and cellular accumulation of 9-OH-E and NMHE by DC-3F/9-OH-E cells. Same legend as Fig. 1. Because the maximum solubility of NMHE in the cell growth medium is close to 100 μM, the survival curve of DC-3F/9-OH-E cells in the presence of this drug was limited to the concentrations shown on the graph. B and D: the cells were incubated with 11 μM (3 μg/ml) 9-OH-E or 87 μM (24 μg/ml) NMHE: ● and ▲, with verapamil; ○ and △, no verapamil.

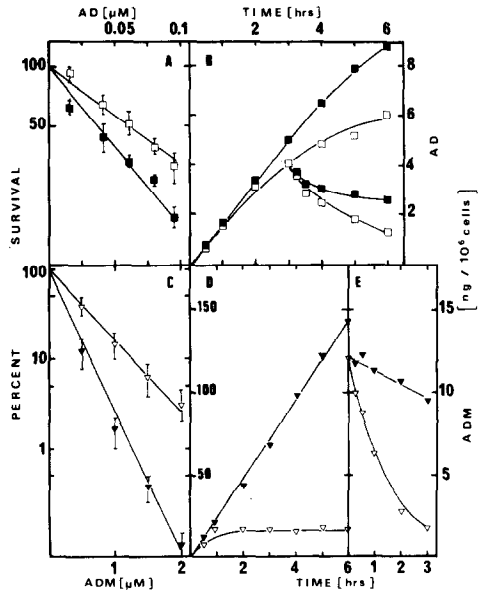

Fig. 4. Effect of verapamil on the toxicity and cellular accumulation of AD and ADM by DC-3F/9-OH-E cells. Same legend as Fig. 1. B and D: the cells were incubated with 0.06 μM (0.08 μg/ml) AD or 0.5 μM (0.3 μg/ml) ADM. E, enlarged scale for the ADM efflux kinetics; ■ and ▼ with verapamil; □ and ▽, no verapamil.

hand, after 3 hr of efflux, the cells still retained 60% of the drug instead of 30% in the absence of verapamil. The effects of verapamil on the ADM accumulation by DC-3F/9-OH-E cells were much more pronounced: while, in the control, a steady state was reached after 2 hr of incubation, the ADM uptake was still linearly increasing after 6 hr of treatment with verapamil. Besides, in these conditions, the ADM efflux was strongly inhibited: after 3 hr of efflux, 80% of the drug remained associated with the cells in the presence of verapamil instead of 15% in its absence. As a result, verapamil provoked a 5-fold increase of the ADM cellular concentration after 3 hr of treatment. However, for both AD and ADM, the cellular toxicity was only about 2-fold increased.

Effect of verapamil on drug toxicity and cellular accumulation in DC-3F/AD cells

Figure 5 shows that the effects of verapamil on the toxicity and cellular accumulation of 9-OH-E and NMHE by DC-3F/AD cells were markedly different. Verapamil did not change the toxicity of 9-OH-E on these cells. A slightly decreased cellular concentration of this drug was observed and may be related to both a decreased uptake (in the presence of verapamil, the plateau was reached at a concentration about 15% lower than the control) and an increased efflux (the initial rate was higher and the amount of drug remaining tightly associated with the cells was about 2 ng/10^6 cells as compared to about 4 ng/10^6 cells in the control). In contrast, verapamil provoked an 8-fold increase of NMHE toxicity. In the meantime, the uptake of this drug was about 3-fold increased, whereas the efflux was inhibited.

As expected from previously reported results [22, 23], Fig. 6 shows that the effects of verapamil were most pronounced on the toxicity and cellular accumulation of AD and ADM by DC-3F/AD cells. In these cells, the resistance to AD was lowered in the presence of verapamil from 4200-fold to about 54-fold. Correspondingly, the uptake of AD was about 14-fold increased whereas the efflux was strongly inhibited since the amount of the drug retained by the cells after 3 hr efflux was about 10-fold greater in the presence of verapamil (because of the much decreased permeability of DC-3F/AD cells to AD and ADM, the efflux kinetics were determined on cells loaded with either one drug in the presence of verapamil). The cross-resistance to ADM was also markedly decreased, from about 106-fold to 5-fold, and the uptake and efflux modifications were very close to those observed with AD (about a 10-fold increased uptake and a corresponding 10-fold greater amount of the drug associated with the cells after 3 hr of efflux in the presence of verapamil).

DISCUSSION

In addition to the alteration of a specific intracellular target which is responsible for the resistance to DNA topoisomerase II inhibitors [3, 9], the 9-OH-E resistant cells are also cross-resistant to other antitumor agents such as AD, ADM, methotrexate and vincristine [1, 9]. For both AD and ADM, reduced cellular accumulation appears to account for the resistance [1, 9]. These data, together with other properties, demonstrated that membrane alterations, reminiscent of those associated with the MDR phenotype, are also observed in cells resistant

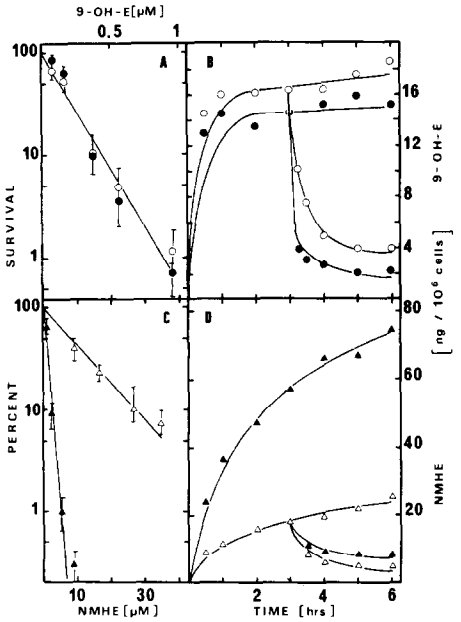

Fig. 5. Effect of verapamil on the toxicity and cellular accumulation of 9-OH-E and NMHE by DC-3F/AD cells. Same legend as Fig. 1. B and D: the cells were incubated with 0.15 μM (0.04 μg/ml) 9-OH-E or 1 μM (0.3 μg/ml) NMHE. The concentration of NMHE used is about 1/10 of the ED_{50} in the absence of verapamil in order to get about 50% survival of cells treated with verapamil: ● and ▲, with verapamil; ○ and △, no verapamil.

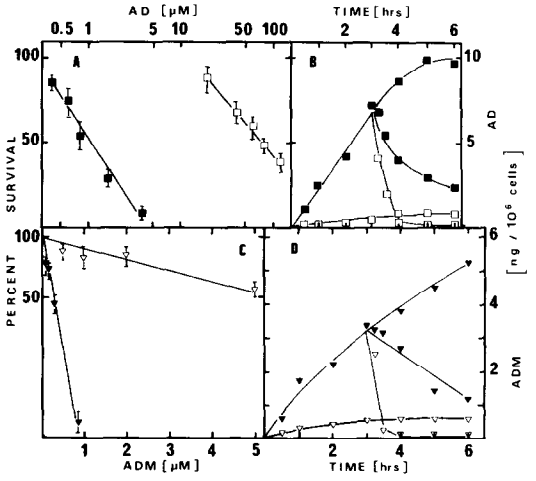

Fig. 6. Effect of verapamil on the toxicity and cellular accumulation of AD and ADM by DC-3F/AD cells. Same legend as Fig. 1. B and D: the cells were incubated with 0.8 μM (1 μg/ml) AD or 0.16 μM (0.1 μg/ml); the efflux rates were measured on cells previously loaded with the drug in the presence of verapamil. The concentrations of AD and ADM were chosen in order to get about 50% survival of cells treated with verapamil. ■ and ▼, with verapamil; □ and, ▽, no verapamil.

Table 2. Relative uptake and toxicities of the different drugs in the presence of verapamil

Drug	DC-3F uptake*	DC-3F toxicity†	DC-3F/9-OH-E uptake	DC-3F/9-OH-E toxicity	DC-3F/AD uptake	DC-3F/AD toxicity
9-OH-E	0.8	0.5	1	1	1	1
NMHE	0.55	0.5	1.4	1	3	8
AD	1	2	1.25	2	14	80
ADM	1.6	2	4.6	2	6	30

* Relative uptake is defined as the ratio of the uptake observed after 3 hr of treatment with the drug in the presence of 10 μg/ml (22 μM) verapamil to the uptake in absence of verapamil (see legend to Fig. 1 for accuracy).

† Relative toxicity is defined as the ratio of the reciprocal of ED_{50} determined in the presence of verapamil to the ED_{50} in absence of verapamil (a value >1 corresponds to an increased toxicity in the presence of VPM, whereas a value <1 corresponds to a decreased toxicity). All the ratios which are different from 1 correspond to significant differences ($P < 0.05$) between the two ED_{50} values as based on the *t*-test for independent random samples.

to ellipticine derivatives. In this work, we have examined the effects of verapamil, a molecule known to overcome the MDR [24, 25], on the toxicity and cellular acccumulation of four drugs either on ellipticine or on multidrug resistant cells. The results are gathered in Table 2.

The most striking features of this study are the following.

(1) In the sensitive DC-3F cells, verapamil provoked a decreased uptake with a corresponding decreased toxicity of the ellipticine derivatives. Current hypotheses on the mechanism of action of verapamil do not provide a simple interpretation for this unusual observation.

(2) In DC-3F/AD cells, a direct relationship between the level of cross-resistance and the molecular weight of the drug has been demonstrated [11]. In general, the greater the molecular weight, the higher the cross-resistance of these cells. Therefore, the lack of cross-resistance to 9-OH-E, which has a molecular weight of 263, was not unexpected. In contrast, the DC-3F/AD cells are about 8-fold cross-resistant to NMHE which has a molecular weight of 277. Verapamil, which had no effect on the uptake of 9-OH-E, provoked a 3-fold increase of the NMHE cellular accumulation by DC-3F/AD cells, and this resulted in a complete reversion of the cross-resistance. These data indicate that, at least to some extent, the mechanism involved in the MDR phenotype is able to protect the DC-3F/AD cells against NMHE but not against 9-OH-E. The only structural trait which might account for this difference is the presence of a methyl group on the nitrogen at position 2 which makes NMHE a quaternary ammonium compound as compared to 9-OH-E.

(3) Verapamil made the cellular accumulation of AD and ADM by DC-3F/9-OH-E cells about identical to what it would be in the parental cells at the same external drug concentration (uptakes of these drugs are proportional to extracellular drug concentrations over the range used, data not shown). However, the toxicity of these drugs is not increased in the same proportion as it is in the DC-3F/AD cells. This is most spectacular with ADM: an almost 5-fold increased uptake of this drug by DC-3F/9-OH-E cells resulted only in a 2-fold increased toxicity, whereas a 6-fold increased uptake by DC-3F/AD cells is associated with a 30-fold increased toxicity (Table 2). Since AD and ADM are also DNA topoisomerase II inhibitors [8, 27], a possible interpretation is that the alteration of this enzymatic activity [9, 10] in the ellipticine resistant cells might account for these results. VPM would restore a normal cellular accumulation of these drugs in the DC-3F/9-OH-E cells, but they would not recognize the modified enzyme. These data would thus indicate that the interaction of AD and ADM with DNA topoisomerase II play a part in the mechanism of the cellular toxicity of these drugs.

The effects of verapamil on the DC-3F/9-OH-E cells are consistent with our previous conclusions [1, 9] showing an alteration of the cellular membrane in these cells. This observation raises such questions as: (i) Is there any relationship between the membrane alteration in DC-3F/9-OH-E cells and any of the membrane modifications which lead to the MDR phenotype? (ii) How such biochemically different events as a membrane alteration and a modification of the DNA topoisomerase II activity can be asssociated in the ellipticine resistant cells. This latter question is particularly puzzling since these cells have been selected for resistance to a drug, the 9-OH-E, which is neither sensitive to the membrane modification which is responsible for the decreased accumulation of other drugs in the DC-3F/9-OH-E cells, nor to the MDR phenotype in the DC-3F/AD cells. We already demonstrated that two of the DC-3F/9-OH-E phenotypic traits, the ellipticine resistance and the loss of tumorigenicity, are the consequences of independent biochemical modifications [28].

It is possible that, during the long term selection process required for the isolation of the DC-3F/9-OH-E cells, multiple biochemical changes accumulated in these cells. Alternatively, these various phenotypic changes, some of which were found to be associated in other antitumor drug resistant cells [11], might be genetically linked.

Further experiments are now in progress to answer these questions.

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