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UPTAKE AND CYTOFLUORESCENCE LOCALIZATION OF ELLIPTICINE DERIVATIVES IN SENSITIVE AND RESISTANT CHINESE HAMSTER LUNG CELLS

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Abstract—Uptake of two ellipticine derivatives, 2-*N*-methyl-ellipticinium (NME) and 2-*N*-methyl-9-hydroxy-ellipticinium, by sensitive and resistant Chinese hamster lung cells was studied. The results show that uptake and retention of these molecules by both types of cells were identical, thus indicating that the resistance to ellipticines, in this system, is not related to an impaired permeability of the cells to the drugs. However, influx and efflux kinetics, as well as experiments at increasing external concns, showed that both drugs accumulate within the cells in different ways. A cellular overconcentration of the drugs, which does not require an energy-dependent process, is observed. Fluorescence microscopy showed that, in both sensitive and resistant cells, NME is mainly, if not entirely, located in the cytoplasm.

Chinese hamster lung cells resistant to the DNA-intercalating antitumoral drugs from the ellipticine series have been selected by growing the cells in the presence of step-wise increasing concns of 9-OH-E‡ [1]. In these conditions, two sublines, with respectively about 10- and 12-fold resistance to this drug, were isolated. Our results showed that, in parallel to the development of resistance to 9-OH-E, the resistant cells undergo several changes: among them were modifications of the morphology and growth parameters, a decreased oncogenic potential, and a cross-resistance to a variety of anti-tumoral agents. In the same cellular system, resistance to actinomycin D and anthracyclin antibiotics was associated with similar modifications of the cell properties [2, 3], and in these cases the drug resistance was attributed to a change in the cell membrane that resulted in a decreased permeability of the cells to the drug.

In order to study the mechanism of cellular resistance to ellipticine derivatives, we investigated the uptake of two ellipticine derivatives by the sensitive and resistant cells: one, NME, is a fluorescent molecule, and the other, NMHE, was radioactively labeled. Uptake and retention of these molecules by the sensitive and resistant cells were identical, thus indicating that the resistance mechanism is not related to an impaired permeability of the cells to the drugs. The uptake kinetics of these derivatives, which only differ from one another by one hydroxyl group at position 9, are markedly different. Yet, both drugs appear to be overconcentrated in the cells by an energy-independent process. Finally, the cellular localization of NME was studied by fluorescence microscopy which showed that, in both sensitive and resistant cells, most, if not all the drug was located in cytoplasm.

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‡ Abbreviations: 9-OH-E, 9-hydroxy-ellipticine; NME, 2-*N*-methyl-ellipticinium acetate; NMHE, 2-*N*-methyl-9-hydroxy-ellipticinium acetate; TPB, tetraphenylboron; MEM, Eagle's minimum essential medium modified; PBS, phosphate buffer saline.

MATERIALS AND METHODS

Cells and culture medium. The Chinese hamster lung cells, DC-3F, and the 9-OH-E resistant sublines have been previously described [1]. Monolayer cultures were maintained in MEM, supplemented with 7% fetal calf serum, streptomycin (50 μg/ml) and penicillin (100 I.U./ml). Resistant sublines DC-3F/9-OH-E 0.3 and 9-OH-E 0.6 were permanently grown respectively in the presence of 0.3 and 0.6 μg/ml 9-OH-E. The drug was removed from the medium for 10–15 days before each experiment.

Chemicals. The structures of both ellipticine derivatives used in this work are shown in Fig. 1. NME was a generous gift from Drs Nguyen Dat-Xuong and E. Lescot (Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France). NMHE, carrying a ^{14}C-labeled methyl group at position 2 (2 mCi/mmole, 600 μg/ml), was synthetized and kindly provided by Dr Van Bac [4] (Institut de Chimie des Substances Naturelles). All chemicals were of reagent grade and obtained from commercial sources.

Uptake of drugs. For kinetics experiments, the cells were plated, about 16 hr before the beginning of the assay, on 35-mm dia. tissue culture dishes (Corning, No. 25000), containing 2 ml of growth medium. The number of plated cells was adjusted to have about 1×10^6 cells/dish at the time of drug exposure. At zero time, the medium was replaced by 1 ml of drug-containing medium [NME or [^{14}C]NMHE (1 μg/ml)]. The cells were then incubated either at 4° or 37° in a humidified incubator

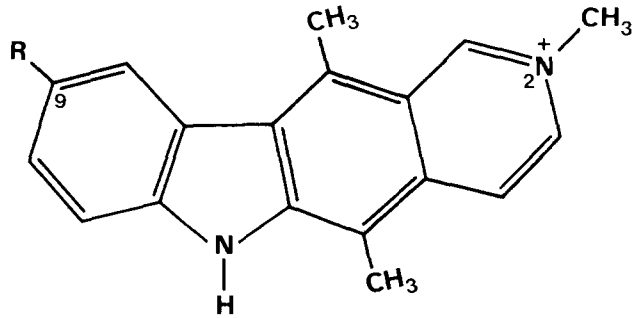

Fig. 1. NME and NMHE structures. Numbering of atoms at positions 2 and 9 are indicated. R = H: 2-*N*-methyl-ellipticinium (NME). R = OH: 2-*N*-methyl-9-hydroxy-ellipticinium (NMHE). Counter ion is acetate for both molecules.

with 5% CO_2 in air. At the indicated times, the cells were washed 3 times with 1 ml of 9% NaCl at 4°, and harvested by adding 1 ml of 0.05% trypsin–0.05% EDTA for 3 min at 37°. The cells were separated from the bulk of surrounding solution by means of a three-component filter assembly derived from that described by Conrad and Singer [5]. The top filter was a 25-mm dia. polycarbonate membrane with an average pore dia. of 2 μm (Nuclepore Corp., No. 110611). the middle filter was a 25-mm dia. glass fiber paper GF/C (Whatman Ltd, U.K.). The bottom filter was a 4 mm thick pad of absorbent white cellulose. The three filters were held together by a weighted stainless steel cylinder with a central core (dia. 19 mm). The cell suspension was placed on the top filter, which retains the cells, whereas the external fluid is rapidly absorbed into the bottom filter. This device allows us to avoid suction which has been shown to provoke a loss of cell cytoplasmic content [5].

For determination of the NME uptake, each filter was transferred to a 25-ml Corex capped tube, and incubated for 1 hr with 2 ml of 0.5 N sodium hydroxide and 20 μl of 0.01 M TPB. Four millilitres of ethyl acetate were then added and mixed. After 1 hr resting, the phases were separated by centrifugation for 5 min. A 3-ml aliquot of the ethyl acetate phase, containing the NME–TPB complex, was evaporated to dryness at 45° under a nitrogen stream, and the residue was dissolved in 0.1 ml of methanol. NME concn was then measured by high-performance liquid chromatography coupled to fluorescence detection, under the conditions described by Muzard and Le Pecq [6].

For [^{14}C]NMHE determination, the filters were transferred, after drying, to scintillation vials. 4.5 ml of permafluor III (Packard Instruments Co.) were added, and the radioactivity was measured in an Inter-technique SL32 liquid scintillation spectrometer.

Efflux kinetics. The cells were plated as described earlier, and incubated for 4 hr in growth medium containing 1 μg/ml NME or NMHE. After washing 3 times with 1 ml of 9‰ NaCl at 4°, the cells were incubated at 37° in 5 ml of drug-free medium. At the indicated times, the amount of drug which remained associated with the cells was determined.

Cell size determination. The average cell size was determined by flow cytometry analysis, carried out with a 6300 A Cytograf coupled to a 2100 Distribution Analyser (Biophysics System Inc.). Polystyrene microspheres of 5.10 and 10.18 μm dias from Duke Scientific (catalogue Nos 269 and 270) were used as reference standards.

Drug metabolism. In order to increase the sensitivity of the analysis, the hydroxylated metabolites of NME must be acetylated to become fluorescent.

After incubation with the drug, the cells were lysed in 1 ml of a solution containing 0.1 M NaCl, 0.01 M Tris–HCl, 0.001 M EDTA and 0.5% SDS. This lysate was then mixed with 2 ml of ethyl acetate and 20 μl of 0.01 M TPB. After 1 hr resting, 2 ml of the ethyl acetate phase were mixed with 300 μl of pyridine, and 150 μl of acetic anhydride, as described by Muzard and Le Pecq [6]. After 10 min at 20°, the yield of the acetylation reaction was close to 100%. Ethyl acetate phase was then evaporated to dryness and the samples were analysed by high-performance liquid chromatography as described earlier. Using pure 9-acetoxy- and 7-acetoxy-ellipticinium as standards, the best resolution was obtained by isocratic elution with a mixture of methanol–ammonium acetate buffer (0.01 M, pH 5.4) (70:30). In these conditions, the limit of detection of the acetoxy derivatives is about 10 ng/ml of the cellular extract.

Fluorescence microscopy. The day before the experiment, 8×10^5 cells were plated on a glass lamella (34 × 34 mm) in a 60-mm dia. Petri dish containing 6 ml of growth medium. After transfer of the lamellas to chambers (5.3 cm^2, 2 ml), the cells were incubated with NME (10 or 20 μg/ml) at 37° for the indicated times. Because we observed that prolonged exposure under the light of the microscope may induce modifications of the intracellular fluorescence distribution, different lamellas were used for each incubation times. Multiple photos of each lamella were taken in a very short time (less than 1 min). Although, under these conditions, they were identical to one another, only the first taken is shown in the Results. These experiments were carried out on a Zeiss photomicroscope III, with a high-pressure mercury source (HBO 50), and the 48 77 06 filters combination ($\lambda_{exc.}$ = 436 nm, $\lambda_{em.} \geq$ 475 nm). Photomicrographs were made either on Kodak Recording Film 2475 (catalogue No. 503 1992) or Ilford HP5 (ASA 400), exposed and developed at ASA 1600.

Isolation of nuclei. Nuclei were isolated by deter-

gent treatment in isotonic medium as described by Vogelstein and Hunt [7]. These nuclei remained attached to the support, and studies on drug accumulation were performed by fluorescence microscopy as described for whole cells. The same method was used to isolate the nuclei from drug treated cells.

RESULTS

Problems raised by the unspecific binding of the drugs

Ellipticine derivatives display a high unspecific binding to the growth supports of the cells. In order to lower as much as possible the resulting background, we examined a number of washing procedures. Although rather drastic, methanol was found to be the most efficient, and was used in preliminary experiments [1]. However, examination of the cells treated with NME in the fluorescence microscope revealed that, as will be discussed later, most of the drug fluorescence is located in extranuclear structures (cytoplasm and/or membranes). In contrast, after washing of the cells with methanol under the conditions used for the uptake experiments, nearly all the drug fluorescence was located in the nucleus (Fig. 2A and B). This observation suggested that, in addition to the removal of the unspecific binding, the methanol treatment was able to concentrate the drug into the nucleus. Such an effect made the interpretation of the uptake and efflux experiments very difficult and, therefore, this procedure was abandoned.

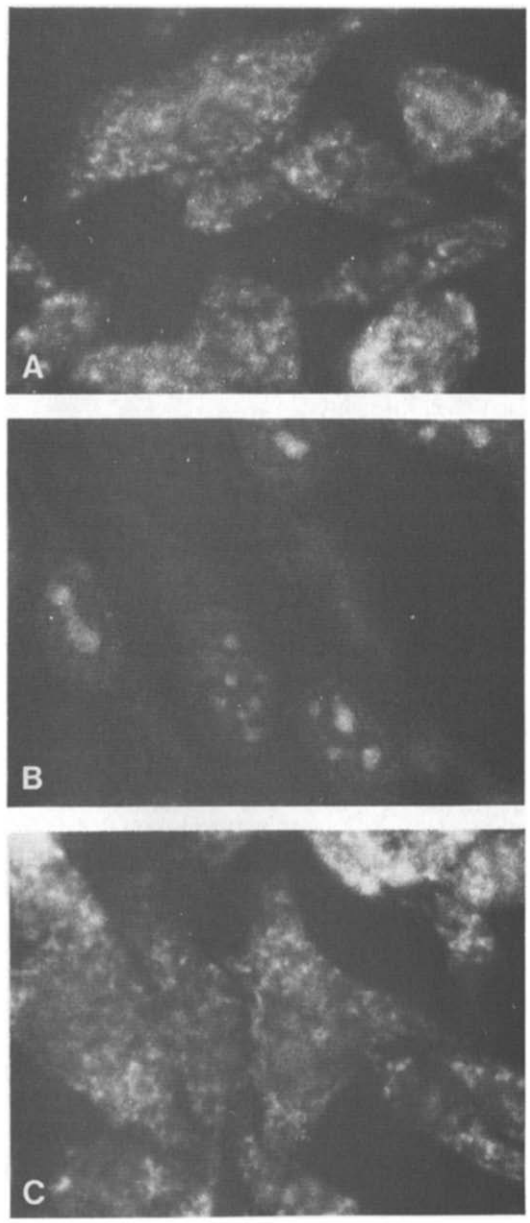

Fig. 2. Fluorescence microscopy of DC-3F cells incubated with NME (A), and then washed 3 times with 1 ml of either ice-cold methanol (B) or 9% NaCl (C). Experimental conditions are given in Materials and Methods. Kodak Recording Film 2475. × 800.

Another washing procedure, combining the use of an aqueous solvent and the detachment of the cells from the support, which was not the case in the methanol procedure [1], was then developed. Among other solvents tested, including MEM and PBS, 9‰ NaCl was found to be acceptable, yielding a background of unspecific absorption lower than 5%. In addition, Fig. 2C shows that the fluorescence pattern of the cells treated with NME was not changed after washing with 9‰ NaCl.

Time-course of NME and NMHE uptake

The time-course of uptake of NME and NMHE by sensitive and 9-OH-E resistant cells was measured by incubating the cells with either one of the drugs at a concn of 1 μg/ml. Fig. 3A shows that, in all cell lines, NME uptake reaches a plateau after about 3 hr of incubation. This plateau is about 15% higher in the resistant than in the sensitive cells. Fig. 3B shows that during the first 2 hr the uptake kinetics of NMHE is identical to the one of NME. After this period, the net uptake rate falls to a level which remains constant for the next 4 hr. Again, similar uptake kinetics were observed in the three cell lines. After 4 hr of incubation, about 80 μg of NMHE and 70 μg of NME were bound per 10^6 sensitive or resistant cells.

In order to refer this amount of drug to the cellular vol., the sizes of the sensitive and resistant cells were determined by flow cytometry. After trypsinization, the average cell dias of the different cell lines were identical, and close to 8 μm. Based on this value, and the assumption that the trypsinized cell is spheric, it was calculated that, after 4 hr of incubation, NME and NMHE intracellular concns were about 300 μg/ml, which represents an overconcentration of about 300-fold, as compared to the external medium.

Effect of temperature and sodium azide on NME and NMHE uptake

The cellular uptake of actinomycin D and several anthracycline antibiotics has previously been reported to be temp-dependent [8–10]. Fig. 3A and B shows that the cellular accumulation of NME and NMHE by sensitive and resistant cells is also temp-sensitive. After 6 hr of incubation with the drugs, the uptakes of NME and NMHE were about

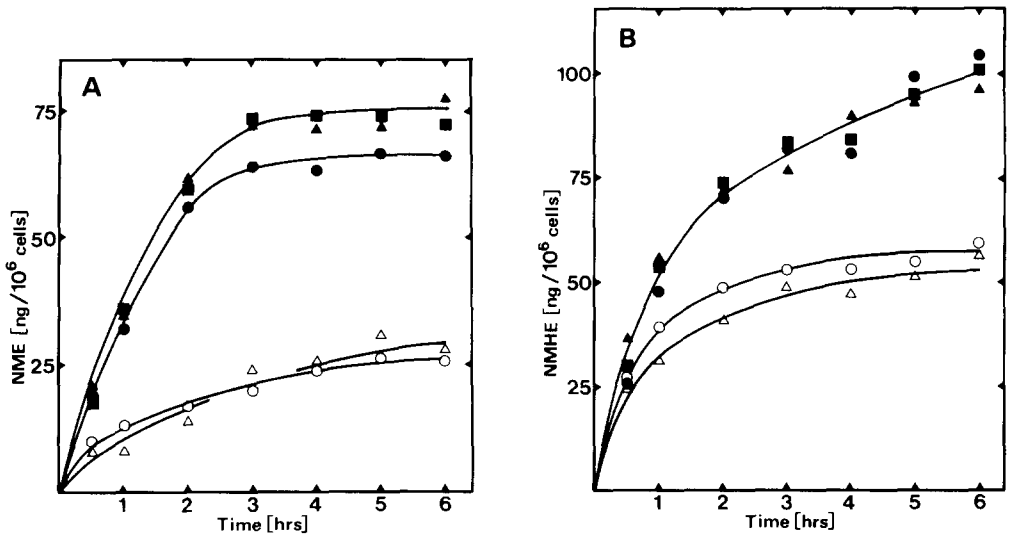

Fig. 3. Time-course uptake of NME (A) and NMHE (B) by sensitive and resistant cells at 4 and 37°. The conditions of the experiments are described in Materials and Methods. Each point represents the mean value of two independent determinations. (●) DC-3F; (■) DC-3F/9-OH-E 0.3; (▲) DC-3F/9-OH-E 0.6. Open symbols: same experiments at 4°.

three- and two-fold lower at 4° than at 37°. However, this residual uptake, at such a low temp, is somewhat higher than the results reported for other DNA-intercalating agents.

Sodium azide (10 mM) had no effect on the uptake of either one of these drugs by sensitive and resistant cells (data not shown), suggesting that the cellular accumulation of NME and NMHE is not under the control of an energy-requiring process.

Uptake of the drugs as a function of cell number

Increasing DC-3F cell numbers were incubated for 4 hr with 1 μg/ml NME or NMHE in 35-mm dia. Petri dishes. Fig. 4 shows that, in the range of 0.5×10^6 to 2×10^6 cells per dish, the drug levels were linearly related to the cell numbers. In both cases, above 2×10^6 cells per dish, this linear relationship was lost, possibly because, under these conditions, cells are approaching saturation density. For this reason, all experiments were carried out at about 1×10^6 cells per dish.

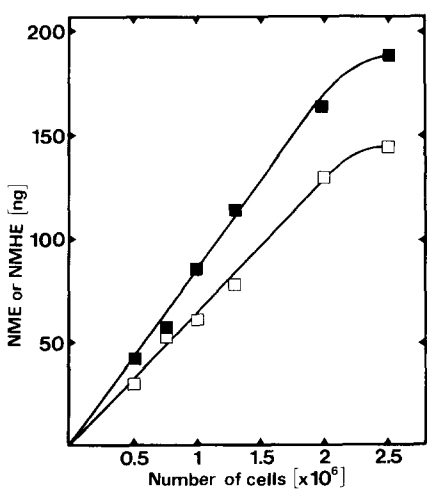

Fig. 4. Effect of cell number on NME and NMHE accumulation. DC-3F cells were incubated either with NME (□) or NMHE (■) at 1 μg/ml for 4 hr. Each point is the mean value of two independent determinations.

Efflux kinetics

NME and NMHE kinetics were measured on the DC-3F parental line and the DC-3F/9-OH-E 0.6 subline, previously loaded for 4 hr with either drug. Fig. 5 shows that the efflux kinetics of NME and NMHE are similar, and identical on both cell lines: after a rapid initial loss during the first 10 min, the efflux slowed down and became negligible for the next 3 hr. This indicates that a major part of these drugs was loosely bound to the cells, while only about 10–15% of NME and 30% of NMHE remained tightly associated with both sensitive and resistant cells. Sodium azide (10 mM) had no effect on the efflux kinetics of either one of these molecules.

Drug uptake at different external concns

DC-3F and DC-3F/9-OH-E 0.6 cells were incubated for 4 hr in growth medium containing increasing NME or NMHE concns. As shown on Fig. 6, the uptake of both drugs by sensitive and resistant cells increased linearly from 1 to 10 μg/ml. Above this concn, NME uptake reached a plateau, up to 50 μg/ml, the maximum tested concn. In contrast, NMHE uptake continued to increase, although with a slower slope, up to 30 μg/ml. However, because of the rather low concn of the radioactively labeled compound, it was not possible to further increase the drug concn in the medium, and to determine the NMHE saturation level in these cells.

Drug metabolism

NME can be metabolized both *in vitro*, in the presence of rat liver microsomes, and *in vivo*, after intravenous administration [11]. Several metabolites

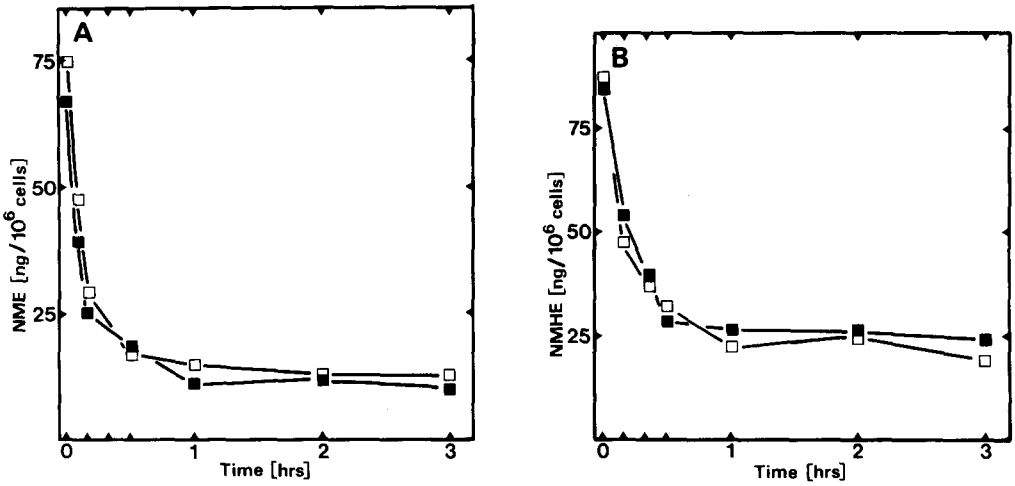

Fig. 5. Efflux kinetics of cellular NME and NMHE. Cells, loaded with NME (A) or NMHE (B) at 1 μg/ml, were then incubated with drug-free medium at 37°. At given times, samples were processed as described in Materials and Methods. Each point is the mean value of two independent experiments. (■) DC-3F; (□) DC-3F/9-OH-E 0.6.

are produced: the major one is NMHE, and small amounts of 2-methyl-7-hydroxy-ellipticinium can also be observed. We have been looking for the presence of these hydroxylated derivatives in cells treated with NME for 2 or 3 hr, at concns of 1, 2, 5 and 10 μg/ml. After extraction, these derivatives were made fluorescent by acetylation, and analysed by high-performance liquid chromatography. Under elution conditions, settled to separate NME from its 7- or 9-acetylated derivatives, the presence of these molecules was undetectable. This indicates that in DC-3F cells the metabolisation of NME to 7- or 9-hydroxylated derivatives, if any, does not exceed the limits of the method, i.e. about 1%.

Cytofluorescence localization

The intracellular distribution of NME was studied by fluorescence microscopy. DC-3F and DC-3F/9-OH-E 0.6 cells were incubated with the drug at 20 μg/ml for different times. Fig. 7A and B shows the patterns of cellular fluorescence in the sensitive and resistant cells after 4 hr treatment. In both cases, the drug fluorescence was essentially located in the cell cytoplasm, while the nuclei appeared as dark cores under these conditions, corresponding to a high drug concn, and to the plateau of penetration. In contrast, at early times, the fluorescence was relatively homogeneously distributed (Fig. 2 shows the pattern observed after 30 min). Similar results were obtained when the cells were treated with the drug at 10 μg/ml (results not shown).

Cells loaded with the drug for 4 hr were then incubated in drug-free medium for three additional hours, and the fluorescence of the non-exchangeable drug was examined. Fig. 7C and D shows that, again in both DC-3F and DC-3F/9-OH-E 0.6, the remain-

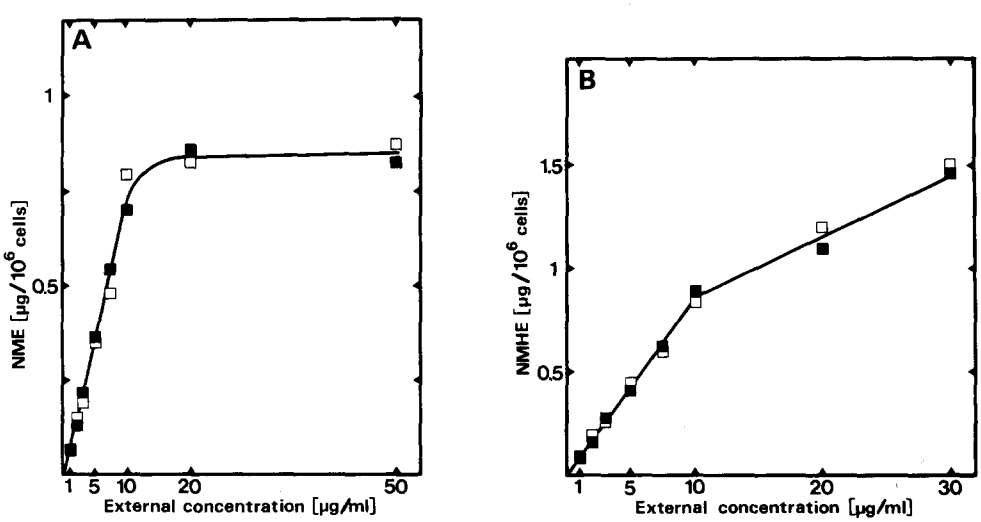

Fig. 6. Uptake of NME (A) and NMHE (B) at different external concns by sensitive and resistant cells. The cells were incubated for 4 hr with the drug at the indicated concns. The amount of accumulated drug was then determined as described in Materials and Methods. (■) DC-3F; (□) DC-3F/9-OH-E 0.6.

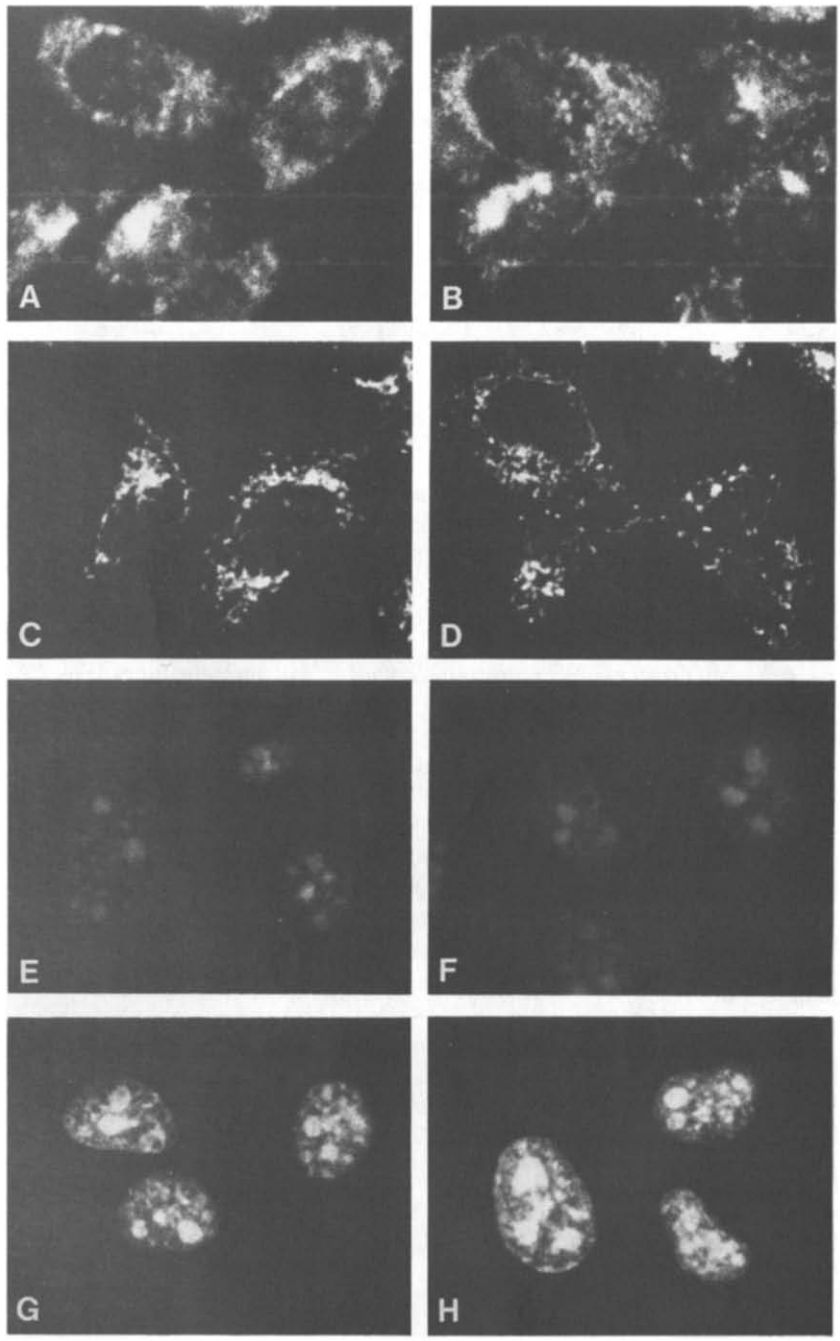

Fig. 7. Cytofluorescence analysis of NME in sensitive (left) and resistant (right) cells. The whole cells were incubated with NME at 20 μg/ml for 4 hr (A–D, G, H). A and B: DC-3F and DC-3F/9-OH-E 0.6 after drug uptake; C and D: DC-3F and DC-3F/9-OH-E 0.6 after 3 hr efflux; G and H: nuclei isolated either from DC-3F or DC-3F/9-OH-E 0.6 as described in Ref. 7; E and F: isolated nuclei from DC-3F and DC-3F/9-OH-E 0.6 were incubated with 0.1 μg/ml NME. For experimental details, see Materials and Methods. Ilford HP5. × 800.

ing drug appeared as fluorescent granulations in the cell cytoplasm. Clearly, efflux resulted in the disappearance of the diffused cytoplasmic fluorescence, observed in Fig. 7A and B, while the fluorescence intensity of the granulations, which represent the non-exchangeable fraction of the drug, remained almost unchanged. So far, the identity of these granulations, which behave as strong binding sites of the drug, has not been established.

Isolated nuclei were then incubated with NME at different concns, ranging from 0.1 to 20 μg/ml. After 1 min of incubation, at the lowest concn (0.1 μg/ml), Fig. 7E and F shows that the nuclei isolated from sensitive and resistant cells displayed already a similar and clearly detectable fluorescence. Furthermore, this fluorescence progressively increased with the drug concn. This indicates that the lack of intranuclear fluorescence in the living cells is not likely

to result from a fluorescence quenching in the nuclei. It then follows that the NME intranuclear concn remains very small as compared to the cytoplasmic concn.

Although the technique does not allow a precise quantitation, one can estimate, from the variation in fluorescence intensity observed in the range of drug concns applied to the isolated nuclei, that the intranuclear concn in whole cells should not exceed about 1%.

Finally, cells loaded with the drug, as described in Fig. 7A and B, were treated with the non-ionic detergent NP-40 for 3 min at 4°. As previously described [7] under defined conditions, the cell membranes and cytoplasm are removed, while the nuclei remained attached to the support. Fluorescence microscopy then showed that these nuclei displayed an intense fluorescence, specially emphasized in nucleolar structures. This experiment shows that detergent treatments, which are usually involved in subcellular fractionation, provoke a drug translocation from the cytoplasm to the nucleus, and then may lead to artefactual results.

As already mentioned in Materials and Methods comparable drug translocation can also be observed after prolonged exposure of the cells under the light of the microscope. Whether it results from a light and/or a temp effect is now under investigation. Yet this observation indicates that, when the drug is present, an intranuclear fluorescence is visible *in situ*, in the absence of any modification provoked by a detergent.

DISCUSSION

Cellular resistance to numerous DNA-intercalating agents has been developed in a variety of cell lines and experimental tumors. In many cases, development of resistance to these drugs was accompanied by a reduction in the uptake and/or the retention of the drug by the cells (reviewed in Refs 2, 3 and 12). Therefore, the most straightforward approach to the understanding of the mechanism of resistance to 9-OH-E was to compare the cellular drug accumulation by the sensitive and resistant cells. For analytical reasons, this study was carried out on two ellipticine derivatives to which the cells are cross-resistant: NME was chosen because its fluorescence properties allow measurements of the cellular binding with the same order of sensitivity as that obtained with the radioactively labeled NMHE.

This study shows that each of these drugs is accumulated in sensitive and resistant cells at the same level, thus indicating that, in these cell lines, resistance to ellipticine derivatives is not related to a decreased penetration and/or retention of the drug. *In vitro* selection of resistant cells by continuous exposure to increasing 9-OH-E concns makes it unlikely that one could select for a subline with a decreased retention which, under these conditions, would not be a critical factor. In contrast, the absence of uptake modifications was somewhat unexpected. As already pointed out, analysis of the properties of the 9-OH-E resistant cells indicates that the cell membrane has been modified in such a way that, among other things, uptake of actinomycin D by these cells was impaired. Alternatively, uptake of NMHE by D/AD X, a highly actinomycin D resistant subline [2], which is also about 10-fold cross-resistant to ellipticine derivatives, was not modified [1]. These results suggest that the cells cannot become resistant to ellipticine derivatives by decreasing their ability to take up these drugs. Such a resistance might rely on a mechanism more closely related to the precise mechanism of action of ellipticines, which is as yet unknown.

Cellular accumulations of NME and NMHE, which only differ from one another by one hydroxyl group, are markedly different. Thus, NME uptake is saturable, while NMHE uptake appears as a biphasic process. On the other hand, as already demonstrated with actinomycin D [13] and anthracyclines [10], uptake of these drugs is temp-dependent, although to a lesser extent. Physical basis and physiological consequences of these differences are unknown. Both drugs display about the same DNA binding parameters (affinity constants and unwinding angles [14]), and comparable cytotoxicities to the sensitive as well as to the resistant cells [1].

An intracellular metabolism of these drugs might provide a clue to explain these differences. Since the hydroxylation of NME at position 9, and eventually 7, had been demonstrated in other *in vitro* and *in vivo* systems, we attempted to detect these derivatives in NME-treated cells. Considering the sensitivity of the technique, failure to detect these molecules shows that such a metabolism, if any, should be very limited, and cannot account for the differences between the drugs. Although they cannot be presently excluded, other types of metabolic transformations of these molecules have not yet been observed.

The results presented in this paper also show that, at the equilibrium corresponding to our experimental conditions, NME cellular uptake represents an overconcentration of about 300-fold, and NMHE can even reach higher levels. This observation is not peculiar to ellipticine derivatives, and comparable values can be obtained with other DNA-intercalating agents, such as actinomycin D [1, 2] and daunomycin [10]. The uptakes of NME and NMHE are not sensitive to sodium azide, a most usual metabolic inhibitor, thus suggesting that they do not involve an energy-requiring process. This indicates that, once inside the cells, the drugs have to be bound to structures which display a high drug affinity and/or numerous binding sites. It was, then, of interest to study the subcellular localization of the ellipticine derivatives. Cytofluorescence analysis has shown that most, if not all the NME is located in the cytoplasm of the living cell, yet this technique does not allow us to exclude the possibility that a small fraction of the drug, which would be physiologically important, might be located in the nucleus. Detection of such a fraction would require quantitative techniques, involving subcellular fractionation. However, this approach is usually based on the use of detergents. We have shown here that NP-40 is able to induce changes in the drug cellular distribution, the most obvious being the drug translocation to the nucleus. Therefore, these fractionation techniques cannot be used in our system. In fact, the

same effect was also observed with other fluorescent derivatives in the ellipticine series, thus suggesting that detergents should generally be used with caution in this type of studies.

The cytoplasmic localization of these ellipticine derivatives indicates that, in addition to nucleic acids, other potential binding sites should be considered. For example, the cytoplasmic membrane network might be an important binding site for drugs which are known to be lipophilic [15, 16]. Examples of such a behavior for lipophilic basic drugs have been previously described [17, 18]. However, extention of these results to other ellipticine derivatives, as well as the role that such a binding could play in the mechanism of action of these drugs, remains to be studied. For example, Egorin *et al.* [19], who studied the intracellular localization of a series of anthracycline antibiotics by fluorescence microscopy, have shown a great variability between the different drugs, and found it very difficult to predict the consequences of this variability on the pharmacological properties of these molecules.

In conclusion, our results show that drug accumulation by the cells does not by itself account for cellular sensitivity or resistance to antitumoral agents in the ellipticine series. According to cytofluorescence analysis, most of the drug appears to be located in the cell cytoplasm. However, the development of cellular fractionation techniques, avoiding the detergent artefacts outlined earlier, which are now in progress in our laboratory, should provide a more accurate knowledge of the cellular localization of these drugs.

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