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EFFECTS OF LYSOSOMOTROPIC DETERGENTS ON THE HUMAN MALARIAL PARASITE *PLASMODIUM FALCIPARUM* IN *IN VITRO* CULTURE

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Abstract—Various lysosomotropic detergents were tested in this work on *in vitro* cultures of *Plasmodium falciparum* and are shown to be potent antimalarial agents. The order of antimalarial potency was similar to that of cell toxicity on mammalian cells in culture (Miller DK *et al.*, *J Cell Biol* **97**, 1841–51 (1983)). The most efficient agents, *N*-dodecyl-imidazole (NDI) and *N*-dodecyl morpholine (NDM) displayed IC_{50} values of 6.7 ± 0.7 μM and 23 ± 5 μM. The mechanism of action of NDI measured at IC_{50} concentrations displayed the following features: irreversible antimalarial effect after 15 min exposure of cells to drug; alkalinization of the parasite food vacuole; inhibition of protein synthesis; inhibition of host cell protein digestion by the parasite; lack of vacuolar membrane disruption; lack of effect on the rate of constitutive autoproteolysis. No biochemical or ultrastructural indications were found to support a detergent-like action of NDI and its structural congeners on the major acidic compartment of the parasite, the food vacuole. Rather, alkalinization of that compartment by weak-base accumulation properties of the amphiphilic drugs and ensuing protonophoric effect are likely to play a major role in the various parasite-associated properties affected by these drugs.

Intraerythrocytic malarial parasites digest their host cell cytosol for the provision of amino acids needed for protein synthesis [1]. The human parasite *Plasmodium falciparum* does so by endocytosis into small vesicles, which migrate from the parasite surface to the central food vacuole, where the vesicle contents are delivered and hydrolysis takes place [2]. As the pH of the vacuole is demonstrably acidic (pH 5–5.2) [3, 4], it serves as the major accumulation site for weak bases, including some of the most important antimalarial drugs, such as quinine and chloroquine (see Ref. 5 for a recent review). High level accumulation of antimalarial drugs in parasites causes inhibition of feeding [6] and of protein synthesis [7]. Other weak bases are also expected to accumulate in this organelle, including the amphiphilic bases thought to possess potential detergent activity, first introduced by Firestone *et al.* and coined as lysosomotropic detergents [8].

Lysosomotropic detergents were studied in various mammalian cells [9], and were shown to accumulate inside their acid lysosomes, causing their pH to rise, the synthesis of protein and RNA to decrease and macromolecules to be released from the cells, leading eventually to cell death. It was suggested that the amphiphilic amines could accumulate inside the lysosomes and reach their critical micellar concentration. At those levels they could cause disruption of the lysosomal membrane, thus leading to the release of hydrolases into the cytosol and to cell death. Based on these properties, lysosomotropic detergents were thought to possess potential antimalarial activity.

In the present work we tested the effects of various lysosomotropic detergents on *P. falciparum* grown in culture. We show that they inhibit, efficiently, parasite growth, they inhibit protein synthesis, and they increase the pH of the parasite food vacuole and inhibit the degradation of host cell cytosol. No evidence was found to support the idea that any of the above properties were caused by major distruption of organellar membranes as a result of detergent-like action.

MATERIALS AND METHODS

Cultures. Cultures of *P. falciparum* (FCR_3 uncloned strain from Gambia, obtained from Dr. J. B. Jensen, and ITG-2F6 cloned strain from Brazil, obtained from Dr. L. H. Miller) were grown in culture flasks containing growth medium (RPMI-1640 from Gibco, supplemented with 25 mM HEPES, 32 mM Na-bicarbonate, 10 mM glucose and 10% heat-inactivated AB^+ or A^+ plasma) and either O^+ or A^+ washed human erythrocytes at 2–2.5% hematocrit. The growth medium was replaced daily and the cultures were gassed with a mixture of 90% N_2, 5% CO_2, and 5% O_2. Cells were normally harvested or subcultured when reaching 15–20% parasitemia (determined microscopically by thin blood smears stained with Giemsa). Trophozoite and early schizont stages were routinely isolated by the gelatin flotation method as described elsewhere [10].

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Chemicals. *N*-Dodecyl imidazole (NDI), *N*-dodecyl morpholine (NDM), *N*-dodecyl, *N*-(2,2-difluoroethyl)amine (NDFEA) and *N*-dodecyl, *N*-(2,2,2-trifluoroethyl)amine (NDTFEA) were synthesized as described in detail elsewhere [8]. Solutions of 1–10 mg/ml were prepared as stocks in DMSO just before use. Preweighed samples of the various agents were kept dry in sealed ampoules and reconstituted immediately before use. Cyclohexamide (CHX) and Acridine Orange (AO) were from Sigma Chemical Co. and all other chemicals were of the best available grade.

Tritium labeled isoleucine, [^{3}H]Ileu (2 Ci/mmol), was obtained from the Radiochemical Centre (Amersham, U.K.). All solutions were prepared in deionized and glass-distilled water and the osmolarities were determined on a Wescor osmometer.

Effect on parasite growth. Cultures of parasites were synchronized according to the method of Lambros and Vanderberg [11] except that sorbitol was substituted by the relatively less toxic alanine. After reaching the trophozoite stage (10–20% parasitemia), the cells were washed aseptically with growth medium, resuspended to a 2% hematocrit (1–2% parasitemia) and distributed into wells of a 24-well microtiter plate (0.6 ml/well). Inhibitors were added to the indicated concentrations, the plate transferred to a candle jar and incubated at 37° for the indicated periods of time (0–4 hr). After that period, the supernatant was removed, the cells were washed with fresh growth medium and returned to culture conditions (without inhibitor) for an additional 18 hr period. Samples were withdrawn for microscopic estimation of the newly formed rings and [^{3}H]Ileu was added to a final activity of 4 μCi/ml. Following an additional 24 hr incubation period, cells were transferred in triplicate samples to 96 well plates and harvested in a Cell Harvester (Dynatech Inc). The filters were washed with distilled water, then dried for 2 hr at 60° and transferred into toluene-based scintillation fluid for counting of radioactivity. Control samples of uninfected cells and of infected cells exposed to different concentrations as in the different systems, were subjected to the same treatments as those described above. Where indicated, cells were exposed to inhibitors for a continuous 22 hr period.

The IC_{50} values of inhibition of growth were calculated from the profiles of parasite growth obtained in the presence of various concentrations of inhibitor using the equation:

$$y_i = y_{i\infty} + (y_{i0} - y_{i\infty})/[1 + (i/IC_{50})^f]$$

where y_i is the amount of [^{3}H]Ileu incorporated into protein in the system subjected to a given concentration of inhibitor i, y_{i0} is at $i = 0$, $y_{i\infty}$ is at that i concentration where the inhibitory effect is maximal and f is a scaling factor [12]. The IC_{50} ± SD and the correlation coefficients values were obtained by non-linear regression analysis of the data according to the above equation, based on the Lavenberg–Marquardt algorithm. The analysis was done on a Wang PCSII system.

Effect on protein synthesis. The effect of NDI on [^{3}H]Ileu incorporation into parasite proteins was assessed essentially as described above for parasite growth, except that [^{3}H]Ileu and either the test agent in DMSO or DMSO alone, were added to the cultures and were present during the indicated time of incubation (0–4 hr). Triplicate samples from each system were processed for determination of the amount of [^{3}H]Ileu incorporation into proteins as described above.

Effect on protein degradation. Cultures which had reached the ring stage (50 ml, 2% hematocrit, 25% parasitemia) were supplemented with [^{3}H]Ileu (4 μCi/ml final radioactivity) and grown in culture conditions for 18 hr. The cells were washed 4 times with PBS containing 10 mM glucose (PBS-G) prewarmed to 37°, and resuspended to a 5% hematocrit suspension in the presence of NDI (0 or 20 μg/ml) ± cycloheximide (5 μg/ml). At the indicated times of incubation at 37°, triplicate samples of 50 μl were withdrawn from the suspension and added to 5 μl of TCA (50% w/v) placed in an Eppendorf centrifuge tube. After centrifugation, an aliquot of 40 μl of the supernatant was taken from each system and the radioactivity counted. The total radioactivity of the suspension was obtained by diluting 50 μl of the suspension in 1.5 ml distilled water, taking 300 μl of the lysate supplemented with 25 μl of 30% H_2O_2 and counting the radioactivity.

Effect on parasite acidic compartments. Cultures of parasitized cells which had reached the trophozoite stage (25% parasitemia) or were enriched in trophozoites (85% parasitemia) by the gelatin flotation method [10], were washed in RPMI 1640 medium lacking bicarbonate and Phenol Red (spectroscopy medium, SM). An aliquot of cells was suspended in a cuvette containing 2.5 ml SM (0.2–0.8% hematocrit) and placed in Spex Fluorolog II spectrofluorometer thermostated at 37° with continuous stirring. Time dependent changes in fluorescence were monitored at 495 nm excitation and 525 nm emmission in a ratio mode. Acridine Orange was added to a final concentration of 1 μM and 1–10 μl aliquots of NDI or NDM were added from a stock solution of 1.0 mg/ml (in DMSO). At the end of the experiment, carbonylcyanide-*m*-chlorophenyl hydrazone (CCCP) was added to a final concentration of 10 μM. The pH difference between the parasite food vacuole and the extracellular medium was calculated as described before [3], considering that Acridine Orange is dibasic and taking into account the hematocrit, the parasitemia and the relative volume of the food vacuole [2].

Effects on parasite feeding. Cultures at 20–25% parasitemia, trophozoite stage, were washed twice with PBS-G. Cells were resuspended in the same medium at 2–2.5% hematocrit, without any additives, with 10 μg/ml NDI, or with 25 μg/ml NDM, or with the equivalent volume of DMSO alone, and incubated at 37°. Aliquots of 300 μl were taken at 0, 30, 60 and 90 min and mixed with 15 μl of 100% (w/v) trichloroacetic acid (TCA). The concentration of amino acids in the supernatant was determined by the fluorescamine method [13].

Electron microscopy. Cells (1 ml, 2% hematocrit, 90% parasitemia) were treated for up to 4 hr with NDI (50 μg/ml) under conditions analogous to those described for parasite growth. After centrifugation and washing with phosphate buffered saline (10 mM

Na–phosphate, 145 mM NaCl, pH 7.4), the cells were washed again and resuspended in isotonic sucrose buffered with cacodylate. The cells were fixed with 2% glutaraldehyde for 1 hr, washed thrice with the sucrose based buffer and fixed again with OsO_4 (1%) at 5° for an overnight period. After washing with distilled water, the cells were stained with uranyl acetate (1%) in buffer lacking sucrose (30 min at room temperature), washed, dehydrated with ethanol, embedded in Epon for microtome sectioning and contrast-stained with uranyl acetate–lead citrate, essentially as described previously [2]. The samples were examined with a Philips 300 electron microscope.

RESULTS

Dose–response relationships of acidotropic detergents

When cultures of *P. falciparum* were asynchronously grown in the presence of the various *N*-dodecyl derivatives (0.5–500 μg/ml final concentration), parasite development was markedly reduced. The IC_{50} values of [^{3}H]Ileu incorporation into cultures exposed for 24 hr to the various derivatives increased in the order NDI < NDM < NDDFEA < NDTFEA as given in Fig. 1. With NDI, the most potent of the agents tested (IC_{50} = 6.7 ± 0.7 μg/ml (=28.4 μM)), significant hemolysis (5%/hr) of both uninfected and infected cells was noticed at concentrations >80 μg/ml. In systems consisting of only DMSO added, i.e. in the absence of inhibitor, up to 30–40% increase in [^{3}H]Ileu incorporation was repeatedly seen and was taken into consideration for the calculation of % inhibition.

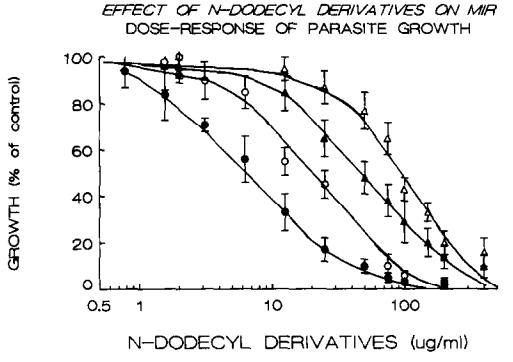

Fig. 1. Dose–response curves of *N*-dodecyl derivatives on parasite growth. Cultures of *P. falciparum* which have reached the trophozoite stage (2% hematocrit, 1% parasitemia), were grown in culture conditions in the presence of the indicated concentrations of drugs. After 22 hr, [^{3}H]Ileu was supplemented and the cultures incubated overnight as described in Methods. Cells were harvested and the [^{3}H]Ileu incorporated into protein was determined. Data are given as % incorporation of Ileu into cells exposed to the corresponding concentration of drug relative to control cells (no drug, but the same final concentration of DMSO as added with the drug). Symbols are: (●) NDI, (○) NDM, (▲) NDDFEA and (△) NDTFEA. The lines were drawn through points generated by the non-linear regression fit of:

$$y_i = y_i + (y_{i0} - y_i)/[1 + (i/IC_{50})^f]$$

(see Methods). The IC_{50} ± SD values (in μg/ml) for the different drugs were: NDI: 6.7 ± 0.7; NDM: 23 ± 5; NDDFEA: 51 ± 8; NDTFEA: 98 ± 12.

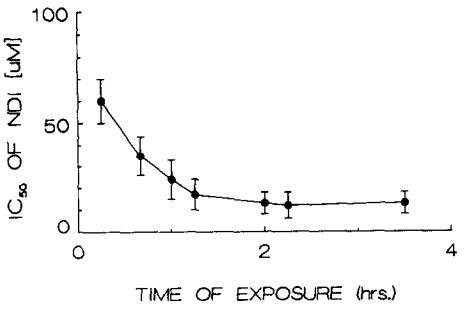

Fig. 2. Time dependence of growth inhibition by NDI. Cells (2% trophozoites, 2% hematocrit) were exposed to different concentrations of NDI for different periods of time as described in Fig. 1, washed of NDI and returned to culture conditions. The IC_{50} values of NDI on parasite growth were obtained from inhibition profiles analogous to those shown in Fig. 1.

Time dependence of inhibitory effect

Because of the good reproducibility of results obtained with NDI and because it is the most potent of all the agents tested in the work, we concentrated most of our effort on determining its *modus operandi*. We first determined the relationship between the inhibition of parasite growth and the time of exposure to the drug and the reversibility of the inhibitory effect. Cultures containing trophozoites were treated with various concentrations of NDI for various periods of time, washed extensively and put back into culture conditions. As shown in Fig. 2, irreversible inhibition was observed already at the shortest time of exposure (15 min) (IC_{50} = 60 ± 10 μg/ml). The inhibitory effect increased progressively for the first 1.5 hr. The value of IC_{50} of 6.7 ± 0.7 μg/ml was apparently stable for 2 to 48 hr exposure to NDI. The results indicate that the extent of irreversible damage to parasite growth by NDI depended not only on the concentration of drug but also on the time the cells were exposed to the drug.

Effect of NDI on parasite ultrastructure

The acidotropic NDI is considered to act as a lysosomotropic detergent in fibroblasts [9] as a result of its weak base properties and amphilic character. So as to ascertain whether NDI accumulation in the parasite food vacuole could lead to structural damage of the food vacuole membrane, cells exposed for 2 hr to 100 μg/ml drug were examined by electron microscopy. As seen in Fig. 3, no detectable alterations were apparent in the food vacuole compartment and no major damage could be observed in the cytosol of either the host or the parasite. The only ostensible change observed in 10–15% of the sections was in the organization of the ribosomes, which appeared aggregated as a collar around the food vacuole (Fig. 3, inset). Similar ultrastructural changes were observed in parasites treated with the antimalarial drug mefloquine [14].

Effect of NDI on degradation of parasite proteins

Although no disruption of the food vacuole was apparent at the ultrastructural level, it seemed

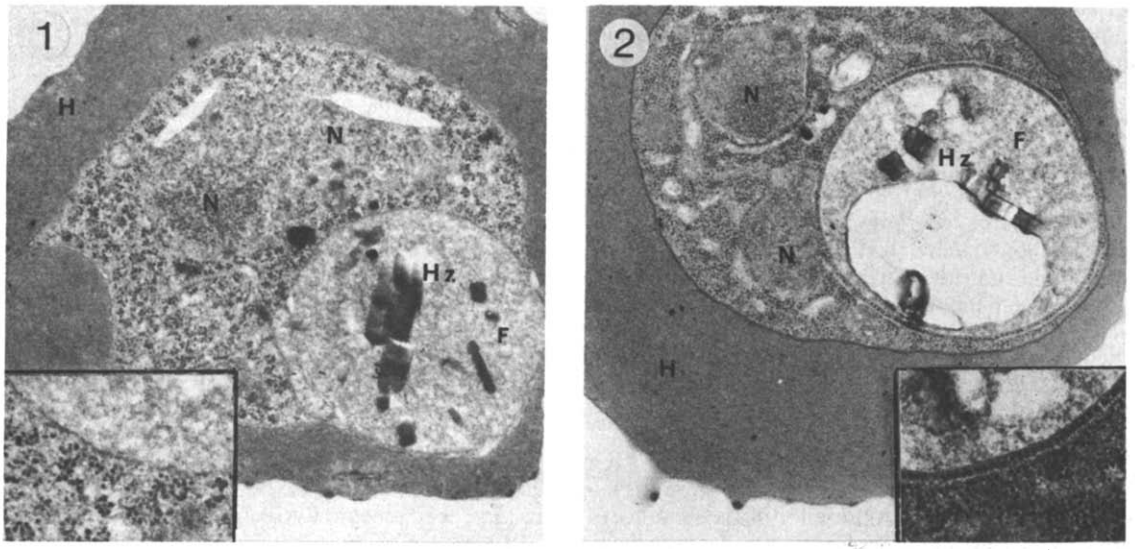

Fig. 3. Ultrastructure of NDI-treated cells. Left (1): untreated cell; right (2): cell treated with 100 μg/ml NDI. Magnification: 52,500. F: parasite food vacuole with hemozoin crystals (Hz); N: parasite nucleus; H: red cell hemoglobin. The inset (150,000) depicts an enlarged area of the parasite vacuole membrane.

important to ascertain that this was also true at the biochemical level, i.e. that proteases were not released with consequent autoproteolysis. So as to test this possibility, parasites were metabolically labeled with [^{3}H]Ileu and subsequently incubated in buffered medium supplemented with glucose. As seen in Fig. 4, the rate of autoproteolysis was relatively low in the untreated system, whereas in the presence of cycloheximide, the rate increased by almost 12-fold. This clearly indicates that most of the released [^{3}H]Ileu is recycled back into proteins, unless the latter process is blocked. Addition of NDI led to a 5-fold increase in amino acid release, but in the presence of cycloheximide and NDI additional release was observed only to the level induced by cycloheximide alone.

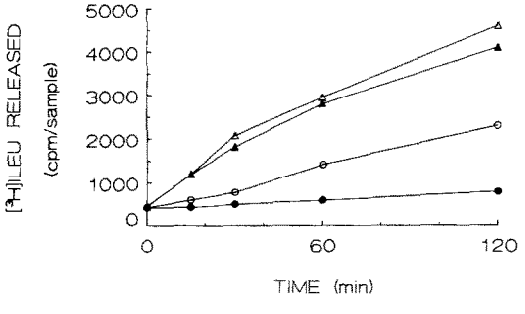

Fig. 4. Effect of NDI on protein degradation. Cells prelabelled with [^{3}H]Ileu were harvested at the trophozoite stage as described in Methods, washed of extracellular label and resuspended in phosphate buffered saline supplemented with 10 mM glucose (●) and: 20 μg/ml NDI (○), or 5 μg/ml cycloheximide (△), or both NDI and cycloheximide (▲). Data are given as TCA soluble label released from cells as a function of time of incubation.

Effect of NDI on protein synthesis

Although no molecular interpretation could be attached to the apparent structural modification caused by NDI treatment of cells, it was reasoned that morphological changes in ribosomal organization could be associated with alterations in the protein synthetic capacity of the parasites. As seen in Fig. 5 (inset), exposure to NDI led to a marked inhibition of protein synthesis. The inhibitory effect was apparent at the earliest time of sampling (30 min) and at the lowest concentration of drug (10 μg/ml). As previously shown [15], the rate of protein synthesis was clearly biphasic in the control system, the latter displaying a 5-fold increase in rate after the

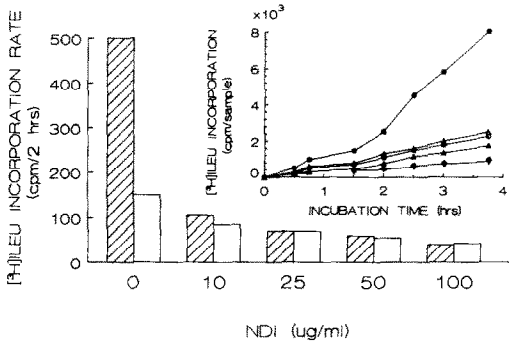

Fig. 5. Effect of NDI on protein synthesis. Cells were exposed to various concentrations of NDI as described in Fig. 2, except that [^{3}H]Ileu (1 μCi/ml) was present during the incubation periods indicated in the figure inset; NDI (μg/ml) : 0 (●), 10 (△), 25 (○), 50 (▲) and 100 (◆). Data are given as [^{3}H]Ileu incorporation (cpm/sample, inset) and as rate of incorporation (cpm/2 hr) in the first 2-hr period (blank box) and the second 2-hr period (hatched box).

initial 2 hr period of incubation (Fig. 5). On the other hand, the NDI-treated systems showed a steady but reduced rate of protein synthesis over the entire 4 hr period of incubation. Therefore, the inhibitory effect was apparently more pronounced in the second phase (2–4 hr).

Effect of NDI on parasite food vacuole pH

NDI was designed as an acidotropic agent and as such could alkalinize the major acidic compartment of the parasite, i.e. the food vacuole, and compromise the feeding process. We therefore assessed its ability to dissipate the pH gradient between the food vacuole and the extracellular medium. Using Acridine Orange as a fluorescent weak base, we observed that its accumulation into cells of a trophozoite enriched culture (85% parasitemia) was markedly reduced as a result of adding NDI (Fig. 6). Since similar changes in dye accumulation could be obtained by adding to cells either the protonophore CCCP or other weak bases (not shown), we interpreted the effects of NDI as alkalinization of the vacuolar contents. The transvacuolar pH gradient calculated from AO accumulation is some 0.4 pH unit smaller than that determined previously by other methods [3, 4], suggesting that AO accumulation may not be adequate for precise determination of vacuolar pH. Nevertheless, its response to CCCP indicates that it is reflecting changes in the vacuolar pH. As seen in Fig. 5, significant alkalinization was accomplished already at 0.4 μg/ml NDI and the maximal effect was obtained at 15 μg/ml NDI. With normal cultures (25% parasitemia), a similar picture

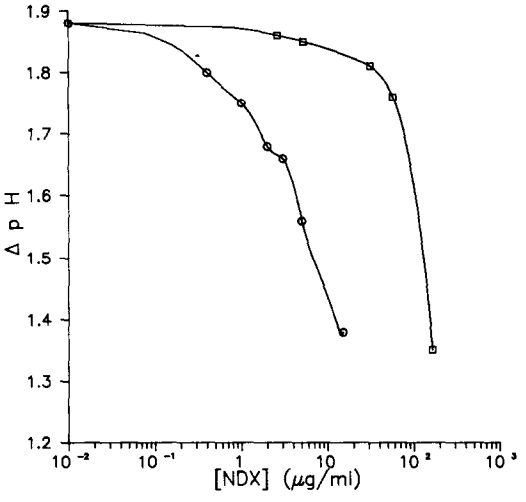

Fig. 6. Effect of NDI and NDM on the pH of the parasite food vacuole. Accumulation of Acridine Orange into trophozoites (0.2% hematocrit, 85% parasitemia) was monitored as described in Methods. Aliquots of NDI and NDM were added from stock solutions and the change in the fluorescence signal was recorded. The pH difference between the parasite food vacuole and the extracellular medium was calculated, considering the relative volume of the food vacuole (the site of dye accumulation) and the change in the fluorescence signal. NDX represents the *N*-dodecyl-derivative of imidazole (NDI) (○) or morphine (NDM) (□).

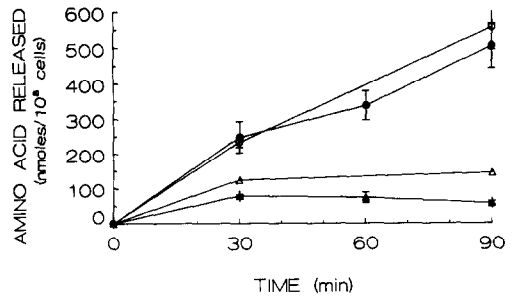

Fig. 7. Effect of NDI and NDM on parasite feeding as followed by amino acid release. Cultures (20–25% parasitemia, 2–2.5% hematocrit) were incubated at 37° in PBS-G (●), supplemented with 1% DMSO (○) and either 10 μg/ml NDI (▲) or 25 μg/ml NDM (△). Samples were taken at different time intervals and the concentration of free amino acids was determined by the fluorescamine method.

was obtained (not shown), except that alkalinization was less pronounced (maximal effect was observed at approximately 30 μg/ml NDI). That difference is accounted for by an apparent binding of NDI to uninfected cells, a property which would affect the effective concentration of drug in the medium. Similar experiments with NDM resulted in a much smaller effect: slight alkalinization was achieved only above 3 μg/ml and the full effect was observed above 160 μg/ml.

Effect on parasite feeding

Malarial parasites digest their host cell cytosol by producing amino acids, most of which egress from the infected cell [6]. Assay of amino acid production by infected cells serves as a measure of the feeding process. In the presence of NDI or NDM at their respective IC_{50} concentrations, the production of amino acids by infected cells is immediately and totally blocked (Fig. 7). DMSO used as a solvent for the acidotropic detergents had no effect on this process.

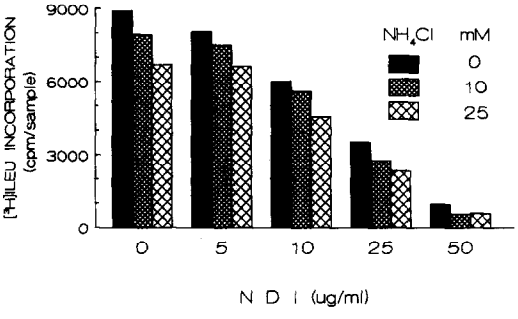

Fig. 8. Effect of ammonium chloride on NDI inhibition of parasite growth. Cells (hematocrit 2%, trophozoites 85%) were treated for 1 hr with the indicated concentration of NDI in the presence of several concentrations of NH_4Cl (AmCl). After washing, the cells were returned to culture conditions and processed for measurements of [^{3}H]Ileu incorporation as shown in previous figures.

Effect of NH_4Cl pretreatment of cells on NDI inhibition of growth

The possible association of growth inhibition of parasites by NDI with NDI acidotropic effects was assessed also by a method known to reduce weak base accumulation in acidic compartments without compromising parasite viability [16]. Pretreating parasitized cells with NH_4Cl (25 mM) for a 1 hr period of incubation had a minor effect on subsequent parasite growth whereas pretreatment with NDI (0.212 mM) was highly effective in reducing parasite viability (Fig. 8) as it did with BHK cells [9]. However, in the presence of both agents, no apparent protection from NDI was obtained. On the contrary, some additive effect of both agents was demonstrated, suggesting that NDI inhibition of parasite growth was probably not associated exclusively with the acidotropic effect of the drug.

DISCUSSION

The effects of lysosomotropic detergents on cultivated human malarial parasites are in various respects similar to their effects on mammalian cells [9]: (1) They inhibit the growth and propagation of the parasite (Fig. 1); (2) they inhibit parasite protein synthesis (Fig. 5); (3) they raise the pH in the parasite food vacuole, the equivalent of the mammalian phagolysosome (Fig. 6); (4) the order of antimalarial potency is similar to the order of their toxicity to mammalian cells; (5) the inhibitory effects show a time/dose relationship, inasmuch as higher doses require relatively shorter exposure times to exert similar inhibitory effects (Fig. 2). Moreover, they reduce the digestion of host cell cytosol by inhibiting parasite hydrolase activity (Fig. 7). This could result from an upward shift in vacuolar pH and/or from a direct action of the drugs on the vacuolar enzymes. Although it was initially suggested that lysosomotropic detergents exert their cytotoxic effect by accumulating in intracellular acidic compartments due to their weak base properties, and detergent-mediated disruption of these organelles, their antimalarial activity is only in part in line with this hypothesis. First, according to the proposed mechanism, basic amphiphiles having higher pK_a's are expected to accumulate to higher levels inside acidic compartments and thus be relatively more potent inhibitors. Yet, NDI (pK_a of 6.3), is considerably more cytotoxic and antimalarial than its two structural analogs which have demonstrably higher pK_a values, i.e. NDDFEA ($pK_a = 7.5$) and NDM ($pK_a = 7.6$) (Fig. 1). Second, the proposed release of hydrolases from lysosomes [9, 17] should have resulted in a similar release from the food vacuole with concomitant proteolysis of the parasite cytosolic proteins. However, we have failed to notice such an effect both by ultrastructural and biochemical means (Figs 3 and 4). Our results seem to be more in line with those recently reported for mammalian lysosomes [18]. In the latter case, no apparent disruption of lysosomal membranes occurred as a result of the direct action of the putative lysosomotropic detergents on the isolated lysosomes. In the malaria system, the slight NDI-induced increase of [^{3}H]Ileu release is apparently not due to enhanced autoproteolysis, but to reduced incorporation of recycled label due to inhibition of protein synthesis. The latter is probably due to the demonstrable inhibition of the feeding process by NDI, and hence, to a shortage of amino acid supply. Similar effects have been shown recently for quinoline-containing antimalarial drugs, methylamine and NH_4Cl [7].

The effect of the lysosomotropic detergents on the pH gradient between the parasite's food vacuole and the extracellular medium (Fig. 6) with NDI ($pK_a = 6.3$) being more effective than NDM ($pK_a = 7.6$), suggests that intravacuolar detergent accumulation to critical micellar concentration (CMC) levels, with ensuing solubilization of surrounding membranes, is probably not the cause for the dissipation of the pH gradient. Based on pK_a values alone, NDM should have been 20-fold more effective than NDI. Yet it is about 3.5-fold less effective as an antimalarial and *ca.* 10-fold less effective than NDI in dissipating the transvacuolar pH gradient. Our view is that the amphiphilic agents enter into the acidic food vacuole as free base, become protonated, accumulate in the vacuole and interact with the food vacuole membrane, as do many other amphiphiles [19]. The drugs could cause leakage of ions through the lysosomal membrane either by mild detergent action or could cause a specific egress of protons by the fact that they are highly lipophilic bases (protonophoric effect). With either mechanism, the drugs will cause a proton leak across the vacuolar membrane with consequent alkalinization of the food vacuole. This nonspecific leakage or protonophoric effect would then depend not only on the vacuolar concentration of the detergent (which is pK_a-dependent) but also on the lipophilicity of the compound. As the Hansch constant of imidazole is almost an order of magnitude larger than that of morpholine [20], NDI is expected to be relatively more lipophilic than NDM and the combined effect of lipophilicity and pK_a would render it *ca.* 10–50-fold more potent in dissipating the transvacuolar pH gradient. Furthermore, a central argument in favour of the detergent mode of action of these compounds was based on the decrease in their potency with reduction in the length of the side chain [9]. Yet, a decrease in chain length also results in decreased lipophilicity and hence, in a reduced proton gradient dissipating activity. Nonetheless, irrespective of the mode by which the drugs cause vacuolar alkalinization, the end result is inhibition of parasite feeding (Fig. 7) with concomitant reduction of protein synthesis and parasite growth. This effect need not be accompanied by disruption of the parasite food vacuole integrity nor by release of hydrolases into the cytosol and parasite autoproteolysis. Finally, although acidotropic detergents are potent antimalarial compounds, their use in chemotherapy of malaria is yet to be established as their toxicity to the parasite is very similar to that of mammalian cells. This is in sharp distinction with chloroquine, which is also an acidotropic agent in the 10–100 μM range [19], but is an efficient antimalarial at 10–500 nM [21].

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