Microtubules in *Plasmodium falciparum* merozoites and their importance for invasion of erythrocytes

R. E. FOWLER[1,2], R. E. FOOKES[1], F. LAVIN[1], L. H. BANNISTER[2] *and* G. H. MITCHELL[1]*

[1] *Department of Immunology, UMDS, The Medical School, Guy's Hospital, London SE1 9RT, UK*
[2] *Department of Anatomy and Cell Biology, UMDS, The Medical School, Guy's Hospital, London SE1 9RT, UK*

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SUMMARY

Plasmodium falciparum merozoites have an array of 2–3 subpellicular microtubules, designated f-MAST. We have previously shown that colchicine inhibits merozoite invasion of erythrocytes, indicating a microtubular involvement in this process. Colchicine inhibition of invasion was reduced by the Taxol®-stabilization of merozoite microtubules prior to colchicine exposure. Immunofluorescence assays showed that the number and length of f-MASTs were reduced in colchicine-treated merozoites, confirming that microtubules were the target of colchicine inhibition. The dinitroaniline drugs, trifluralin and pendimethalin, were shown by immunofluorescence to depolymerize the f-MAST and both drugs were inhibitory in invasion assays. These results demonstrate that the integrity of the f-MAST is important for successful invasion. Fluorescence imaging demonstrated the alignment of mitochondria to f-MAST, suggesting that mitochondrial transport might be perturbed in merozoites with disorganized f-MAST. Depolymerizing mt in late-stage schizonts did not affect the allocation of mitochondria to merozoites.

Key words: *Plasmodium falciparum*, merozoites, microtubules, invasion, colchicine, dinitroanilines.

INTRODUCTION

P. falciparum merozoites have a narrow band (Read *et al*. 1993) of 2 or 3 subpellicullar microtubules (mt) (Bannister & Mitchell, 1995). We have called this structure the *P. falciparum* merozoite assemblage of subpellicular microtubules, and abbreviated this to f-MAST (Fowler *et al*. 1998). We have proposed that these mt are important for the successful invasion of red blood cells (RBC) (Bejon *et al*. 1997) because low concentrations of colchicine (10 μM–1 mM) significantly inhibited invasion. We concluded that colchicine action was likely to be related to the merozoite mt rather than any other factor. However, mt show variable sensitivities to colchicine, and colchicine has the potential to affect other cellular activities (see Hommel & Schrével, 1998).

We have therefore re-examined this question using 3 other antimicrotubule drugs with differing actions. Taxol® (paclitaxel) and its derivative Taxotere® (docetaxel) stabilize microtubules and inhibit their disassembly (Schiff, Fant & Horwitz, 1979; Manfredi, Parness & Horwitz, 1982; Taraschi *et al*. 1998) and they have recently been shown to block schizont mitosis (Schrével *et al*. 1994; Sinou, Grellier & Schrével, 1996). However, Pouvelle *et al*. (1994) showed that *P. falciparum* blood stages were insensitive to 1 μM Taxol® administered 40–48 h post-invasion, corresponding, presumably, to post-mitotic, mature schizont stages. We have exploited this 'window' to pre-treat mature schizonts with Taxol® immediately prior to using them as a merozoite source in invasion assays. By Taxol®-stabilizing merozoite mt, and thus protecting mt against colchicine action, we have been able to assess whether or not the f-MAST is the colchicine target.

The dinitroaniline compounds, trifluralin and pendimethalin, bind to tubulin in trypanosomatids (Chan & Fong, 1990; Chan *et al*. 1993*a*) and Kaidoh *et al*. (1995) showed that trifluralin bound to and fragmented subpellicular mt in *P. falciparum* gametocytes. We have used these drugs, again exploiting the post-mitotic 'window', to examine the effects of disassembling the f-MAST on invasion. In addition we have investigated the possibility that mts are involved in moving mitochondria into developing merozoites. This mechanism has been proposed for dividing axonemal cells (Heggeness, Simon & Singer, 1978) and, from electron microscope (EM) images, is indicated for *P. falciparum* merozoites by the proximity of the mitochondrion to the f-MAST (unpublished observations).

These experiments have confirmed that tubulin and the integrity of f-MAST is important for successful invasion, but indicate that mt are not responsible for the inclusion of mitochondria into merozoites.

* Corresponding author: Department of Immunology, UMDS, The Medical School, Guy's Hospital, London SE1 9RT. Tel: +0171 955 4421. Fax: +0171 955 8894. E-mail: ghm57@umds.ac.uk.

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MATERIALS AND METHODS

Parasites

P. falciparum (C10 strain) were maintained in continuous culture using standard medium, atmosphere and techniques (see Webb *et al*. 1996) and synchronized using either sorbitol lysis or Percoll® density cushions (Dluzewski *et al*. 1984).

Drug preparations

1 mM Taxol®, 1 mM colchicine (both Sigma Chemicals, UK), 1 M trifluralin and 1 M pendimethalin (both Riedel deHaën, Phillip Harris, London) stock solutions in AnalaR dimethylsulfoxide (Me_2SO) were either used immediately or stored at −20 °C until required. Solubility in culture medium of both trifluralin and pendimethalin was limited, and drug precipitate was seen at 10 mM concentrations and above.

Invasion assays

Two variations of the standard invasion assay were used. Synchronized cultures which predominantly consisted of mature schizonts were added in a 1:1 ratio to wells in 24-well plates (Costar, UK) containing culture medium, the appropriate concentrations of drug or control Me_2SO solvent, and sufficient RBC to give a final haematocrit of 1%. A temperature of 37 °C was maintained throughout. Each experiment was carried out in triplicate. Cultures were incubated under normal culture conditions. Experiments were terminated after 3–4 h, so that all rings counted were derived from schizonts which were post-mitotic at the start of the incubation. Thin blood smears were taken from each treatment at the beginning and end of each experiment (3–4 h duration under normal culture conditions) and stained with Giemsa.

Invasion assays were also carried out using schizonts pretreated with Taxol®. Schizonts were purified on Percoll® cushions, washed and resuspended in complete culture medium. Then 1 mM Taxol® in Me_2SO was added to half of the schizont suspension to give a final concentration of 1 μM and solvent alone added to the other half as a control. The parasites were returned to culture for 1 h at 37 °C, without the addition of fresh RBC. The Taxol® and control cultures were then each split into 2 equal volumes, which were washed and resuspended in equal volumes of culture medium, with erythrocytes present at 1% haematocrit. Colchicine was added at 25 μM (Experiment 1), 100 μM (Experiment 2) or 500 μM (Experiments 3 and 4) to 1 of the Taxol® and 1 of the Me_2SO aliquots, and solvent Me_2SO to the 2 remaining aliquots. Each aliquot was divided amongst 3 wells in 24-well plates, to give triplicate subcultures.

48 h invasion assays

Assays for the effects of 0·1, 10 and 1000 μM pendimethalin and trifluralin over 1 complete asexual cycle were carried out essentially as outlined for the invasion assays above. Parasite cultures were incubated for 48 h under standard culture conditions (starting from late schizogony), with 1 change of medium (containing either drug or control Me_2SO at the appropriate concentration) after 24 h. Samples for blood films were taken at 0, 18, 24, 42 and 48 h after addition of the drugs.

Statistics

Probability (*P*) was estimated using Microsoft Excel's 2-tailed Student's *t*-test for 2 samples of equal variance.

Immunofluorescence assays (*IFA*)

Parasites from Percoll® layers were resuspended in culture medium (without additional erythrocytes), and cultured for a further 3–4 h on glass slides coated with poly-L-lysine. These slides had been prepared by flooding them with 100 μg/ml poly-L-lysine solution for at least 1 h, tipping off the excess solution and then allowing the slides to dry overnight at room temperature, or for 1 h at 60 °C. Adapting the method of Read *et al*. (1993), these cultures were fixed by diluting their medium with freshly prepared, 3·7% paraformaldehyde in phosphate-buffered saline (PBS) in a 1:1 ratio; the slides were left overnight at 4 °C and further diluted with paraformaldehyde, to give approximately 1:10 dilution, and again left overnight at 4 °C. Cells were extracted in 0·5% Triton X–100 for 30 sec at room temperature, or in cold (−20 °C) acetone for 10 min. Slides and cover-slips were washed in PBS–Tween (0·05%), incubated with 1:500 anti-α-tubulin mouse monoclonal (Sigma Chemicals, UK – catalogue no. T9026) as the primary antibody and 1:10 FITC-conjugated rabbit anti-mouse immunoglobulin as the second antibody (Dako Ltd, UK). Parasite nuclei were stained with 1 μg/ml diamidino phenylindole (DAPI) (Sigma Chemicals, UK) for 30 sec. The slides were mounted in 'Pro-long' (Molecular Probes, Leiden), and examined and photographed with Nikon Optiphot or Olympus PROVIS AX70 microscopes, using appropriate filters.

Staining mitochondria

Mitotracker™ Orange CMTMRos (Molecular Probes, Leiden) was prepared as a 1 mM stock solution in Me_2SO, and added at 0·25 μl/ml to cultures, to give a 250 nM concentration. These cultures, predominantly consisting of mature schizonts (schizonts with a full complement of nuclei),

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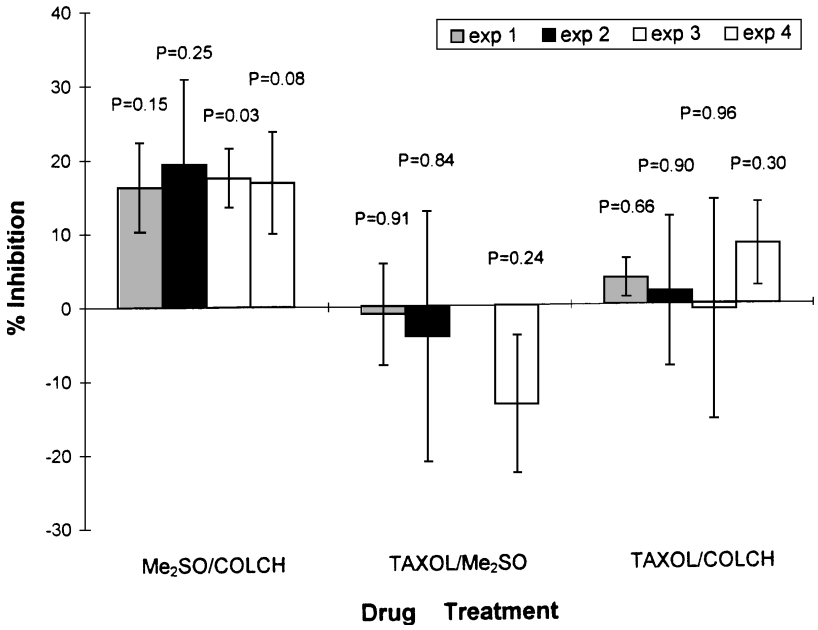

Fig. 1. Mature schizonts were exposed to Taxol® or control solvent (Me_2SO) prior to invasion assays in the presence of colchicine (25, 100 or 500 μM in Exps 1, 2, and both 3 and 4 respectively) or diluent. Blood films from triplicate (Exps 1, 2, and 3) and 5 replicate wells (Exp. 4) were assayed for the number of ring stages/100 RBC. The histogram shows mean percentage inhibition from the mean control values. Error bars represent $\pm$S.E. and the numbers above each bar represent the statistical probability that these values differ from the controls (see Materials and Methods section). Where there are negative values for inhibition, ring counts were greater than those of the controls (see Results section).

were incubated under normal culture conditions for 1 h. Schizonts were concentrated on a Percoll® cushion, washed and resuspended in culture medium containing either 1 mM pendimethalin or Me_2SO solvent (duplicate cultures), and cultured for 5 h on poly-L-lysine coated slides. The slides were fixed and stained for α-tubulin immunofluorescence as above.

IFA and Mitotracker™ scoring

To assess the effect of colchicine, trifluralin and pendimethalin on f-MAST in merozoites, the number of merozoites in at least 15 fields was estimated by counting DAPI-stained nuclei and the FITC fluorescence image for each field was used to assess the number of merozoites with tubulin polymerized in the f-MAST form (see Fig. 4A).

In pendimethalin-treated and control cultures the number of merozoites with a mitochondrion was assayed, using poly-L-lysine preparations stained with DAPI and Mitotracker™. The proportion of merozoites with a mitochondrion was estimated by scoring the number of (a) DAPI-stained nuclei and (b) Mitotracker™-stained mitochondria per field (see Fig. 6). A range of late schizont and merozoite stages was examined. Parasites were categorized under the FITC filter and identified as: 'schizonts' – multinucleate parasites still bound by the erythrocyte membrane; 'released schizonts' – multinucleate parasites without the erythrocyte membrane, surrounding a residual body; 'merozoite clusters' – groups of 2–12 released merozoites without a residual body; and 'single merozoites' – released, isolated merozoites. Each field contained 1–3 parasites. Approximately 100 fields were examined for each treatment.

RESULTS

Effect of Taxol® pre-incubation on colchicine inhibition

To determine whether the inhibitory effects of colchicine on invasion (Bejon *et al.* 1997) could be prevented by the action of Taxol®, 4 experiments, using increasing concentrations of colchicine in 3–4 h invasion assays, were performed (see Materials and Methods section). Their results are shown separately in Fig. 1. Where appropriate, the results from these 4 experiments have been pooled in the following text and given ($\pm$) standard error (S.E.). Control cultures had mean ring counts/100 RBC of 4·31 ($\pm$0·31), 3·96 ($\pm$0·34), 4·36 ($\pm$0·14), 3·89 ($\pm$0·18) (Experiments 1, 2, 3 and 4 respectively). All inhibition values are expressed as a percentage of the controls.

Pre-treatment of mature schizonts with either 1 μM Taxol® for 1 h, followed by washing, did not adversely affect numbers of new rings in the invasion assays. In the Taxol® pre-treatment group, the mean

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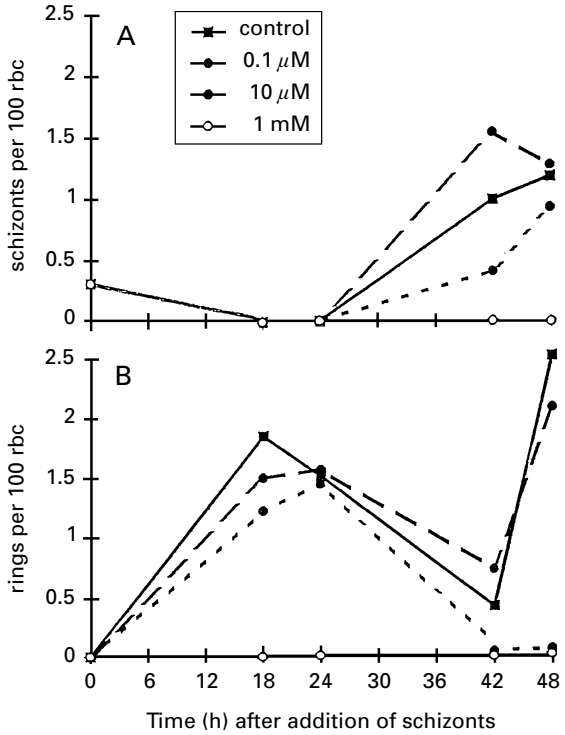

Fig. 2. Schizont counts (A) and ring counts (B) over time for pendimethalin treated and control cultures. Mature schizonts were used to initiate cultures over 1 asexual cycle i.e. 48 h. Cultures were treated with either 0·1 μM, 10 μM or 1 mM pendimethalin. Control cultures contained the Me_2SO solvent. Blood films from the zero, 18, 24, 42 and 48 h time-points were assayed for the number of ring-stage parasites and schizonts/100 RBC.

count of ring stages/100 RBC was 7·58 % (±11·69) higher than the control group, but this was not statistically significant ($P = 0·29$). In colchicine (25, 100 or 500 μM) treated cultures, initiated with schizonts from the control pre-treatment group, the mean inhibition values ranged from 16·3 % (±6·07) to 19·3 % (±11·6). In cultures initiated with schizonts from the Taxol® pre-treatment group and similarly incubated with 25, 100 and 500 μM colchicine, mean inhibition values fell to a range of −0·84 % (±14·9) to 8·06 % (±7·20) (see Fig. 1).

Effect of dinitroanilines on cultures

Both trifluralin and pendimethalin were assayed during a 48 h period of continuous drug culture for their effects on *P. falciparum* cultures initiated with mature schizonts. No parasites were seen in cultures dosed with 1 mM trifluralin or pendimethalin. For both drugs, the number of new rings in the cultures treated with 10 μM concentrations was similar to control levels at the 18 h time-point (Fig. 2), but the older parasites observed at the 42 and 48 h time-points were not multi-nucleate, despite being the size of schizonts, and showed diffuse nuclear staining throughout the parasite. At 48 h, cultures containing 10 μM trifluralin were devoid of rings and those containing 10 μM pendimethalin had very few (98 % inhibition) (Table 1). Cultures dosed with 0·1 μM trifluralin or pendimethalin closely followed the control parasitaemia (Fig. 2).

Effects of dinitroanilines on invasion

Both trifluralin and pendimethalin significantly inhibited ring stage numbers in 3–4 h invasion assays initiated with late schizonts (Fig. 3). At concentrations of 0·5 and 1 mM, counts were reduced by 61 % (S.E. = ±11·7, $P = 0·01$) and 99·4 % (S.E. = ±3·06, $P = 6·2 \times 10^{-5}$) respectively in trifluralin treated cultures, and by 38·4 % (S.E. = ±5·8, $P = 0·02$) and 69·8 % (S.E. = ±2·9, $P = 0·02$) respectively in pendimethalin-treated cultures. There was no significant effect on schizont numbers in these experiments, at any of the concentrations tested (data not shown).

Immunofluorescence

In merozoites released from mature schizonts, and allowed to settle on poly-L-lysine coated slides, the f-MAST was visualized by indirect immunofluorescence, using the anti-α-tubulin monoclonal antibody (see Materials and Methods section). The f-MAST appeared as a single band of fluorescence, extending from the apical region of the merozoite toward the posterior of the cell and, depending on the orientation of the cell, as an arc which followed the periphery of the cell (Fig. 4A). In very mature schizonts, these bands of fluorescence were seen in random orientation within the parasite, but in schizonts where the merozoites were still attached to the residual body, the f-MAST bands radiated out from the residual body in a very distinct pattern (Fig. 6B) (as also noted by Read *et al.* 1993).

In control cultures, approximately 28–45 % of free merozoites counted had a discernible f-MAST. The lack of the characteristic band in the other merozoites was probably due to the disintegration of the f-MAST in old and dead merozoites. In cultures treated with 1 μM Taxol® over the culture period (3–4 h), 99 % or more of the free merozoites showed the characteristic pattern, often as longer and brighter bands, and with less diffuse fluorescence in the merozoite cytoplasm than the controls (Fig. 4B). Colchicine treatment (500 μM) reduced the number of discernible f-MAST counts by 25 % of control values (Table 2), and appeared to shorten, or break up the bands of fluorescence (Fig. 5A). Both pendimethalin and trifluralin drastically diminished the number of merozoites showing f-MAST at

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Table 1. Percentage inhibition of ring and schizont parasitaemia in synchronized *Plasmodium falciparum* cultures after 48 h (1 asexual cycle) exposure to dinitroaniline compounds

	Ring counts			Schizont counts		
Compound/conc. (μM)	Mean inhibition (%)	S.E.	*P**	Mean inhibition (%)	S.E.	*P**
Pendimethalin						
0·1	17·60	7·45	0·61	− 8·08	9·82	0·48
10	98·09	1·91	0·03	22·75	12·90	0·16
1000	100	0	0·03	100	0	6×10^{-6}
Trifluralin						
0·1	31·30	11·06	0·11	−14·04	12·65	0·62
10	100	0	6×10^{-4}	−16·67	39·30	0·73
1000	100	0	6×10^{-4}	100	0	0·01

* *P*, statistical probability that the parasitaemia values for the drug-treated cultures differ from those of the controls, estimated using Student's *t*-test.

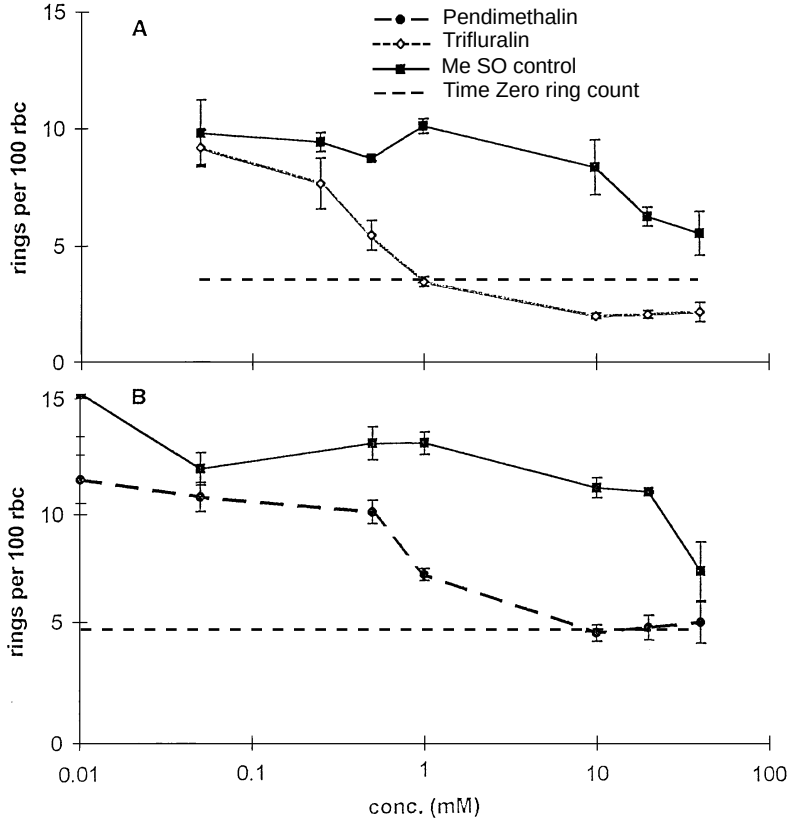

Fig. 3. The effect of increasing concentrations of trifluralin (A) and pendimethalin (B) on ring counts compared to controls (cultures containing equivalent volumes of Me_2SO solvent). Triplicate cultures were initiated with synchronized cultures consisting of mature schizonts, but also some rings, represented on the graphs as 'Time Zero ring count'. Both experiments were terminated after 4 h, and blood films assayed for number of ring stage parasites/100 RBC.

concentrations of 1 mM and 0·1 mM respectively. At lower concentrations, reduced levels of inhibition were seen (Table 2). Tubulin-associated fluorescence appeared as a single apical dot in the affected merozoites (Fig. 5C), as opposed to the f-MAST band seen in the controls.

Merozoite mitochondria and f-MASTs

We investigated the possibility that the f-MAST was involved in translocating mitochondria into budding merozoites, using anti-α-tubulin immunofluorescence and Mitotracker™ (see Materials and Methods

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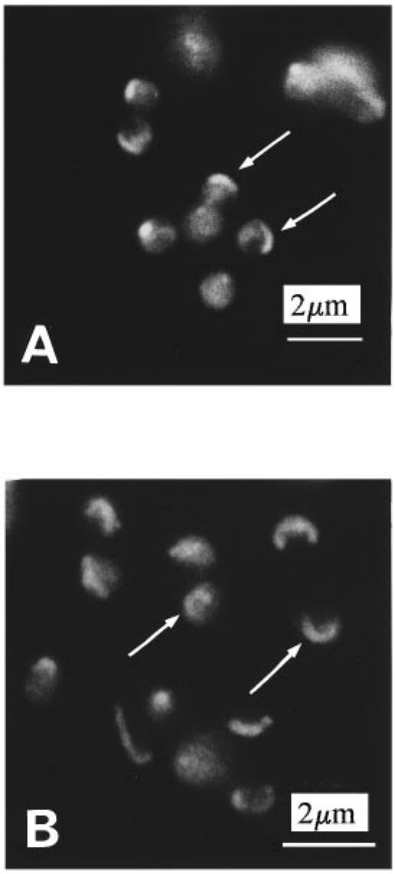

Fig. 4. Free *Plasmodium falciparum* merozoites released from schizonts cultured in the presence of Me_2SO diluent (controls) (A), or 1 μM Taxol® (B) on poly-L-lysine coated slides, and stained with anti-α-tubulin immunofluorescence. The arc of the f-MAST can clearly be seen (arrows) following the perimeter of the merozoites.

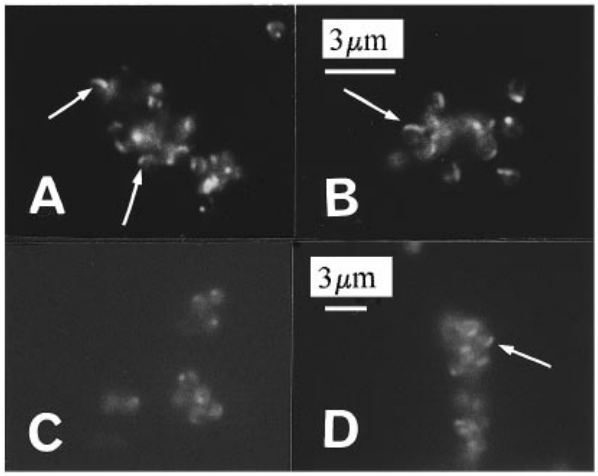

Fig. 5. *Plasmodium falciparum* schizonts, released from the RBC membrane, cultured on poly-L-lysine treated slides in the presence of 500 μM colchicine (A), the equivalent volume of Me_2SO solvent (B), 100 μM trifluralin (C) or, again the equivalent volume of Me_2SO (D). The slides were fixed with paraformaldehyde and stained with anti-α-tubulin immunofluorescence. Some f-MAST bands can still be seen in the merozoites of the colchicine-treated schizont (arrows) (A); only dots of tubulin fluorescence can be seen in the merozoites of the trifluralin-treated schizont (C), whereas the f-MAST is clearly visible in most of the merozoites of the control schizonts (arrows) (B and D).

section). Parasites in mid-schizogony had a single, long mitochondrion as reported by Divo *et al.* (1985). Schizonts with merozoites budding off from the residual body appeared with bright spots of Mitotracker™ staining, aligned circumferentially around the residual body (Fig. 6A), in parallel with f-MAST 'spokes' also radiating out from the circumference (Fig. 6B). Mature schizonts, with discrete merozoite progeny showed f-MAST bands and Mitotracker™ spots of fluorescence arranged randomly within the parasitized erythrocyte, 1 of each/DAPI-staining nucleus. Merozoites released from schizonts had single bright spots of mitochondrial staining which, in some merozoites at least, appeared to be situated midway along the f-MAST.

Schizont-rich cultures were incubated with Mitotracker™, treated with 1 mM pendimethalin and then fixed and immuno-stained with anti-α-tubulin. They were examined under DAPI, rhodamine and FITC viewing filters to see if de-polymerizing the f-MAST during development prevented the incorporation of mitochondria into mature merozoites. A range of parasite stages was examined and scored for DAPI-staining nuclei (Fig. 6E), and Mitotracker™

Table 2. The effect of anti-microtubule drugs administered to cultures of mature schizonts on the percentage of merozoites with discernible f-MAST bands shown by anti-tubulin immunofluorescence

Drug	Concentration	Merozoites with f-MAST (%)		Inhibition (%)
		Drug-treated	Control	
Colchicine	(500 μM)	21·1	28·1	24·9
Trifluralin	(100 μM)	3·4	33·9	90·0
Pendimethalin	(10 μM)	25·9	55·7	53·5
Pendimethalin	(50 μM)	15·7	45·0	65·1
Pendimethalin	(1 mM)	0	34·0	100

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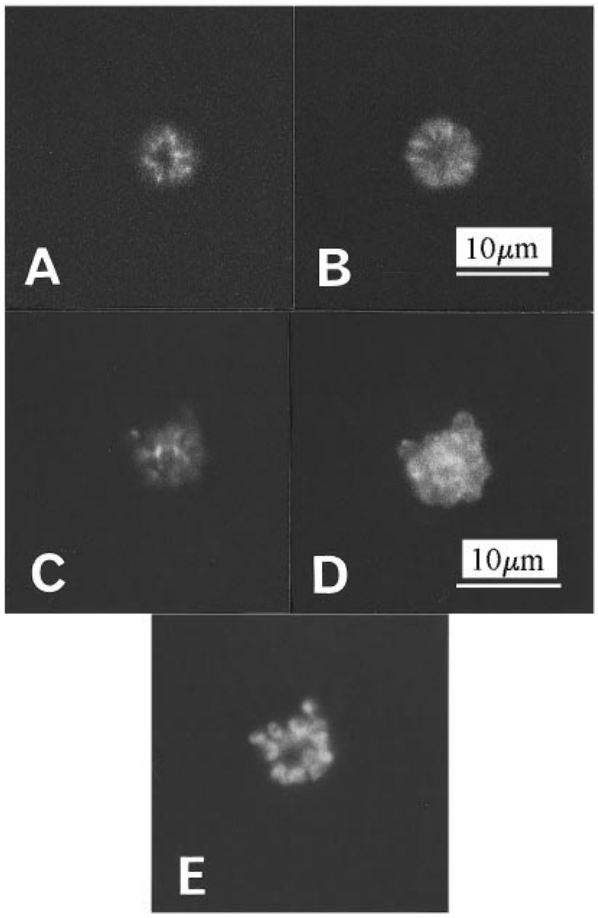

Fig. 6. Schizont from a control culture showing mitochondria stained with Mitotracker™ (A) radiating out from the centre of the schizont, in parallel with f-MAST bands stained with tubulin immunofluorescence (B). Schizonts from cultures treated with 1 mM pendimethalin were scored for Mitotracker™ staining mitochondria (C) and DAPI stained nuclei (E). These parasites had no discernible f-MASTs; only diffuse staining and dots of tubulin immunofluorescence were seen (D).

dots (Fig. 6C). No difference was seen in any developmental category between the Mitotracker™ scores (dots/nuclei) for control and pendimethalin treated cultures (Fig. 7).

DISCUSSION

We concluded in a previous paper that treating schizonts with colchicine inhibits merozoite invasion (Bejon *et al*. 1996). It has been suggested (Hommel & Schrével, 1998; Bell, 1998) that this effect may be due to colchicine effects other than poisoning of mt polymerization, such as rigidification of the RBC membrane. We have exposed post-mitotic schizonts to Taxol®, which polymerizes and stabilizes mt, prior to colchicine exposure during invasion assays. This pre-treatment raised invasion rates in colchicine-treated cultures, often close to control values. These results and the 25% reduction in f-MAST numbers in colchicine-treated cultures indicated that poisoning of the f-MAST was responsible for the colchicine inhibition of invasion. However, the invasion rates were low in these assays due, we presume, to the premature rupture of schizonts during centrifugation washes after the initial incubation (with Taxol® or control Me_2SO). Colchicine exposures were shorter compared to those used by Bejon *et al*. (1997) and both of these factors probably reduced the level of inhibition in these experiments.

To explore further whether f-MAST has a role in merozoite invasion we used 2 dinitroanilines, trifluralin and pendimethalin, which depolymerizes mt. Trifluralin has activity against *Leishmania* spp. (Chan & Fong, 1990), *P. falciparum* asexual blood stages (Nath & Schneider, 1992), gametocytes (Kaidoh *et al*. 1995), *Trypanosoma brucei* (Chan *et al*. 1993*b*) and *Toxoplasma gondii* (Stokkermans *et al*. 1996). Much of this activity has now been attributed to choralin, an impurity from trifluralin synthesis (Chan *et al*. 1993*a*; Callahan *et al*. 1996). Pendimethalin was found to be more potent than trifluralin against *L. mexicana* (Chan *et al*. 1993*a*) and to be the most effective dinitroaniline tested against *Cryptosporidium parvum* (Arrowood *et al*. 1996).

These 2 drugs were assessed for their effects on *P. falciparum in vitro* for up to 48 h post-invasion. At concentrations of 10 μM, cultures initiated with mature schizonts went on to produce new rings, but both drugs prevented the next cycle of mitosis and thus merozoite generation. These results agree with those of Nath & Schneider (1992) who reported inhibition of *P. falciparum* growth and differentiation *in vitro*, at trifluralin concentrations between 1 and 5 μM. The loss of parasites before 18 h post-invasion from 1 mM pendimethalin and trifluralin cultures parallels the results of the invasion assays, where 0·5 and 1 mM concentrations reduced the number of new rings. It seems unlikely that either late schizogony or the release of merozoites from schizonts was affected, since this would have resulted in greater numbers of schizonts in treated cultures than the controls, which was not the case (data not shown). Either merozoite development or the process of RBC-invasion (including merozoite attachment) must therefore have been affected by the drugs. Although the possibility of drug effects on the RBC have not been ruled out, the results of the IFA studies confirmed that both trifluralin and pendimethalin depolymerized the f-MAST of merozoites. However, this depolymerization was not complete and a residuum of tubulin fluorescence remained at the anterior pole.

Why is the f-MAST important for invasion? The f-MAST in Taxol® treated merozoites is stabilized, often appearing longer than in control cells. Despite the stabilization of the mt, these merozoites invaded RBC efficiently (Pouvelle *et al*. 1994; Bejon *et al*. 1997), and were protected, to a large extent, from the

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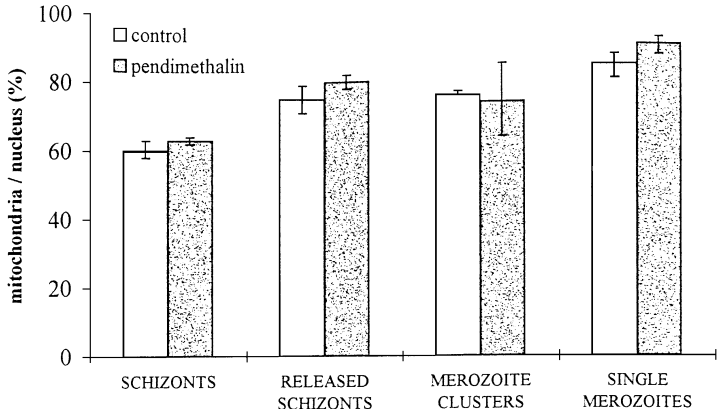

Fig. 7. Schizonts were incubated with 250 nM Mitotracker™ mitochondrial stain, and then cultured on polylysine coated slides for 4 h in the presence of either 1 mM pendimethalin, or Me_2SO diluent (controls). Slides were fixed and stained by tubulin immunofluorescence and DAPI nuclear stain. Parasite stage was assessed under the FITC filter and then the number of nuclei and mitochondria scored. Approximately 100 fields on 2 slides were examined for both treatments. The histogram shows the mean percentage of merozoites with mitochondria in each developmental stage category. The high and low values in each category (i.e. the range) are represented by the error bars.

inhibitory action of colchicine. Motility generated by polymerization and depolymerization of the mts themselves, 'treadmilling', therefore, cannot be essential for invasion. It is possible for stabilized mt to act as tracks for dynein or kinesin motor molecules. One possibility is that organelle transport, a known function of mt-associated motors in dividing cells (Brady, 1991; Bloom, 1992), is disrupted by the dinitroanilines and colchicine. We have previously shown that the apical organelles of merozoites treated with inhibitory concentrations of colchicine appear normal under the electron microscope (Bejon *et al*. 1997). Here we examined the possibility that the allocation of mitochondria was disrupted by the depolymerization of merozoite microtubules, using immunofluorescence and a fluorescent, mitochondrion-selective 'Mitotracker™' probe (Nangaku *et al*. 1994). Despite the damage to the f-MAST structure in pendimethalin-treated mature schizonts, we could find no evidence that this prevented mitochondria, aligned at the base of the budding merozoites, from being packaged inside each cell. However, we have not excluded the possibility that the absence of the f-MAST may disrupt fine placement or function of mitochondria, and further work is needed to ascertain whether organelle translocation is affected by mt depolymerization at an early stage of merozoite development. These studies are in progress.

It may be that the f-MAST simply functions as a structural brace against the forces exerted by the RBC. Microtubule-associated ATPases, such as dynein or kinesin may operate as motors along the f-MAST. They may be responsible for motility of the merozoite as it orientates itself on the RBC surface, and an active role in invasion itself cannot be ruled out. Another possibility is that the mts are associated with cell signalling processes (e.g. Jasper *et al*. 1995) which allow invasion to proceed. The molecules which are associated with f-MAST and their locations and functions are currently under investigation in our laboratory.

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