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Carrageenans inhibit the in vitro growth of *Plasmodium falciparum* and cytoadhesion to CD36

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Abstract Carbohydrates are implicated in many of the invasive and adhesive interactions that occur between *Plasmodium falciparum* malaria parasites and human host cells, including invasion of sporozoites into hepatocytes, entry of merozoites into new host erythrocytes during asexual blood-stage replication, adhesion of infected erythrocytes to uninfected erythrocytes (rosetting) and to a number of host endothelial receptors including ICAM-1, CD36 and chondroitin-4-sulphate. In addition to increasing our understanding of host–parasite interactions, the investigation of carbohydrates with differing levels and patterns of sulphation as inhibitors may contribute to the development of novel therapeutics targeting malaria. Here we show that three polysaccharides derived from seaweed (carrageenans) with differing sulphation levels and patterns can inhibit the in vitro erythrocytic invasion and growth of both drug sensitive and drug resistant *P. falciparum* lines and the adhesion of parasitized erythrocytes to the human glycoprotein CD36.

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Introduction

Malaria is a parasitic disease of worldwide importance resulting in more than 300 million clinical cases and 2–3 million deaths each year, mainly in sub-Saharan Africa. With resistance to commonly used anti-malarials growing (Baird 2005) and no vaccines available at present, the need for new drugs that target novel parasite pathways or parasite-specific processes is clear. During the asexual stage of its life cycle, *Plasmodium falciparum* parasites interact with human host cells in a number of ways and many of these interactions have been shown to be carbohydrate mediated. For example, after injection into the hosts' blood circulation, sporozoite-stage parasites travel to the liver and migrate into hepatocytes (Frevert et al. 1993). A sporozoite surface protein, termed the circumsporozoite (CS) protein (Nussenzweig and Nussenzweig 1985), has been shown to be involved in the binding to heparin sulphate proteoglycans expressed on the hepatocytes surface membrane (Cerami et al. 1992; Frevert et al. 1993; Ying et al. 1997). During the intra-erythrocytic stages, the invasion of merozoites into erythrocytes has been shown to involve a merozoite stage *P. falciparum* erythrocyte-binding antigen 175 (EBA-175) which binds to the sialoglycoprotein glycophorin A on human erythrocytes in a sialic acid dependent manner (Ockenhouse et al. 2001; Duraisingh et al. 2003). Interactions between parasitized erythrocytes and human tissues have also been shown to involve carbohydrate interactions, including parasite sequestration in the placenta during maternal malaria where the glycosaminoglycans chondroitin-4-sulphate (CSA) and hyaluronic acid have been identified as receptors (Rogerson et al. 1995; Fried and Duffy 1996; Beeson et al. 1998, 2000).

Given that many of the interactions between *P. falciparum* parasites and host cells are carbohydrate mediated, agents that block these interactions will help to define these biological processes and may contribute to the development of anti-malarial therapies. Large polysaccharides like dextran sulphate and fucoidan have been found to inhibit the binding of sporozoites to the surface of hepatocytes and thus invasion into these cells (Pancake et al. 1992). These same compounds inhibit both the asexual in vitro invasion and growth of *P. falciparum* and the adhesion of parasitized erythrocytes to host receptors such as CSA and CD36 (Butcher et al. 1988; Xiao et al. 1996; Clark et al. 1997). We have previously demonstrated that various polysaccharides with differing levels and patterns of sulphation, including carrageenans and cellulose sulphate derivatives, inhibit CSA-specific adhesion of *P. falciparum* infected erythrocytes to in vitro cultured mammalian cells expressing CSA and to ex vivo human placenta (Andrews et al. 2005). In this report, we extend these finding and show that carrageenans inhibit in vitro anti-malarial activity and can interfere with adhesion to the human host receptor CD36.

Materials and methods

Compounds

A panel of seven polysaccharides were tested; carrageenans LP-42 (Iota), CSW-2 (Lambda) and MB73-F (Kappa) were kindly donated by CP Kelco, Denmark. Dextran sulphate 500 kDa from *Leuconostoc* ssp., chondroitin-4-sulfate from Bovine trachea (Sigma, Deisenhofen, Germany), and fucoidan from *Fucus vesiculosus*, were all purchased from Sigma (St. Louis, MO, USA).

In vitro culture *of P. falciparum* and C32 melanoma cells

In vitro culture of the drug sensitive 3D7 (Walliker et al. 1987) and multi-drug resistant Dd2 (Wellems et al. 1988) *P. falciparum* lines was carried out as previously described (Trager 1976). Parasites were grown in RPMI 1640 media supplemented with 10% heat-inactivated pooled human serum and using O+ human erythrocytes. Parasites were maintained in a synchronous state by sorbitol treatment (Lambros 1979) or gelatine floatation (Jensen 1978). The C32 melanoma cells (DSMZ, Braunschweig, Germany) were cultured in Dulbeccos modified eagle's media (JRH Biosciences, PA, USA) supplemented with 10% heat inactivated foetal calf serum (Gibco, Heidelberg, Germany) and 1% penicillin/streptomycin (Gibco). All cultures were Mycoplasma free, as determined using a Mycoplamsa Plus PCR Primer Set (Stratagene, La Jolla, CA, USA).

P. falciparum growth/invasion inhibition assays

The effect of polysaccharides on inhibition of parasite growth or invasion was tested radiometrically using ^{3}H-hypoxanthine incorporation, as previously described (Andrews et al. 2000). Briefly, *P. falciparum* infected erythrocytes at 0.25% parasitemia and 5% haematocrit were grown in 96 well-tissue culture plates in hypoxanthine free RPMI 1640 media (Gibco) supplemented with 10% pooled human serum and 0.01 mg/mL gentamicin (Sigma). Polysaccharides were prepared in hypoxanthine free RPMI 1640 media (Gibco) supplemented with 10% pooled human serum and 0.01 mg/mL gentamicin (Sigma). All compounds were tested in triplicate, in at least three independent experiments. Growth inhibition assays were carried out over 72 h, starting with ring stages. Invasion inhibition, assays were carried out over 36 h, beginning with schizont-stage parasites. Concentrations of drug that inhibited parasite growth by 50% compared to controls (EC_{50}) were determined by linear interpolation of inhibition curves (Huber 1993). Statistical significance was determined using two-tailed Student's *t*-test. In some cases, growth or invasion inhibition was monitored non-radiometrically via microscopic examination of Giemsa-stained thin blood smears and percentage parasitemia determined compared to controls.

Cytoadhesion-inhibition assays

Polysaccharides were tested for the ability to inhibit adhesion of *P. falciparum* line 3D7 infected erythrocytes to CD36 expressed on C32 melanoma cells. Trophozoite-infected erythrocytes, purified by gelatine flotation, were resuspended at 5×10^{6} infected-erythrocytes/mL in RPMI 1640 (pH 6.8) supplemented with 10% human serum. Trophozoite-stage infected erythrocytes were pre-incubated with test substances for 15 min at room temperature prior to incubation on C32 cell monolayers (>70% confluency) for 1 h, with gentle agitation every 15 min, before washing away unbound erythrocytes. De-adhesion assays were carried out in a similar fashion (Andrews et al. 2005), however, in this case, infected-erythrocytes were allowed to bind to C32 cells for 30 min and unbound infected erythrocytes were washed away before adding the test compounds. Adherent infected erythrocytes were fixed in 2% glutaraldehyde (Sigma), before staining with Giemsa. In each assay, CD36-specific binding was determined by inclusion of 0.2 mg/mL FA6/152 anti-CD36 monoclonal antibody (Immunotech, Heidelberg, Germany). Soluble chondroitin-4-sulphate (CSA) at 100 μg/mL was included as a negative control in each assay. The number of adherent infected erythrocytes bound to at least 500 cells was determined for three independent experiments. Statistical significance was determined using two-tailed Student's *t*-test.

Results and discussion

Carbohydrates mediate a number of interactions between malaria parasites and host cells, and molecules that inhibit these interactions may have a role to play in our understanding of host/parasite biology. In this study, we have investigated the anti-malarial activity and anti-adhesive capacity of a panel of polysaccharides including four seaweed extracts; fucoidan, carrageenan CSW-2 (λ), LP-42 (ι), and MB73-F (κ). The carrageenans have previously been shown to interfere with adhesion of *Helicobacter pylori* to heparan sulphate and have demonstrated antiviral activity against herpes simplex (1 and 2) and hepatitis A (Girond et al. 1991; Utt 1997; Carlucci et al. 1999).

In order to examine the effect of these polysaccharides on the invasion of *P. falciparum* into erythrocytes, we cultured mature schizont-stage parasitized erythrocytes in vitro with varying concentrations of each polysaccharide for 36 h during which time control schizonts ruptured and progeny merozoites were released and invaded new erythrocytes. We also investigated the ability of polysaccharides to interfere with parasite growth and development over a 72 h period, starting with early ring stages. Of the three carrageenans tested, CSW-2 appeared most effective at inhibiting the in vitro invasion and growth of both a drug-sensitive (3D7) and multi-drug resistant (Dd2) *P. falciparum* line (Table 1). There was no significant difference in invasion inhibition EC_{50}s between Dd2 and 3D7, however Dd2 appeared more sensitive to CSW-2 over a 72 h-growth period (EC_{50} Dd2, 6 μg/mL (±3 μg/mL) compared to EC_{50}, 3D7 44 μg/mL (±8 μg/mL) $P=0.02$) (Table1). While carrageenan LP-42 was less effective than CSW-2 at inhibiting in vitro *P. falciparum* inhibition and growth, again Dd2 appeared to be more sensitive to this carrageenan (invasion inhibition 3D7 EC_{50}, 85 (±1 μg/mL) compared to Dd2 EC_{50} 29 (±19 μg/mL); $P=0.02$) (Table1). Likewise, Dd2 was more sensitive to MB73-F displaying an EC_{50} of 11 μg/mL (±3 μg/mL) for the 72 h-growth-inhibition assay, but no inhibition of either growth or invasion of 3D7 occurred at the highest concentration tested (100 μg/mL). We observed similar levels of in vitro anti-malarial activity for two control polysaccharides, fucoidan and dextran sulphate 500 kDa. These findings were similar to those of Butcher et al. (1988), however in contrast to those of Xiao et al. (1996) who found that dextran sulphate 500 kDa and fucoidan had no significant effect on the growth of ring-stage parasites (12.5–200 μg/mL). These authors did, however, find that both of these high molecular weight polysaccharides inhibited invasion. These conflicting reports may be due to strain-specific effects, although we obtained similar EC_{50}s for two different parasite lines (Table1).

During asexual blood stage development, *P. falciparum* parasites modify the host erythrocyte and insert parasite derived proteins into the erythrocyte membrane, such as variant surface antigen *P. falciparum* erythrocyte protein 1 (PfEMP1) (Sharma 1991). PfEMP1 has been shown to mediate interactions with a number of host endothelial receptors, including ICAM-1 in the brain microvasculature, which is linked with cerebral involvement (Berendt 1989; Ockenhouse et al.

Table 1 In vitro anti-malarial efficacy of carrageenans against *Plasmodium falciparum*

		Lambda CSW-2	Iota LP-42	Kappa MB73-F	Fucoidan	Dextran sulphate (500 kDa)
Molecular weight (kDa)[a]		230–790	230–790	230–790	180	500
Gross structure		→ 3)-β-D-Gal *p*-(1 → 4)-α-D-Gal *p*-(1-[A → B]	→ 3)-β-D-Gal *p*-(1 → 4)-3.6-anh-α-D-Gal *p*-(1[A → B]	→ 3)-β-D-Gal *p*-(1 → 4)-3.6-anh-α-D-Gal *p*-(1-[A → B]	Mainly: → 2)-α-L-Fuc *p*-(1 in addition: D-Gal, D-Xyl, D-Hex *A*	→ 6-α-D-Glc *p*-(1-
$DS^{b}_{sulphate}$		1.35	1.00	0.50	~0.90	–
Position of sulphation in the sugar unit ($S=SO_3^-$)		A: 70% 2-S, B: 2,6-di-S	A: 4-S, B: 2-S	A: 4-S, B: -	Mainly 4-S-Fuc	
Charge density[c]		2.07	1.53	0.92	Nd	Nd
Growth inhibition EC_{50} (μg/ml)	3D7	44 (±8)	>100	>100	26(±8)	53 (±8)
	Dd2	6 (±3)	37 (±9)	>100	16 (±12)	4 (±3)
Invasion inhibition EC_{50} (μg/ml)	3D7	34 (±20)	85 (±1)	>100	nd	17 (±15)
	Dd2	14 (±11)	29 (±19)	11 (±3)	25 (±15)	3 (±1)

[a] As given by the supplier (dextran and fucoidan)/calculated from the DP_{Cu} of the polysaccharide before sulphation (celluloses)/literature data (carrageenans, H. Scherz, Hydrokolloide)

[b] *DS* degree of substitution = average number of modified OH/sugar unit

[c] Sulphate groups per disaccharide unit

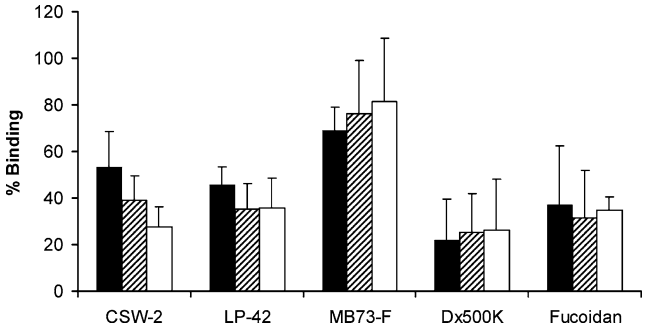

Fig. 1 Effect of polysaccharides on CD36-specific adhesion of *P. falciparum* infected erythrocytes to C32 melanoma cells. Carrageenans (CSW-2, LP-42, and MB73-F), dextran sulphate 500 K (Dx500 K) and fucoidan were tested at 25 μg/mL (*solid bars*), 50 μg/mL (*stripped bars*) and 100 μg/mL (*open bars*). The mean % binding (±SD) compared to controls is shown for three independent experiments

1992; Beg et al. 2002) and adhesion to CSA in the placenta which is associated with pregnancy malaria (Fried and Duffy 1996). Pregnancy associated malaria is of particular interest as, due to the strain-independent nature of the immune response to CSA-binding parasites from different geographical locations (Fried et al. 1998), the parasite adhesin mediating binding to CSA is a prime therapeutic target (Costa et al. 2003). Studies into the adhesive interactions between the parasitized erythrocytes and CSA have demonstrated that sulphated polysaccharides not only inhibit binding to CSA (Xiao et al. 1996; Clark et al. 1997; Andrews et al. 2005), but they can also be utilized to de-adhere sequestered parasites from the placenta (Fried and Duffy 1996; Gysin, Pouvelle et al. 1999).

Another major host receptor to which infected erythrocytes can adhere is the glycoprotein CD36, which is expressed on a vast number of endothelial cells (Barnwell et al. 1989; Oquendo et al. 1989; Ockenhouse et al. 1992) and is associated with non-severe disease outcomes (Newbold et al. 1997; McGilvray et al. 2000). When we examined the ability of the three carrageenans to inhibit binding to CD36 we found that, as we have recently shown for adhesion to CSA (Xiao et al. 1996; Clark et al. 1997; Andrews et al. 2005), carrageenans CSW-2 and LP-42 were more effective than MB73-F (Fig.1). However, unlike CSW-2 which inhibited all binding to CSA at 50 μg/mL (Andrews et al. 2005), neither CSW-2 nor LP-42 was able to completely inhibit binding of 3D7 to C32 cells, even at 100 μg/mL, although inhibition was still statistically significant (CSW-2 $P=0.02$; LP-42 $P=0.05$). As expected, an anti-CD36 monoclonal antibody (FA6/152) included as a control in each assay completely inhibited adhesion to C32 cells (not shown), confirming the CD36-specific binding phenotype of these parasitized erythrocytes. Adhesion to C32 cells was significantly reduced by two control polysaccharides, dextran sulphate 500 kDa (25, 50 and 100 μg/mL, $P=0.02$) and fucoidan (25 μg/mL, $P=0.03$; 50 μg/mL, $P=0.02$; 100 μg/mL, $P=0.04$). While CSW-2 displayed some dose-dependant inhibitory effect, there was little difference in inhibition levels across the range of concentrations tested for LP-42, MB73-F and the two control inhibitors. Similar findings have been previously reported for dextran sulphate 500 kDa and fucoidan in a study examining inhibition of *P. falciparum* HB3C32-6 infected erythrocytes to C32 (Xiao et al. 1996).

Here we have demonstrated that carrageenans display in vitro anti-malarial properties and that there may be some strain-specific dependence on their activity as Dd2 parasites were found to be more sensitive than 3D7 parasites. However, given the poor potency of these compounds, it is unlikely that they would be a useful anti-malarial therapy. As in our previous study on the effects of these carbohydrates on CSA-specific adhesion, the CD36-specific inhibition profiles for the carrageenans would seem to indicate a dependence on the level of sulphation of these high molecular weight compounds. Carrageenan MB73-F, which did not inhibit CSA or CD36-specific adhesion, has a lower sulphation level and thus a lower overall negative charge, than CSW-2 or LP-42, both of which inhibit CD36-specific adhesion to C32 cells (Table1). These findings support previous work on the importance of sulphation and charge on the inhibition of parasite adhesion to glycoconjugate molecules.

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References

Andrews KT, Walduck A et al (2000) Anti-malarial effect of histone deacetylation inhibitors and mammalian tumour cytodifferentiating agents. Int J Parasitol 30(6):761–768

Andrews KT, Klatt N, Adams Y, Mischnick P, Schwatrz-Albiez R (2005) Inhibition of chondroitin-4-sulfate-specific adhesion of *Plasmodium falciparum* infected erythrocytes by polysaccharides. Infect Immun 73(7):4288–4294

Baird JK (2005) Effectiveness of antimalarial drugs. N Engl J Med 352(15):1565–1577

Barnwell JW, Asch AS, Nachman RL, Yamaya M, Aikawa M, Ingravello P (1989) A human 88-kDa glycoprotien (CD36) functions in vitro as a receptor for a cytoadherence ligand on *Plasmodium falciparum*-infected Eryhtrocytes. J Clin Invest 84:765–772

Beeson JG, Chai W et al (1998). Inhibition of binding of malaria-infected erythrocytes by a tetradecasaccharide fraction from chondroitin sulfate A. Infect Immun 66(7):3397–3402

Beeson J, Rogerson S et al (2000) Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. Nat Med 6:86–90

Beg M, Khan R et al (2002) Cerebral involvement in benign tertian malaria. Am J Trop Med Hyg 67(3):230–232

Berendt AR, Simmons DL, Tansey J, Newbold CI, Marsh K (1989) Intracellular adhesion molecule-1 is an endothelial cell adhesion receptor for *Plasmodium falciparum*. Nature 341:57–59

Butcher GA, Parish CR et al (1988) Inhibition of growth in vitro of *Plasmodium falciparum* by complex polysaccharides. Trans R Soc Trop Med Hyg 82(4):558–559

Carlucci MJ, Ciancia M et al (1999) Antiherpetic activity and mode of action of natural carrageenans of diverse structural types. Antiviral Res 43(2):93–102

Cerami C, Frevert U et al (1992). The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of *Plasmodium falciparum* sporozoites. Cell 70(6):1021–1033

Clark DL, Su S et al (1997) Saccharide anions as inhibitors of the malaria parasite. Glycoconj J 14(4):473–479

Costa FTM, Fusaï T, Parzy D, Sterkers Y, Torrentino M, Lekana Douki J-B, Traoré B, Petres S, Scherf A, Gysin J (2003). Immunization with recombinant duffy binding-like-γ-3 induces pan-reactive and adhesion-blocking antibodies against placental chondroitin sulfate A binding *Plasmodium falciparum* parasites. J Infect Dis 188:153–164

Duraisingh MT, Maier AG et al (2003) Erythrocyte-binding antigen 175 mediates invasion in *Plasmodium falciparum* utilizing sialic acid-dependent and -independent pathways. PNAS 100(8):4796–4801

Frevert U, Sinnis P et al (1993) Malaria circumsporozoite protein binds to heparan sulfate proteoglycans associated with the surface membrane of hepatocytes. J Exp Med 177(5):1287–1298

Fried M, Duffy P (1996) Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta. Science 272:1502–1504

Fried M, Nosten F et al (1998) Maternal antibodies block malaria. Nature 395:851–852

Girond S, Crance JM et al (1991) Antiviral activity of carrageenan on hepatitis A virus replication in cell culture. Res Virol 142(4):261–270

Gysin J, Pouvelle B et al (1999) Ex vivo desequestration of *Plasmodium falciparum*-infected erythrocytes from human placenta by chondroitin sulfate A. Infect Immun 67:6596–6602

Huber W, KJ (1993) A comparison of three methods of estimating EC50 in studies of drug resistance of malaria parasites. Acta Tropica 55(4):257–261

Lambros A, VP (1979) Synchronisation of *Plasmodium falciparum* stages in culture. J Parasitol 65(3):418–420

Jensen JB (1978) Concentration from continuous culture of erythrocytes infected with trophozoites and schizonts of *Plasmodium falciparum*. Am J Trop Med Hyg 27(6):1274–1276

McGilvray ID, Serghides L et al (2000) Nonopsonic monocyte/macrophage phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes: a role for CD36 in malarial clearance. Blood 96(9):3231–3240

Newbold C, WP, Black G, Berendt A, Craig A, Snow B, Msobo M, Peshu N, Marsh K (1997) Receptor-specific adhesion and clinical disease in *Plasmodium falciparum*. Am J Trop med Hyg 57(4):389–398

Nussenzweig V, Nussenzweig RS (1985) Circumsporozoite proteins of malaria parasites. Cell 42(2):401–403

Ockenhouse CF, Barbosa A et al (2001). Sialic acid-dependent binding of baculovirus-expressed recombinant antigens from *Plasmodium falciparum* EBA-175 to Glycophorin A. Mol Biochem Parasitol 113(1):9–21

Ockenhouse C, Tegoshi T et al (1992) Human vascular endothelial cell adhesion receptors for *Plasmodium falciparum*-infected erythrocytes: roles for endothelial leukocyte adhesion molecule 1 and vascular cell adhesion molecule 1. J Exp Med 176(4):1183–1189

Oquendo P, Hundt E et al (1989) CD36 directly mediates cytoadherence of *Plasmodium falciparum* parasitized erythrocytes. Cell 58(1):95–101

Pancake S, Holt G et al (1992) Malaria sporozoites and circumsporozoite proteins bind specifically to sulfated glycoconjugates. J Cell Biol 117(6):1351–1357

Rogerson S, Chaiyaroj S et al (1995) Chondroitin sulfate A is a cell surface receptor for *Plasmodium falciparum*-infected erythrocytes. J Exp Med 182:15–20

Sharma YD (1991) Knobs, knob proteins and cytoadherence in *falciparum* malaria. Int J Biochem Cell Biol 23(9):775–789

Trager WJJ (1976) Human malaria parasites in continuous culture. Science 193:673–675

Utt M and Wadstrom T (1997) Identification of heparan sulphate binding surface proteins of *Helicobacter pylori*: inhibition of heparan sulphate binding with sulphated carbohydrate polymers. J Med Microbiol 46(7):541–546

Walliker DQI, Wellems TE, McCutchan TF, Szarfman A, London WT, Corcoran LM, Burkot TR, Carter R (1987) Genetic analysis of the human malaria parasite *Plasmodium falciparum*. Science 236(4809):1661–1666

Wellems TE, Oduola AMJ, Fenton B, Desjardins R, Panton LJ, do Rosario VE (1988) Chromosome size variation occurs in cloned *Plasmodium falciparum* on in vitro cultivation. Rev Bras Genet 11:813–825

Xiao L, Yang C et al (1996) Sulfated polyanions inhibit invasion of erythrocytes by plasmodial merozoites and cytoadherence of endothelial cells to parasitized erythrocytes. Infect Immun 64(4):1373–1378

Ying P, Shakibaei M et al (1997) The malaria circumsporozoite protein: interaction of the conserved regions I and II-plus with heparin-like oligosaccharides in heparan sulfate. Exp Parasitol 85(2):168–182