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Inhibition of *Plasmodium falciparum* Growth In Vitro and Adhesion to Chondroitin-4-Sulfate by the Heparan Sulfate Mimetic PI-88 and Other Sulfated Oligosaccharides

Yvonne Adams,[1,2] Craig Freeman,[3] Reinhard Schwartz-Albiez,[4] Vito Ferro,[5] Christopher R. Parish,[3] and Katherine T. Andrews[2]*

Hygiene-Institut, Abteilung Parasitologie, Universität Heidelberg, Im Neuenheimer Feld 324, D-69120 Heidelberg, Germany[1]; Clinical Tropical Medicine Laboratory and the Australian Centre for International and Tropical Health and Nutrition (ACITHN), Queensland Institute of Medical Research, QLD 4006, Australia[2]; Cancer and Vascular Biology Group, Division of Immunology and Genetics, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT 2601, Australia[3]; Deutsches Krebsforschungszentrum, Tumorimmunologie, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany[4]; and Drug Design Group, Progen Industries Ltd., Darra, QLD 4076, Australia[5]

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A panel of sulfated oligosaccharides was tested for antimalarial activity and inhibition of adhesion to the placental malaria receptor chondroitin-4-sulfate (CSA). The heparan sulfate mimetic PI-88, currently undergoing phase II anticancer trials, displayed the greatest in vitro antimalarial activity against *Plasmodium falciparum* (50% inhibitory concentration of 7.4 μM) and demonstrated modest adhesion inhibition to cell surface CSA.

Some of the severe pathophysiological symptoms of *Plasmodium falciparum* infection, the causative agent of the most lethal form of human malaria, involve carbohydrate interactions. For example, infected erythrocytes (IEs) can adhere to the glycosaminoglycans chondroitin-4-sulfate (CSA) and hyaluronic acid (6, 7, 14) in the placenta during pregnancy, and heparan sulfate may be involved in the formation of *P. falciparum* rosettes (5, 9, 10). Negatively charged polysaccharides, such as heparin, CSA, dextran sulfates, cellulose sulfates, fucoidan, and the nonsulfated glycosaminoglycan hyaluronic acid, have been shown experimentally to inhibit the in vitro invasion of *P. falciparum* merozoites into erythrocytes, block cytoadhesion of IEs to various host receptors (3, 11, 26), or disrupt *P. falciparum* rosettes (8, 24). In this study, we have examined a panel of sulfated oligosaccharides, including the heparan sulfate mimetic PI-88 (17), which is currently undergoing phase II clinical trials as an anticancer agent, both for their in vitro antimalarial activities and for their effects on adhesion to the host receptor CSA.

A series of hypersulfated oligosaccharides (di- to hexasaccharide, varying in sugar unit, linkage between sugars, and structure of the reducing terminus sugar) were used (Sigma, MO) (Table 1) (20). Each of the prepared sulfated oligosaccharides, along with dextran sulfate (5 kDa) and PI-88 (average molecular mass of ~2.4 kDa; composed predominately of penta- and tetrasaccharides), possesses between three and five hydroxyl groups per saccharide unit available for sulfation, while pentosan polysulfate (5 kDa) has two. The in vitro antimalarial activities of this panel of sulfated saccharides were examined using the multidrug-resistant *P. falciparum* clone Dd2 and [^{3}H]hypoxanthine incorporation, as previously described (4, 13). Early-ring-stage IEs were incubated with various concentrations of each substance for 48 h before adding [^{3}H]hypoxanthine and culturing for a further 24 h. All of the sulfated saccharides used in this work displayed similar antimalarial 50% inhibitory concentration values, with the larger oligosaccharides, maltopentaose sulfate and maltohexaose sulfate, being more potent than the smaller oligosaccharides

TABLE 1. Effects of sulfated saccharides on *P. falciparum* in vitro growth and cytoadhesion to CSA

Oligosaccharide (molecular mass)	*P. falciparum* antimalarial activity (IC_{50} [μM])[a]	*P. falciparum* CSA-binding inhibition (%)[b]
PI-88	7.4 (±10)	87.6 (±10.5)
Dextran sulfate (5 kDa)	NT	82.8 (±7.4)
Pentosan polysulfate (5 kDa)	NT	86.1 (±0.2)
Maltose sulfate	26.7 (±4)	29.6 (±9.0)
Maltotriose sulfate	20.3 (±5)	19.7 (±9.9)
Maltotetraose sulfate	31.5 (±2)	18.9 (±5.2)
Maltopentaose sulfate	13.3 (±5)	70.7 (±16.5)
Maltohexaose sulfate	13.2 (±7)	76.6 (±52.3)
Isomaltotriose sulfate	35.0 (±10)	42.4 (±4.4)
Maltitol sulfate	36.3 (±10)	28.3 (±13.5)
Maltotriitol sulfate	25.8 (±21)	51.3 (±14.7)
Panose sulfate	NT	42.7 (±3.5)
Lactitol sulfate	23.3 (±7)	39.3 (±5.9)

[a] Average 50% inhibitory concentration values (IC_{50}) (±standard deviations) for at least two independent experiments, each carried out in triplicate, are shown. NT, not tested.

[b] Percentages of inhibition (±standard deviations) compared to that for untreated controls for two independent experiments are shown.

* Corresponding author. Mailing address: Clinical Tropical Medicine Laboratory, Infectious Diseases and Immunology Division, Queensland Institute of Medical Research, Herston, QLD 4029, Australia. Phone: 61 (0)7 3845 3725. Fax: 61 (0)7 3362 0104. E-mail: kathy.andrews@qimr.edu.au.

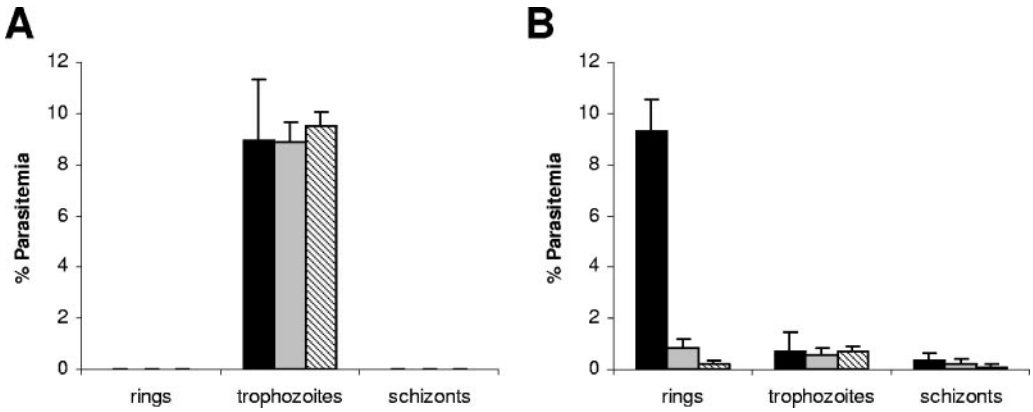

FIG. 1. Stage-specific effects of PI-88 on in vitro development of *P. falciparum* IEs. *P. falciparum* Dd2 IEs were treated with either 100 μg/ml (gray bars) or 50 μg/ml (striped bars) PI-88 or left untreated (black bars) starting at either ring stage (A, 26-h treatment) or trophozoite stage (B, 19-h treatment). Percent parasitemia was determined by microscopic examination of Giemsa-stained thin smears. Representative experiments are shown.

(Table 1). The antimalarial activities obtained for these oligosaccharides are similar to those previously observed for a range of sulfated polysaccharides (1, 3, 11, 26), suggesting promiscuous inhibition by any sulfated polyanion. The most effective compound was PI-88, which gave an antimalarial 50% inhibitory concentration of 7.4 μM (Table 1) and a 90% inhibitory concentration of 13.9 μM. These values are similar to the mean values of maximum concentration of drug in plasma (8.4 to 12.5 μM) which are achieved in cynomolgus monkeys following subcutaneous or intravenous administration of a single dose of PI-88 at 10 mg/kg of body weight (12). Given the extensive work already carried out on the clinical use of PI-88, this compound was more thoroughly studied for its stage-specific activity against malaria. PI-88 did not effect maturation of Dd2 ring-stage parasites to trophozoites (Fig. 1A) but was found to interfere with the invasion of *P. falciparum* merozoites into new erythrocytes (Fig. 1B). Whether this is due to an effect on schizont rupture or a direct effect on merozoite entry into new erythrocytes remains to be determined. Similar results were obtained for the drug-sensitive *P. falciparum* line 3D7 (not shown). At this stage, it is not known if the mechanism of action of PI-88 is due to a specific interaction with a *P. falciparum* molecule or is a nonspecific effect of the high net negative charge of PI-88.

We next tested the effect of the sulfated saccharides in vitro on CSA-specific adhesion of *P. falciparum* IEs. *P. falciparum* clone 3D7 IEs that had been selected more than eight times on CHO-K1 cells for a CSA-binding phenotype ($3D7^{csa}$) (3) were preincubated with 100 μg/ml of saccharide prior to adhesion to CHO-K1 cells. IEs were exposed to cell monolayers (70 to 90% confluence) for 1 h, with gentle agitation every 15 min, before washing away unbound erythrocytes, fixing with 2% glutaraldehyde (Sigma), and staining with Giemsa. CSA-specific adhesion was determined by measuring displacement with 100 μg/ml of soluble CSA (Sigma). The sulfated disaccharides maltose sulfate, maltitol sulfate, and lactitol sulfate were poorer inhibitors than the longer-chain molecules, with PI-88, maltopentaose sulfate, and maltohexaose sulfate being the most potent (Table 1). Again, PI-88 was examined more extensively for its effect on *P. falciparum* cytoadhesion. The effect of PI-88 on CSA-specific adhesion of $3D7^{csa}$ IEs to CHO-K1 cells was tested over a range of concentrations; however, significant inhibition was obtained only at 100 μg/ml and, to a lesser extent, at 10 μg/ml ($P = 0.009$ and 0.086, respectively; two-tailed Student's t test) (Fig. 2). Soluble CSA (100 μg/ml) inhibited binding by more than 90%, confirming CSA-specific binding to CHO-K1 cells (not shown). We next investigated whether PI-88 was able to effect adhesion to the common *P. falciparum* host receptor CD36. Adhesion to CD36 has recently been associated with nonsevere disease (2, 22) and may afford protection via sequestration of IEs in nonvital sites such as the skin and muscle (2, 25). Thus, interfering with CD36 adhesion may promote adhesion to other parasite receptors, such as ICAM-1, which may in turn lead to more-severe disease outcomes, such as cerebral malaria (19). When the effect of PI-88 on CD36-specific adhesion of 3D7 *P. falciparum* IEs to

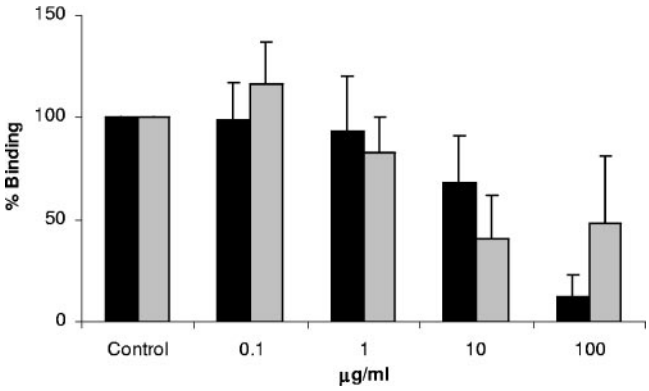

FIG. 2. Effect of PI-88 on CSA- and CD36-specific adhesion of *P. falciparum* IEs. CSA-binding (3D7 selected on CHO-K1 cells [$3D7^{csa}$]) or CD36-binding (unselected 3D7) *P. falciparum* IEs were preincubated with various concentrations of PI-88 prior to adhesion to CHO-K1 (black bars) or C32 (gray bars) cells, respectively. CSA-specific adhesion was determined by the addition of 100 μg/ml soluble CSA, whereas CD36-specific adhesion was determined using 5 μg/ml of the anti-CD36 monoclonal antibody FA6/152. The average percentages of binding (±standard deviations) compared to that for untreated controls for three independent experiments are shown.

C32 melanoma cells was examined (Fig. 2), the inhibition obtained was not significantly different from that obtained with controls ($P > 0.1$; two-tailed Student's t test). An anti-CD36 monoclonal antibody was used as a control for CD36 specificity and, as expected, completely inhibited adhesion of *P. falciparum* IEs to C32 cells (not shown).

Until recently, the only sulfated oligosaccharide to be used clinically against human malaria was heparin (18, 21), which has been discontinued due to heparin-induced hemorrhagic complications. Recently, preclinical and clinical studies have shown that curdlan sulfate, which has a 10-fold-lower anticoagulant activity than heparin, may reduce disease severity in patients with severe and severe/cerebral malaria, although sample sizes in these studies were small (15). PI-88 is a mimetic of heparan sulfate, yet it possesses decreased anticoagulant properties compared with heparin and may therefore be more favorable as an adjunct therapy due to decreased risk of hemorrhagic complications. This oligosaccharide does, however, have limitations; phase I studies indicated that intravenous administration at 2.28 mg/kg/day for 2 weeks resulted in immune-mediated thrombocytopenia in some patients (23), and a short half-life of approximately 1 h has been measured in rats (16), although improvements in pharmacokinetic properties of sulfated oligosaccharides of this type are possible (16). These issues, together with the likelihood that such compounds would be effective only with parenteral administration, will need to be addressed if such a carbohydrate-based drug is to be developed as a novel antimalarial therapy.

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