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The effect of flurbiprofen, a potent non-steroidal anti-inflammatory agent, upon *Trypanosoma vivax* infection in goats

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Platelet aggregation leading to a decreased number of thrombocytes and reduced blood serotonin levels can be correlated with parasitaemia as has been observed in goats and cattle infected with *T. vivax* Y58. Flurbiprofen is a potent anti-inflammatory agent with antipyretic activity. *In vitro*, this agent inhibits platelet aggregation and blocks serotonin release. The results of the present study demonstrated that flurbiprofen inhibited the febrile reactions during the acute phase of *T. vivax* infection, but the drug did not prevent or reverse the associated drop in blood serotonin level during this period. Moreover, it was apparent that flurbiprofen had a deleterious effect on goats infected with *T. vivax* Y58. The infection in the untreated animals (sixteen out of seventeen goats) followed a rather mild and prolonged course with peaks of parasitaemia during the febrile episodes, whereas in flurbiprofen-treated goats (five animals), inoculated with the same number of trypanosomes, the parasitaemia was progressive and terminated in early death with disseminated intravascular coagulation at post mortem examination. These observations would seem to confirm the work of previous investigators, suggesting that anti-inflammatory agents have an aggravatory effect on the course of infection in animals inoculated with various strains of trypanosomes. Important differences exist, however, in the relationship between prostaglandin synthesis in the platelets of the goat and in those of other species.

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INTRODUCTION

Thrombocytopenia is a characteristic feature of acute trypanosomal infections in animals and man (Davis *et al.*, 1974; Robins-Browne, Schneider & Metz, 1975; Veenendaal *et al.*, 1976; van den Ingh *et al.*, 1976a), and platelet aggregation leading to thrombocytopenia and

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reduced blood serotonin levels can be correlated with parasitaemia, as has been observed in goats and cattle infected with *T. vivax* Y58 (Veenendaal *et al.*, 1976; van den Ingh *et al.*, 1976a). The fall in blood serotonin level during temperature peaks, associated with peaks of parasitaemia, and the presence of many platelet thrombi in goats dying during overwhelming parasitaemia suggested a correlation between *T. vivax*, platelet aggregation, and blood serotonin decrease (van den Ingh *et al.*, 1976b). Mixtures of *T. vivax* with plasma containing *T. vivax* antibodies induce release of ^{14}C-serotonin from pre-labelled goat platelets *in vitro* (Slots *et al.*, 1977). *In vivo* studies with *T. brucei* suggest that the rise in body temperature, accompanied by a decrease in blood serotonin level, is related to the formation of immune complexes (Slots *et al.*, 1977).

Recent studies from several laboratories suggest that intraplatelet levels of cyclic AMP and platelet prostaglandin biosynthesis are central factors in platelet aggregation *in vitro* (review: Vermylen, de Gaclano & Verstraete, 1973). Non-steroidal anti-inflammatory and antipyretic agents like flurbiprofen (Adams, McCullough & Nicherson, 1975; van Miert & van Duin, 1977; van Miert, van der Wal-Komproe & van Duin, 1977), which prevent the synthesis of prostaglandins (Flower & Vane, 1972; Vane, 1971; Crook, Collins & Rose, 1976) inhibit human platelet aggregation induced *in vitro* by ADP, adrenaline, collagen and thrombin (Nishizawa, Wynalda & Suydam, 1974; Davies *et al.*, 1974; Sim, McGraw & Sim, 1975). Moreover, flubiprofen blocks the release of ^{14}C-serotonin from human platelets (Davies *et al.*, 1974). This communication reports the effect of flurbiprofen, administered intravenously, in *T. vivax* infected goats.

MATERIALS AND METHODS

These studies were performed using twenty-one crossbred goats of 28–52 kg body weight. *T. vivax* Y58, a pathogenic strain of trypanosomes isolated from cattle at Yakawada, Nigeria has been used throughout this study. This strain has been described by Leeflang, Buys & Blotkamp (1976) and de Gee, Ige & Leeflang (1976). Seventeen goats were infected with *T. vivax* by intravenous inoculation using 10^6 parasites. There is a good correlation between the whole blood serotonin level and the number of thrombocytes in the circulation (Veenendaal *et al.*, 1976, Van den Ingh *et al.*, 1976a and b). Therefore, only whole blood serotonin levels were measured by fluorospectrophotometric analysis using 5-hydroxytryptamine creatinine sulphate as a standard (Das, 1972). The numbers of trypanosomes in the blood were counted (8 × 45 magnification) in wet blood films, using the dipotassium salt of ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. Blood samples, negative for trypanosomes, were re-examined using the haematocrit centrifuge technique (Woo, 1970). Rectal temperature was measured twice daily using a mercury thermometer.

Post-mortem examination was performed on all infected animals treated with flurbiprofen and on one non-infected control. The control was killed at the end of the experiment. Specimens of tissues taken at necropsy were fixed in 10% formalin. After embedding in paraffin, histological sections 5 μm thick were made and stained with haematoxylin-eosin (H and E), periodic acid-Schiff (PAS), phosphotungstic acid haematoxylin (PTAH) and Martius' scarlet blue and by the van Gieson method.

The sodium salt of flurbiprofen was kindly donated by Dr S. S. Adams from the Boots Company Ltd, Nottingham, England. Shortly before use, this agent was dissolved in sterile pyrogen-free saline solution. At the end of the experiments, a number of *T. vivax* infected goats was treated with diminazene aceturate (Berenil, Hoechst Farbwerke A.G., Frankfurt, West Germany).

RESULTS

Experiment 1

Four goats (Nos 1–4) were inoculated intravenously with 10^6 trypanosomes. 3–4 days after the infection febrile reactions were observed coinciding with parasitaemia and associated with decreases of the blood serotonin level (Fig. 1). These phenomena persisted

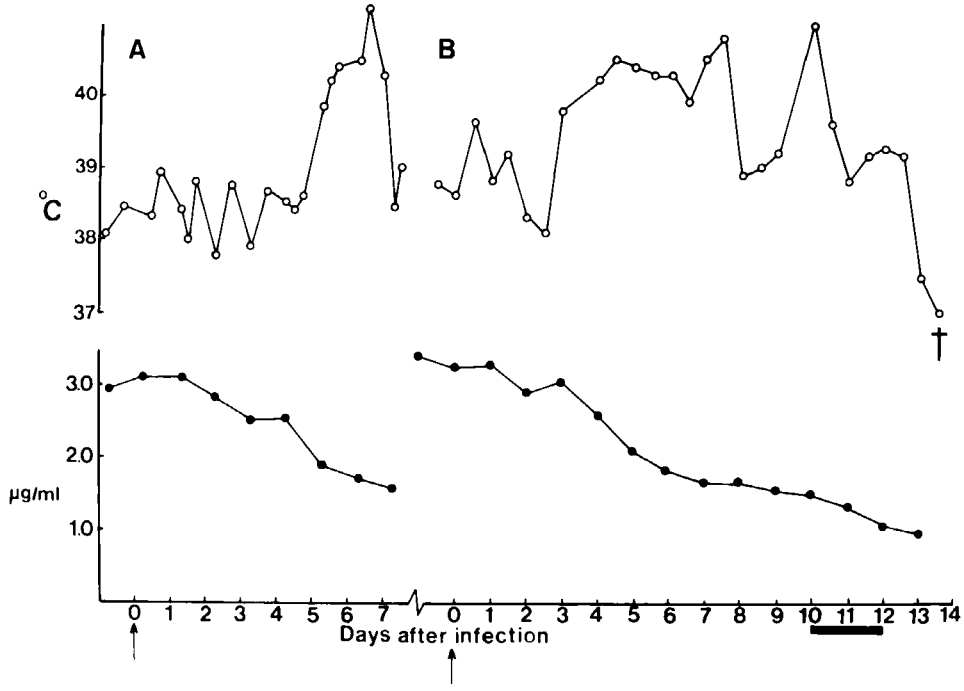

FIG. 1. The effect of flurbiprofen on blood serotonin level and body temperature from goats experimentally infected with *T. vivax*. (A) Control animal (No. 3), not receiving flurbiprofen; (B) One infected goat (No. 1) treated i.v. with flurbiprofen at a dose of 1 mg per kg per day during 3 days (solid bar).

TABLE I. The effect of flurbiprofen on the number of parasites* in the blood from goats experimentally infected with *T. vivax*.

	Goat number									
	Without drug					With drug				
Day	3	4	8	9	10	1	2	5	6	7
0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0
2	ND	ND	0	0	0	+	1-5	ND	ND	0
3	+	1-5	0	0	0	1-5	5-10	1-5	+	0
4	1-5	1-5	0	0	0	5-10	>20	1-5	1-5	0
5	+	+	ND	ND	ND	5-10	>20	>20	>20	ND
6	+	+	0	+	0	1-5	>20	1-5	5-10	+
7	+	+	<1	<1	<1	<1	5-10	10-20	>20	10-20
8	1-5	+	1-5	1-5	<1	>20	1-5	<1	>20	>20
9	ND	ND	10-20	1-5	<1	1-5	>20	ND	ND	>20
10	ND	<1	<1	+	+	+	>20	5-10	1-5	†
11	+	+	+	+	<1	>20	>20	>20	>20	
12	1-5	+	ND	ND	ND	>20	>20	>20	>20	
13	+	+	ND	ND	ND	>20	>20	†	†	
14	+	<1	+	+	+	†	†			
15	5-10	+	<1	<1	1-5					
16	ND	ND								
17	+	+								

*Number of trypanosomes per field (8 × 45). 0, negative; +, positive using the haematocrit centrifuge technique; ND, not determined; †, died.

during the following days. Two of these goats were treated intravenously with flurbiprofen at a dose of 1 mg per kg per day for 3 days (total dose 3 mg per kg) beginning 10 days after inoculation with *T. vivax*. The febrile responses were blocked but the blood serotonin concentrations remained low (Fig. 1). Both animals died on day 14 with high levels of parasitaemia (Table I). The control animals, not receiving flurbiprofen showed intermittent temperature peaks, low blood serotonin levels and a less progressive parasitaemia. These goats were treated with diminazene aceturate, 14 mg per kg body weight intramuscularly on day 19.

Experiment 2

Two goats (Nos 5 and 6) were inoculated intravenously with 10^6 trypanosomes. Beginning 1 day after this inoculation, flurbiprofen was injected intravenously at a dose of 1 mg per kg per day for 5 days. On day 6 the dose of the drug was increased to 2 mg per kg per day, because the duration of antipyresis was less than 12 h. The flurbiprofen was therefore given twice daily from day 6 to day 11 (total dose 15 mg per kg). 4 days after the infection, a progressive parasitaemia developed (Table I) with a concomitant decrease in blood serotonin level. Both animals died during the night following day 12. Blood serotonin concentrations reached minimal values during the terminal stage (0.8 μg per ml; control values, 3.2 and 4.0 μg per ml, respectively).

Experiment 3

Four goats (Nos 7–10) were infected with *T. vivax* on day 0. Only one animal (No. 7) was treated intravenously with flurbiprofen, 1 mg per kg twice daily for 8 days (day 1–9). No febrile reactions were observed in this animal, while the blood serotonin level dropped 7 days after the infection (Fig. 2), with concomitant increase in the number of parasites (Table I). The animal died on day 10. The other three goats (Nos 8–10) showed febrile reactions 6 and 7 days after the infection, while their blood serotonin levels dropped after 5–8 days (Fig. 2). These control animals, not receiving flurbiprofen, showed a less marked parasitaemia (Table I). Nos 9 and 10 were treated with diminazene aceturate, 14 mg per kg body weight intramuscularly on day 16; No. 8 was treated with this drug on day 52.

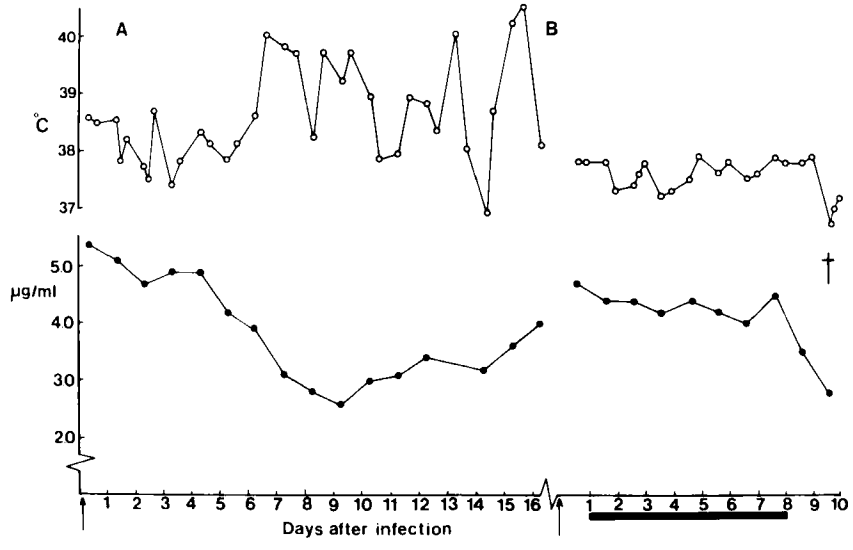

FIG. 2. The effect of flurbiprofen on blood serotonin level and body temperature from goats experimentally infected with *T. vivax* (A) Control animal (No. 9), not receiving flurbiprofen; (B) One infected goat (No. 7) treated with flurbiprofen at a dose of 1 mg per kg, twice daily during 8 days (solid bar).

Experiment 4

Four non-infected goats (Nos 11–14) were treated intravenously with flurbiprofen 1 mg per kg body weight twice daily for 10 days (total dose 20 mg per kg). During this episode no clinical symptoms could be observed and no changes in blood serotonin level could be detected (on day 11, mean value ± SEM: 3.8 ± 0.27 μg per ml blood). One animal (No. 11) was killed on day 11 for post-mortem examination.

Seven other goats (Nos 15–21) not receiving flurbiprofen were inoculated by the intravenous route with 10^6 trypanosomes. Of these animals, one died during the acute phase of *T. vivax* infection (on day 27) and a second one during the chronic phase (on day 56); two were killed after 67 and 132 days, while three were treated with diminazene aceturate after 125, 161 and 168 days respectively.

Pathomorphological observations

The infected, flurbiprofen-treated goats (Nos 1, 2, 5, 6 and 7) all showed at necropsy a markedly enlarged, congested spleen, slight to moderate enlargement of the lymph nodes, petechial or ecchymotic haemorrhages in various organs, and oedema of the lungs. Moreover, goats 5 and 6 had small foci of papillary necrosis in both kidneys, whereas goat 7 showed erosions of the abomasum. Microscopically, severe capillary congestion, intravascular trypanosomes and disseminated intravascular coagulation, as evidenced by hyaline deposition and fibrinous microthrombi, were the most prominent changes. The lungs showed sequestration of granulocytes and macrophages within the capillaries and oedema. The liver showed leukocytosis, proliferation of reticulo-endothelial cells, centrilobular degeneration and solitary liver cell necrosis. The spleen was very congested and several areas of necrosis were found. In the kidneys tubular degeneration, some mononuclear infiltrates and, in goats 5 and 6, papillary necrosis with slight demarcation by neutrophil granulocytes, were observed. In the abomasum of goat 7, the mucosa showed several areas of superficial necrosis and accompanying granulocytic infiltration.

The non-infected, flurbiprofen treated goat (No. 11) killed for pathomorphological examination showed no macroscopic changes except for erosions in the abomasum. Microscopic examination also revealed changes in the renal papillae, consisting of congestion, oedema, focal necrosis, slight infiltration by neutrophil granulocytes, and vacuolisation and regeneration of the epithelium of collecting ducts.

DISCUSSION

The evidence for the existence of a relationship between thrombocytopenia, a decrease in blood serotonin levels, and parasitaemia in trypanosome infections in goats has been mentioned in the introduction to this paper, in which it was suggested that there might also be a relationship with prostaglandin biosynthesis in platelets, as this appears to be a central factor in platelet aggregation *in vitro*. The exact role played by the prostaglandins in the living animal is, however, difficult to work out. In platelets, lung or spleen, arachidonic acid is released from the phospholipid pool by activation of phospholipase A_2 (Flower & Blackwell, 1976). In this context, it is of interest to mention the observation made by Tizard *et al.* (1977), who isolated an active phospholipase A from autolysing *T. congolense*, while the non-pathogenic trypanosome *T. lewisi* did not generate this enzyme on autolysis. Arachidonic acid is converted into prostaglandin endoperoxides by the cyclo-oxygenase system. This step is inhibited by nonsteroidal anti-inflammatory agents (Vane, 1971). In platelets, the prostaglandin endoperoxides are mainly transformed into the unstable thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975), which strongly aggregates platelets. Prostacyclin (prostaglandin X, PGI_2), is a newly discovered prostaglandin (Johnson *et al.*, 1976), which is a very potent inhibitor of blood platelet aggregation (Moncada *et al.*, 1976; Gryglewski *et al.*, 1976). PGI_2 is the predominant product formed from the prostaglandin endoperoxides in the blood vessel linings (Bunting *et al.*, 1976) and the heart (de Deckere, Nugteren & Ten Hoor, 1977).

The cyclo-oxygenase system is inhibited by aspirin and related compounds such as

flurbiprofen (Crook *et al.*, 1976). Flurbiprofen has also been shown to inhibit human platelet aggregation induced *in vitro* by various techniques, and to block the release of ^{14}C-serotonin from human platelets (*vide supra*). Flurbiprofen, like aspirin, inhibits the first step of prostaglandin synthesis from arachidonic acid and thus blocks the formation of both PGI_2 and TXA_2 (thromboxane A_2). Most investigators agree that if, as seems likely, it is the balance between the activities of PGI_2 and TXA_2 that determines whether or not clotting will occur, present information is not adequate to predict the effects of non-steroidal anti-inflammatory agents on the process of platelet aggregation *in vivo*.

In the endotoxin-fevered goat, flurbiprofen is the most potent antipyretic agent yet reported (van Miert *et al.*, 1977; van Miert & van Duin, 1977). The results of the present study demonstrate that flurbiprofen inhibited the febrile reactions during the acute phase of *T. vivax* infection, but the drug did not prevent or reverse the drop in blood serotonin level during this period. Moreover, it is obvious that flurbiprofen had a deleterious effect on goats infected with *T. vivax* Y58. The infection in the untreated animals (sixteen out of seventeen goats) followed a rather mild and prolonged course with peaks of parasitaemia during the febrile episodes, whereas in flurbiprofen-treated goats (five animals) inoculated with the same number of trypanosomes, the parasitaemia was progressive and terminated in early death.

These results resemble the observations by other investigators that anti-inflammatory agents like salicylate (Becker & Gallagher, 1947), cortisone (Petana, 1964) and dexamethasone (Hawking, Wilson & Paris, 1975) have an aggravating effect on the course of infection in animals inoculated with strains of trypanosomes.

It is difficult to find a reasonable explanation for the effect of anti-inflammatory agents like flurbiprofen on trypanosomal infections. The flurbiprofen-treated goats showed parasitaemia of a rapidly progressive type as compared with the intermittent type in the controls. The enhancement of the infection may be due to inhibition of the defence mechanisms of the host (as with steroidal anti-inflammatory drugs) and may lead to acute, fulminating disease. In this acute stage of trypanosomiasis both platelet aggregation and fibrinous microthrombi with concomitant increase of fibrin(ogen) degradation products, have been described (Boreham & Facer, 1974; van den Ingh *et al.*, 1976a and b; Davis *et al.*, 1974). Both mechanisms probably occur during peaks of parasitaemia and may initiate the cascade of an irreversible coagulation of the blood, whereby quantitative differences in the clinicopathological and pathomorphological observations may be found. Flurbiprofen may block the formation of platelet thrombi and thereby possibly potentiate other pathways of thrombus formation, thus leading mainly to fibrinous microthrombi as found in the infected flurbiprofen-treated goats instead of platelet thrombi as mainly found in non-flurbiprofen-treated goats dying during acute, fulminating *T. vivax* infection (van den Ingh *et al.*, 1976b).

The decrease of the blood serotonin level in the flurbiprofen-treated, infected goats suggests that nevertheless platelet aggregation and serotonin release may occur. Intermediates in the biosynthesis of prostaglandins may play an important role in the collagen-induced secretion of platelet granule constituents, such as serotonin. One of the products of the cyclo-oxygenase system is malondialdehyde (MDA) (Flower, Cheung & Cushman, 1973). *In vitro* MDA is produced by thrombocytes from man and rat but not by goat platelets (Akkerman, personal communication). Furthermore, human platelet aggregation and serotonin release can be induced *in vitro* by adrenaline or endotoxin (Hawiger, Hawiger & Timmons, 1975); goat platelets, however, do not respond to these stimuli (Slots, personal communication). It is possible that the prostaglandin-mediated pathway for aggregation does not operate in goat platelets. Vargaftig (1977) suggested that carrageenan and thrombin-induced rabbit platelet aggregation is mediated by non-prostaglandin, phospholipase A_2-derived products. If this is true, non-steroidal anti-inflammatory agents, like flurbiprofen, will only block the synthesis of PGI_2 in favour of clot formation.

The renal papillary necrosis and the abomasal erosions are probable direct toxic effects of flurbiprofen (Woodbury & Fingl,

1975). The absence of coagulopathy in the non-infected flurbiprofen-treated goats on one hand, and the progressive parasitaemia and thrombus formation in flurbiprofen-treated infected goats on the other hand, suggest that the observed changes are not direct toxic effects of the drug.

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