INHIBITION OF HUMAN BLOOD CLOTTING BY EXTRACTS OF ASCARIS SUUM

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ABSTRACT: The effects of soluble extracts of Ascaris suum on human blood coagulation were investigated. Whole worm supernatant solution prolonged the whole blood clotting time and the kaolin-activated, partial thromboplastin time but it did not alter the prothrombin time. These data indicate impairment of the intrinsic pathway of blood coagulation. Whole worm supernate inhibited platelet aggregation induced by ADP and ristocetin. This supernate did not affect fibrinolysis. The maximal concentration of anticoagulant activity was found in the pseudocoelomic fluid of the worm. Activity was also noted in the cuticle and secretory/excretory products. Perhaps inhibition of blood clotting by helminths may facilitate their passage through the blood stream.

The introduction into the circulation of foreign materials such as prosthetic heart valves and arteriovenous fistulae frequently initiates thrombosis. Similarly, microorganisms such as Gram-negative bacteria may activate the coagulation system resulting in disseminated intravascular coagulation. Certain species of helminths such as Ascaris lumbricoides, Strongyloides stercoralis, Trichinella spiralis, and hookworms spend part of their life cycle in the blood circulation whereas others such as filariae and schistosomes may live for many years in the bloodstream.

The mechanisms by which worms avoid stimulating the coagulation system are uncertain. This could be achieved by inhibition of coagulant proteins, inhibition of platelet function, or promotion of fibrinolysis. We undertook preliminary studies using the whole blood clotting time and partial thromboplastin time with kaolin as screening tests. These indicated that anticoagulant properties were associated with extracts of Schistosoma mansoni, Dirofilaria immitis, and A. suum. The latter species was selected for systematic study because it is readily available and permits dissection and preparation of large quantities of material. We now report the effects of A. suum extracts on coagulation, platelet function, and fibrinolysis of human blood.

MATERIALS AND METHODS

Parasite
Live adult A. suum were obtained from a local abattoir for pigs. The worms were homogenized in phosphate buffered saline (7.5 mM NaH2PO4, 0.15 M NaCl) (PBS) at 4°C using a tissue grinder. Solid materials were deposited by centrifugation at 20,000 g at 4°C for 30 min. This supernate of whole worms was collected and stored at −20°C. Worm cuticle, gut, and body fluid (pseudocoelomic fluid) were separated by dissection and supernates were prepared from each fraction. Excretory/secretory products were collected by incubating live worms, washed several times with deionized water in RPMI medium 1640 (GIBCO, Grand Island, New York) containing 100 IU penicillin and 10 μg tobramycin per ml for 24 hr at 37°C. Protein concentrations were determined by the Biuret method (Gornall et al., 1949).

Plasma
Platelet-rich-plasma (PRP) was prepared from volunteer human donors who had received no medications during the previous 2 wk and who had no known defect of platelet function. Blood was anticoagulated immediately with one-tenth volume sodium citrate (final concentration 1.5 mM) and PRP was prepared by centrifugation at 200 g for 20 min at 4°C. The PRP was used immediately after harvesting. Platelet-poor-plasma (PPP) was prepared by centrifugation at 2,000 g for 15 min at 4°C. The PPP was stored frozen at −70°C and thawed at 37°C immediately prior to use. Once-thawed, unused plasma was discarded. Pooled, normal PPP was prepared by mixing equal volumes of PPP from 10 to 15 normal volunteer donors prior to the freezing of the PPP in aliquots.

Coagulation studies
The whole blood clotting time was performed using a modification of the method of Dacie and Lewis (1975) in glass clotting-tubes (Corning Glass Works, Corning, New York). To 0.1 ml of A. suum extract (30 mg per ml), or 0.1 ml PBS as control, was added 0.9 ml blood. After mixing, the clotting time at 37°C was determined. Triplicate samples were performed. The observer was unaware of the contents of each tube. The prothrombin time was performed using a modification of the method of Dacie and Lewis (1975). One-tenth ml pooled PPP and 0.1 ml worm supernate (0.45 mg protein) or 0.1 ml PBS as control, were incubated at 37°C for 5 min prior to the addition of thromboplastin and calcium (Thromborel, Behring Institute, Marburg, West Germany). The time from this addition to clot formation was measured. The partial thromboplastin time with kaolin (PTTK) was measured using a modification of the method of Proctor and Rapaport (1961). One-tenth

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ml plasma was incubated with 0.1 ml worm supernate of varying concentration, or PBS as control, for 4.5 min at 37 C. One-tenth ml platein (General Diagnostics, Morris Plains, New Jersey) and calcium chloride (0.1 ml, 0.025 M) were added in rapid succession and the clotting time measured. Determinations of prothrombin time and PTTK were performed in duplicate.

**Platelet aggregation**

Aggregation was measured in a double-channel aggregometer (Chronolog Corp., Haverton, Pennsylvania). The PRP (0.35 ml), warmed to 37 C, was incubated with 0.1 ml worm supernate, or 0.1 ml PBS as control, for 2 min prior to the addition of aggregating agents. The agents used were epinephrine (David Bull Lab., Melbourne, Australia), adenosine diphosphate (Sigma, St. Louis, Missouri), collagen (Hormann-Chenide, Munich, West Germany), and ristocetin (H. Limdebeck and Co., Copenhagen, Denmark). Test and control samples were performed simultaneously and change in transmission of light was recorded.

**Fibrinolysis**

Fibrinolysis was assessed using unheated fibrin plates (Nilsson and Olow, 1962) made from human fibrinogen (AB Kabi, Stockholm, Sweden) clotted with bovine thrombin (Parke Davis and Co., Detroit, Michigan). Worm supernates (30 μl) ranging in protein concentration from 0.25 to 30 mg per ml were spotted on the surface of the fibrin. Controls (PBS) and standards (Streptokinase, 0.5–5 units per ml, Behring Institute, Marburg, West Germany) were performed concurrently. Fibrinolysis was measured by the square of the diameters of the area of lysis after incubation at 37 C for 24 hr. Enhancement or inhibition of the fibrinolytic activity of streptokinase by the worm extract was studied by mixing the streptokinase and worm extract prior to spotting the plates.

**RESULTS**

**Coagulation studies**

Addition of supernate of *A. suum* to whole blood resulted in a prolongation of the whole blood clotting time by 58.7 ± 1.5 (SD) percent of the control values. Supernate of *A. suum* did not prolong significantly the prothrombin time of 12 human plasmas (mean prolongation 3.6 ± 7.9 [SD] %, paired t-test).

The PTTK of 10 individual plasmas was significantly prolonged (72 ± 20 [SD] %) by the addition of supernate of *A. suum* (P < 0.001, paired t-test). The effects of supernate obtained from cuticle, body fluid, and gut separated by dissection on the PTTK of pooled normal plasma were measured. Maximum anticoagulant effect was detected in the body fluid (Fig. 1). Other fractions possessed much less anticoagulant activity. The supernate prepared from the gut had almost no anticoagulant activity. Prolongation of the PTTK was related exponentially to

![Figure 1. Prolongation of the modified partial thromboplastin time by various concentrations of worm components. Each point represents the mean of at least four determinations (± SD). □ = extract of whole worm; ▲ = cuticle; □ = body fluid.](image)

the worm protein concentration. Alteration of the incubation period of the worm fraction with plasma from 0.5 min to 15 min did not influence the anticoagulant activity. Excretory/secretory products (0.55 mg protein per ml plasma) prolonged the PTTK of pooled normal human plasma (with RPMI medium 1640 as control) from 47 sec to 62 sec (means of duplicates).

**Platelet function**

Platelet aggregation in response to ADP and ristocetin was impaired by the presence of supernate of whole *A. suum*. The impairment of aggregation was variable and ranged from inhibition of the second phase of ADP-induced aggregation to a slowing of the rate of aggregation and a decrease in the change of optical density. The most frequently found changes are illustrated in Figure 2. Aggregation induced by epinephrine and collagen was not affected. Although the degree varied, impairment of aggregation was detected in ADP- and ristocetin-induced aggregation in the PRP of each of four individuals tested.

**Fibrinolysis**

Supernates of whole worm and of body fluid ranging in concentration from 2.5 to 30.0 mg per
prolongation of the PTTK that we observed indicates an effect of A. suum supernate on the intrinsic blood clotting pathway. These results are consistent with those reported with S. mansoni (Tsang and Damian, 1977; Tsang et al., 1977) and T. taeniaeformis (Hammerberg et al., 1980). Indeed, Tsang et al. (1977) concluded that S. mansoni inhibited Coagulation Factor XIIa. We have no direct data as yet to indicate the site or sites of action of the A. suum inhibitor.

The impairment of platelet aggregation by worm products has not been reported previously. This effect may be of particular significance as platelet-surface interaction is thought to be a major precipitant of thrombosis superimposed on introduced surfaces (Weily et al., 1974). Again, the mechanism of this interaction is obscure. It is possible that the effects in platelet aggregation represent a nonspecific interaction with the platelet membrane. Alternatively, the effects on the ristocetin-induced aggregation may be the consequence of an interaction with the Factor VIII molecule, part of which is a requirement for ristocetin-induced platelet aggregation (Howard et al., 1973). Thus, an effect of the worm anticoagulant on Factor VIII could account for the effects noted on the intrinsic pathway of the clotting mechanism and on ristocetin-induced aggregation.

The site of origin of the anticoagulant of A. suum is uncertain. The high concentration of activity in body fluid suggests that this may be the source of the anticoagulant. Because anticoagulant activity was also present in significant amounts in the supernate derived from cuticle there may also be a local concentration of the anticoagulant on the surface of the worm. Furthermore, the anticoagulant effects of the excretory/secretory products indicated that the anticoagulant may diffuse widely.

The observation that helminths have antithrombotic properties should not be unexpected as anticoagulant materials have been obtained from a number of diverse animal species including leeches (hirudin), snakes (ancond), pigs, and cattle (heparin). The precise mechanisms by which helminths inhibit coagulation and the role of these phenomena in determining the nature of the host–parasite relationships merit further investigation. Alternatively, it may be possible to isolate from helminths novel anticoagulant or antithrombotic agents for laboratory or clinical use.

**Discussion**

Any consideration of the life cycle of blood dwelling helminths must take account of the immunological, complement, and hemostatic systems of the host. The present studies have indicated that A. suum may alter the host hemostatic system. Although only the larval stage of A. suum passes through the blood stream, adult worms possess antithrombotic activity and offer a convenient source of material for study.

There have been previous reports that blood dwelling parasites possess anticoagulant properties. Anticoagulant activity has been demonstrated in extracts of S. mansoni (Tsang and Damian, 1977; Tsang et al., 1977) and Taenia taeniaeformis (Hammerberg et al., 1980). The mechanisms of these actions are unclear. The
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LITERATURE CITED


