Antithrombotic Benefits of *Schistosomiasis mansoni* (blood fluke) versus warfarin.

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1. Introduction

This essay compares the benefit of the antithrombotic natures of *Schistosoma Mansoni* (S. Mansoni) and warfarin with the research question: **How does the efficiency (speed) and safety (degree of danger from fibrinolytics versus anticoagulants) of antithrombotic processes of S. mansoni compare to those of existing warfarin medication?** Antithrombotics, although given different meanings, will be the umbrella term for any blood thinning drug or substance in this essay.

1.1 Background information.

Firstly, it is important to understand the hemostatic process - which causes clots that fibrinolytics (drugs that perform the fibrinolysis) must break. The hemostatic process includes a platelet (blood cell) plug that covers the area of injury. The present platelet plug is strengthened, where liquid around the plug turns into a fibrin mesh to seal the damage. This happens when clotting factors such as prothrombinase are made which catalyzes the conversion of prothrombin in the blood into thrombin (Gentry et al). The thrombin enzyme will catalyse the conversion of fibrinogen into fibrin which sticks to everything around it, creating a stable clot (Blanco).

The thrombolytic process is the opposite, often referred to as fibrinolysis. After a blood clot is formed, it must be broken down to prevent it from growing and pressing on important structures or stopping the blood flow. However, this is impeded in patients suffering from deep vein thrombosis or similar illnesses (Wedro). Fibrinolysis consists of the enzyme, plasmin, cleaved from plasminogen (PLMG) using a protease tissue PLMG activator (tPA) breaking down

the clots formed in hemostasis into soluble factors. Drugs that perform this process are referred to as fibrinolytics (Chapin et al.).

S. mansoni are blood dwelling water-borne parasitic worms causing the lethargic disease, schistosomiasis: inflammation due to their movement, harm of organs due to the secretion of certain enzymes, as well as fever, vomiting, and diarrhea due to the eggs ("CDC - Schistosomiasis"). It has induced 290.8 million people to seek treatment in 2018 ("Schistosomiasis"). One of its qualities is the tegument's (coat's) ability to decrease thrombosis in the host, ensuring an optimal range of movement for the parasite. Another of its uniqueness is that they do not produce the vasodilator, bradykinin, which usually causes vessels to dilate during fibrinolysis (SA and HK).

Warfarin, on the other hand, is one of the most popularly used oral anticoagulants - drugs that reduce blood coagulation time, in the world ("About Warfarin"). However, its largest threat has been hemorrhages which are a result of ruptured vessels. 5,000 patients are treated in U.S. emergency departments annually for warfarin-related hemorrhage (Zareh et al.).

1.2 Reasoning

The ability of S. mansoni to avoid vasodilation encourages scientists to reconsider antithrombotic medications such as warfarin, with a low therapeutic index. Safer, and more effective antithrombotics are worth exploring. Additionally, bradykinin is one of the main proteins that causes these hemorrhages. Thus, S. mansoni's thromb cleavage not producing bradykinin has caught the attention of researchers (Harter et al.).

1.3 Hypothesis

S. mansoni's tegumant's strategy for blood cleavage causes fewer vein ruptures and has a more efficient strategy of cleaving the tissue plasminogen activator directly. If the S. mansoni produces a more efficient and safe strategy for antithrombosis, it would suggest that antithrombotics could be developed to a higher standard.

1.4 Research Methodology

This essay utilises data for S. mansoni from researchers at the Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, and Tufts University, (which was found using search terms such as "tegument", "coating", and "enzymes"). Alternatively data for warfarin was taken from Bayer Healthcare (by searching terms such as "INR time" and "side effects"). Moreover, suggested sources from the IB such as NCBI and Researchgate were prioritized when searching. To isolate the effects of the tegument's antithrombotic process, studies on the parasite will be assessed *ex-vivo* - outside the body, to ensure changes in coagulation are not due to side effects of the parasite itself affecting the body. However, the difficulty is the comparison of different types of research on the two methods of anticoagulation. To overcome this issue, two comparison points were used:

- Safety of warfarin considering frequent symptoms (hemorrhages) and rate of reversal, safety of S. mansoni considering alternate fibrinolytics and their frequent symptoms.
- Efficiency based on time to take effect of the S. mansoni and warfarin

This essay tends to provide more information on S. mansoni over warfarin since the parasite is significantly more complex, and requires multiple types of research to understand its process.

2. A comparison of the S. mansoni and warfarin coagulation systems

To begin comparison of S. mansoni and warfarin, it is crucial to understand the difference in the processes they use.

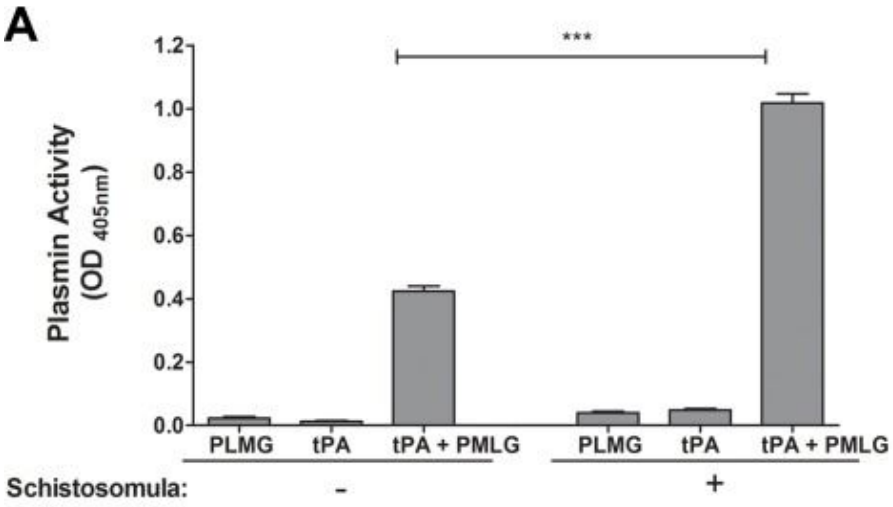
2.1 S. mansoni: Fibrinolytic

S. mansoni has been shown to serve as an antithrombotic similar to a regular fibrinolytic drug (Doenhoff et al.). As stated, this essay will exclusively consider the tegument's enzymes rather than the parasite as whole. S. mansoni's fibrinolysis occurs through cleavage of tPA using enzymes located on the tegument that induce plasmin formation. By cleaving tPA, this allows the tegument to break down blood clots in its host's blood vessels. Note that PLMG and tPA separately have little effect on the generation of plasmin, while together can form significant levels of plasmin through fibrinolysis (Figueiredo et al.).

The occurrence of the relationship of the PLMG and tPA in production of plasmin and the differences in its production with and without S. mansoni were observed. To do this, a plasminogen-activation essay was conducted comparing the plasmin activity with and without a parasite. As plasmin cleaves the synthetic substrate used here: D-Valyl-L-Leucyl-L-Lysine 4-nitroanilide dihydrochloride, it generates a product that is detected at Optical Density of

405nm (OD_{405}) which was used as a method for detecting plasmin activity. A graph is shown below:

Figure 1: A bar graph illustrating PLMG activation by schistosomes in the presence of tPA (Figueiredo et al.).



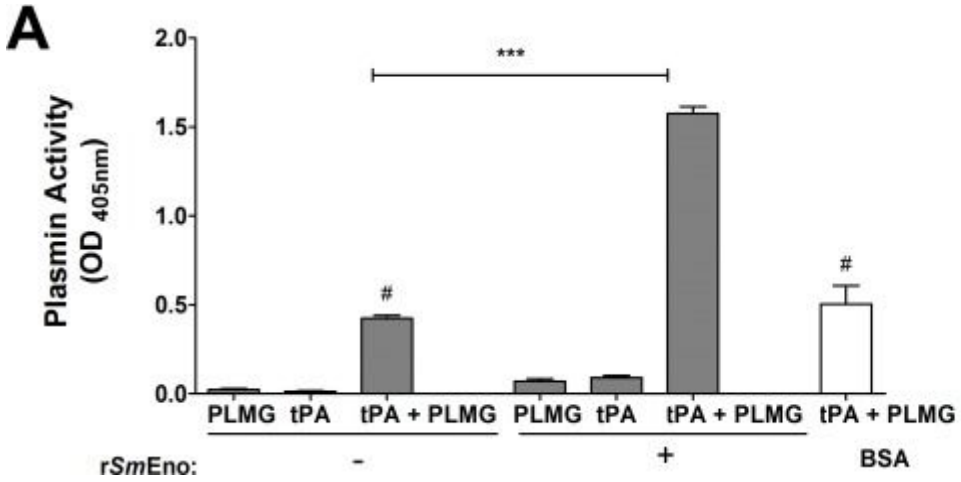
- Figure 1 portrays the plasmin activity (mean OD405 value; 60 min) without a schistosomula (-) and with (+) (1000 parasites per well).

As seen in figure 1, there is only a significant presence of plasmin activation in the presence of tPA and PLMG naturally to fibrinolysis. However, there is also a significant increase of plasmin activity in the presence of S. mansoni, suggesting that it increases plasmin activity, and hence increasing fibrinolysis, serving as a fibrinolytic (Figueiredo et al.).

To emphasize the role of the tegument, it is important to analyze the main enzyme on the coat responsible for fibrinolysis: S. mansoni enolase (SmEno). In this case, an additional investigation was carried out. A purified recombinant SmEno (rSmEno) resembling SmEno

activity was used in a polymerase chain reaction. The rSmEno and an additional control protein BSA were listed as the conditions (Figueiredo et al.):

Figure 2: A bar graph illustrating rSmEno enhancing PLMG activation (Figueiredo et al.).



- Figure 2 portrays the plasmin activity (mean OD405 value) in the presence of rSmEno (+, right gray bars), compared to in the absence of rSmEno (-, left gray bars) and in the presence of a negative control protein BSA (white bar).

Figure 2 shows that in the presence of rSmEno, there is a significant increase of plasmin activity in the presence of tPa and PLMG compared to in the absence of rSmEno. In the presence of a negative control protein BSA, there were no increased effects. This allowed the researchers to conclude that SmEno is an enzyme responsible for inducing plasmin activity in the presence of tPA.

The usage of rSmEno instead of SmEno may be questionable, however the predicted protein sequence was highly conserved as it showed great similarity with other Schistosome species and even human enolase. To ensure that the assumption that SmEno is found exclusively on the tegument is correct, not secreted from the inside of the parasite, an additional experiment can be looked at. The S. mansoni was cultured (which would damage the coat) for 48 hours. This

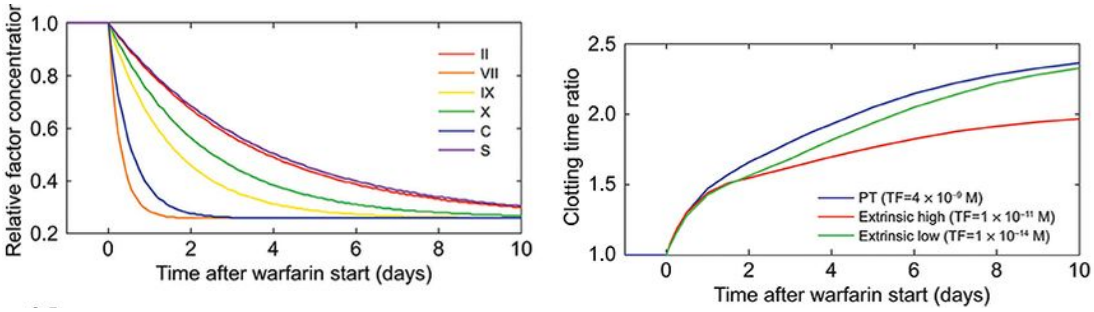
showed no enolase activity, and that although there are high concentrations of enolase in the cytosol of S. mansoni, it did not leak because of damage done to its surface. Hence, it does not display leaking behavior in humans, but is only secreted from the coating (which is damaged here) (Figueiredo et al.). The study's affiliation with the NCBI and many researchers affirmed their results.

2.2: Warfarin: Vitamin K Antagonist

Warfarin is taken by patients often as a way to reduce ischemic stroke due to blood clotting ("Stroke and Blood Clots"). Anticoagulants such as warfarin are referred to as vitamin K antagonists since they reduce vitamin K production by inhibiting the vitamin K reductase enzyme. This leads to a vitamin K decrease in the body, inhibiting the essential process of the gamma-glutamyl carboxylase enzyme converting glutamic acid into gamma carboxyglutamic acid by oxidizing vitamin K. Suppressing the production of this acid decreases the production of clotting factors containing the Gla proteins. This results in a decrease of clotting factors that trigger the production of fibrin from fibrinogen, which results in the inability to form stable clots using fibrin (Harter et al.).

A simulation was used to model the effect of warfarin usage of relative factor concentration and clotting time international normalized ratio (INR) - based on prothrombin time. The amount of warfarin used in the simulation is consistent with the regular dosage a patient receives when taking warfarin as a precautionary heart attack measure (Burghaus et al).

Figure 3: Graphs showing the effect of warfarin on warfarin PK, relative factor concentration, and clotting time ratio (Burghaus et al.).



These graphs show that after warfarin administration, the clotting factors in the blood are reduced, and the time for clot formation increases substantially. This implies that through inhibiting the formation of clotting factors, warfarin decreases clotting and creates an environment where blood clots take a long time to form.

Traditionally, using computational modules to simulate biological phenomena is contentious. However, these models are based on a well-evaluated and peer-reviewed coagulation model by Gaud et al. Furthermore, comparisons of the decaying module of warfarin on INR to data from patients of other peer-reviewed studies have been consistent. Consistency was present for all effects of warfarin (Burghaus et al.).

However, there are limitations. Primarily that the module is based exclusively on Japanese patients. The module doesn't take into account genetic or environmental factors associated with having a uniform sample. The graph also implements new elements like vitamin-K turnover models not part of previous models. Lastly, it is important to consider the publication bias of this module, as it was produced by Bayer Healthcare: a pharmaceutical company producing concurring anticoagulant products to warfarin. Hence, negative effects of warfarin may be amplified by these researchers.

Nevertheless, it can be argued that the general trend remains reliable. The graph is consistent with prior-knowledge regarding the warfarin antagonist action in patients, and the source was published on a reliable journal website: Researchgate. Hence, this essay will continue using this module.

3. Efficiency Comparison

To answer the first part of the research question, the efficiency of both warfarin and S. mansoni will be analyzed

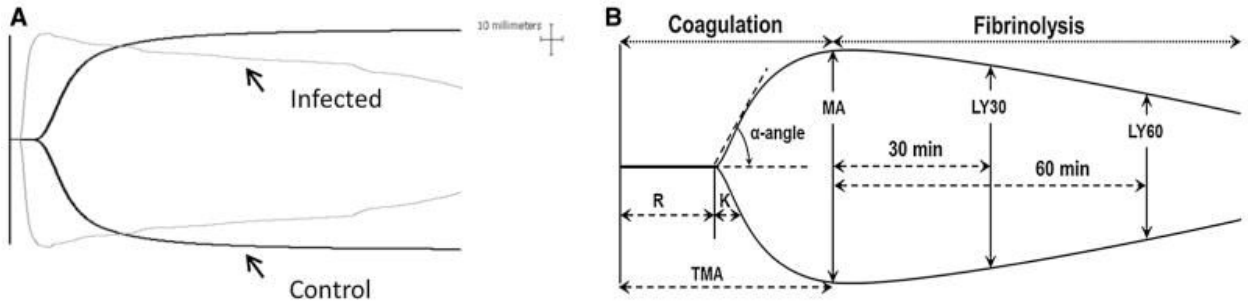
3.1 The S. mansoni

S. mansoni acts similarly to fibrinolytics by breaking down existing blood clots. Fibrinolytics, such as S. mansoni are often considered significantly more effective and rapid in breaking up blood clots as they take immediate effect (Rama et al).

However, it is difficult to perform independent research into S. Mansoni's blood clotting behavior, especially in terms of efficiency. This is the weakness of making definitive statements about S. mansoni's efficiency. Yet, by examining its pathway as a thrombolytic, it does not take days of time as it does for vitamin K antagonists to take action (page 15). They act immediately by breaking down existing blood clots.

To observe the effect of the S. mansoni pathway, this essay uses an experiment that included taking the blood from an S. mansoni infected mouse after 7 weeks and a healthy control mouse, and analyzing their factors. The following thromboelastography (TEG) graphs show the results:

Figure 4: A TEG diagram of infected vs control mice at 7-weeks (Da'dara et al.)



- Figure A shows TEG tracing using a control (the black line) and a 7 week infected mouse (grey line).
- Figure B shows an analysis of the measurements from Figure A's infected sample (the method of reading this diagram is described in appendix i).

The percent lysis of the infected group ranged from 5% to 10% at the 60 minute mark, while consistently remaining 0% in the healthy group. The profile shows an enhanced fibrinolytic state in the blood of infected mice (Da'dara et al).

However, to keep with the integrity of this essay focusing on the tegument exclusively, the graph cannot be considered *in vivo*, as it would be difficult to isolate the effects of enzymes of the tegument from the effects of the parasite itself. *In vivo*, the parasite causes liver fibrosis, which decreases blood coagulation factors (Kamdem et al.), resulting in the effect observed in figure 4, creating a misleading representation of S. mansoni's tegument. Hence, an experiment *ex vivo* should be done additionally to ensure the parasite is not impacting the live mice's circulatory system. In a supplementary investigation, adult parasites were incubated in fresh citrated blood for an hour to observe their direct effects on blood. Another TEG of the *ex vivo* parasites was produced:

Figure 5: A TEG diagram of control murine blood versus murine blood containing adult schistosomes (Da'dara, et al.)

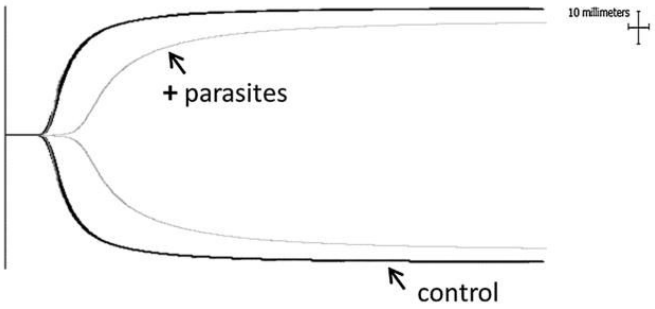
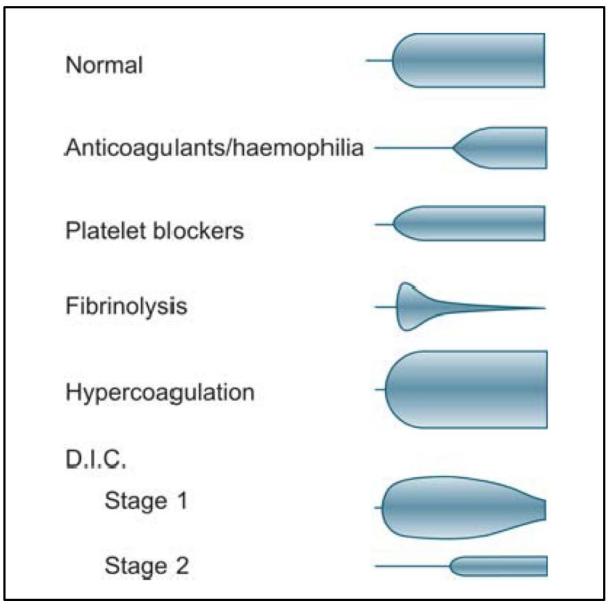


Table 1: A Data table of TEG reaction times of control versus infected plasma, and their complementary P value from a t-test using data from Da'dara, et al.

R time (min)	
Control	5.76 ± 0.85
With Parasites	8.80 ± 1.68
P (t-test)	0.009

This graph shows that the impact of S. mansoni without interaction with the host has a different impact on coagulation. In essence, it shows that S. Mansoni instead increases blood coagulation reaction time (R) - time taken to begin forming a clot. According to literature, the TEG is demonstrably similar to patients that take regular anticoagulant treatment (diagram 1, page 14), indicating that the worms act as anticoagulants, in contrast to *in vivo* experiments.

Diagram 1: A showcase of different TEG waveforms (Simon).



While this study was done on mice, which do not share the same blood as humans, the effects S. mansoni had on blood clearly explain phenomena observed in live patients. These TEGs were compared to 55 infected patient's blood coagulation pathways, and showed great consistency, but with significantly more detail due to fewer ethical barriers (Da'dara, et al.). However, figure 5 is confusing when looking at the analysis of the thrombolytic activity shown in section 2.1, which suggests S. mansoni is a thrombolytic. Yet, thrombolytics show different TEG graphs, as seen in diagram 1, to figure 5. Figure 5 is more similar to an anticoagulant such as warfarin which simply delays thrombus formation. To rationalise this strange difference, the methodology must be reconsidered: the blood analysis was done without the parasites still present (Da'dara, et al.). Yet, for plasminogen binders such as the SmEno to exert effects they need to be ever present in the blood (Gettins and Dolmer). This explains why the thrombolytic pathway isn't prominent in the graph.

Interestingly, this graph gives insight on another property of S. mansoni, which is the ability to delay clotting, and act as a fibrinolytic similar to warfarin. To comprehend this, the tegument can be analyzed further: S. mansoni has shown to be also coated with glycans - polysaccharides and oligosaccharides, which include glycosaminoglycans (polymers of amino sugars) (Mickum et al.). This component contains heparin-like activity of binding to antithrombin and deactivating thrombin - which will impede the coagulation process like other anticoagulants (Bourin and Lindahl). The consistency between the literature and theory confirm the dual function of the parasite (anticoagulant and fibrinolytic).

While it is yet difficult to quantify the fibrinolytic speed, the heparin-like activity of the S. mansoni can now also be compared to heparin. Heparin is an anticoagulant also containing glycosaminoglycans with very similar TEG graphs to figure 5 (Robertson and Cain). Hence, the efficiency of heparin-like activity could be compared to warfarin activity. In fact, 30 participants were given heparin whose effects were analyzed via similar TEG graphs. Heparin showed effect within 4 hours of treatment, showing glycosaminoglycan activity is also faster than warfarin (Tekken et al.). These studies suggest a high efficiency of the S. mansoni.

3.2 Warfarin

Warfarin alone takes around 3 days to reach maximum effect in patients as existing clotting factors need to be used up. Hence, patients with extreme conditions usually need faster-acting anticoagulants such as fibrinolytics or heparin. To observe warfarin's effect, a similar TEG graph would not work as although the INR of warfarin users is increased, their TEG results for warfarin are usually normal (Wysowski et al.). Yet, warfarin is generally produced to

increase INR to 2 or 3 (1 being normal). As shown in figure 3, this process may take days unlike other types of antithrombotics (Jaffer).

Due to the analysis of the listed studies, it can be concluded that warfarin is a slow-acting, and likely passive anticoagulant, taking longer to take effect. On the other hand, as a fibrinolytic, S. mansoni parasites are able to show antithrombotic effects on blood quicker than warfarin. This is since the rSmEno enzyme portrayed in figure 2 is extremely important to the coagulation process, as it inhibits thrombin activity. The heparin effect and process on the coagulation cascade as shown in figure 5 is also significantly faster than the vitamin K antagonist action warfarin utilizes (Garg et al.).

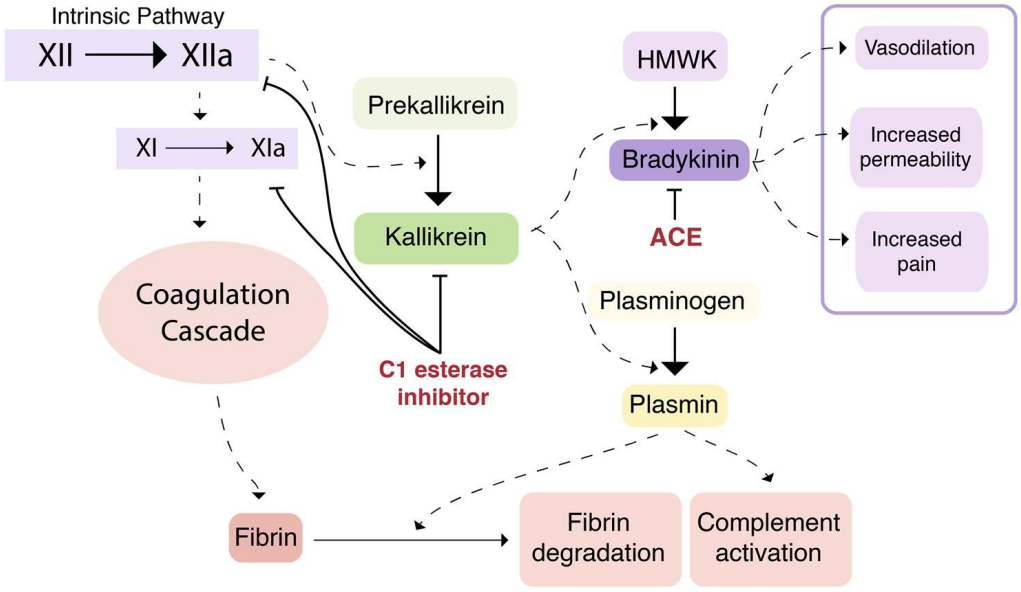
4. Safety Comparison

Next, to answer the second part of the research question, the safety of the S. mansoni and warfarin will be compared.

4.1 Why fibrinolytics are usually more dangerous.

It is easiest to understand the safety concerns with S.mansoni's anticoagulatory pathway by looking at other fibrinolytic treatments used. In this case, the most prominent harm of fibrinolytics is the production of the vasodilator bradykinin throughout their pathway (Gauberti). Bradykinin is a byproduct of the fibrinolytic process, cleaved from high molecular weight kininogen (HMWK) from kallikrein as seen in diagram 2 (Liu). This causes hemorrhages even more often than warfarin (Hoggen). S. mansoni however, has shown to cleave high molecular weight kininogen, without producing bradykinin which will be shown (Hoffmeister et al.).

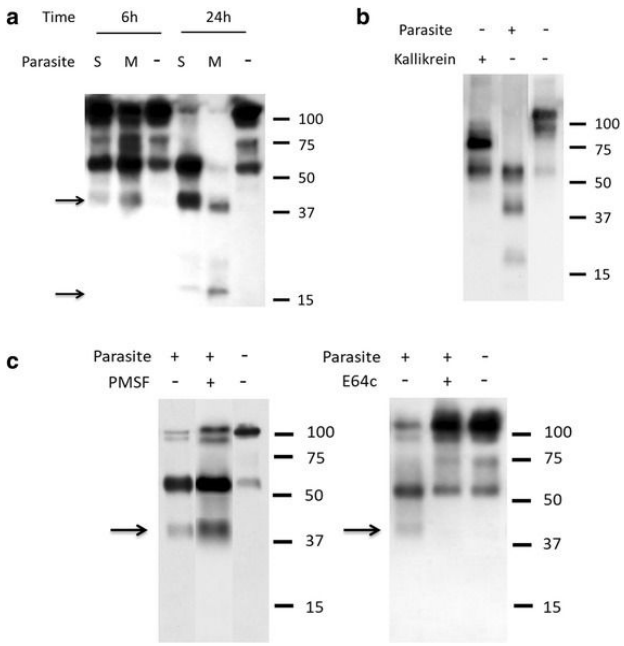
Diagram 2: Kallikrein-Bradykinin Pathway (Liu)



4.2 The S. mansoni

A two-dimensional-differential in-gel electrophoresis (2D-DIGE) was utilized to observe changes in murine plasma proteome (with worms) compared to a control murine plasma (without worms). Specifically changes in high molecular weight kininogen (HK) were looked at. They were observed further through western blot analysis and activity assays. Additionally, a bradykinin detection kit examined the production of Bradykinin from HK (Wang et al.). The western blot analysis used extracted worms and commercial HK. It recovered the samples 6 and 24 hours later, producing the following diagram 7:

Figure 6: Images of the western blot results, analyzing HK cleavage by schistosomes (Wang et al.).



- Part A shows HK either in the presence (S for schistosomula; M for males) or absense (-) of parasites at different times (6 or 24 hours). In this part, there were several products of HK degradation at the arrows, but only where the parasites were present.
- Part B shows cleavage products when parasites and killekarin are either present (+) or absent (-) incubated for 24 hours. The numbers on the side show markers (kDa).
- Part C shows HK incubated for 6 hours

The 2D-DIGE test carried out prior to the western blot analysis test was done on murine plasma, which leads to the question whether the S. mansoni can cleave HK only in murine plasma. To ensure this isn't the case, the worms were also incubated with commercial HK separately, and showed the same results with evidence of HK cleavage as in the murine plasma as seen above (Wang et al.).

After the western blot test, researchers could conclude that the S. Mansoni does indeed cleave HK, however they additionally tested whether it has kallikrein activity that produces bradykinin. The worms were incubated with a kallikrein substrate, and its cleavage was monitored (Wang et al), the following graph was produced:

Figure 7: A line graph representing Kallikrein-Activity with the presence of kallikrein, and schistosomes in the presence/absence of PMSF (Wang et al.)

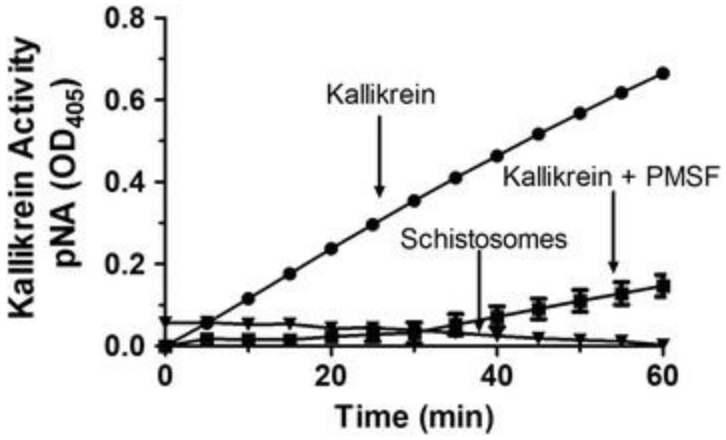


Figure 7 shows there was no cleavage or kallikrein activity in the presence of live parasites. Parasites that were also present for more than one hour still failed the test. However, adding human kallikrein gave a positive trend to generating kallikrein activity. A 2 way ANOVA further shows all of the conditions were very different from the "Kallikrein" one:

$P < 0.001$.

However, it is still possible that without kallikrein activity, the worm is still able to produce bradykinin. For this, a competitive bradykinin ELISA essay was run. In this case, HK was incubated with S. mansoni worms or with Kallikrein for 6 and 24 hours (at which they both cleave HK). The following graph was produced:

Figure 8: A bar graph illustrating generated bradykinin with either male schistosomes or human kallikrein (Wang et al).

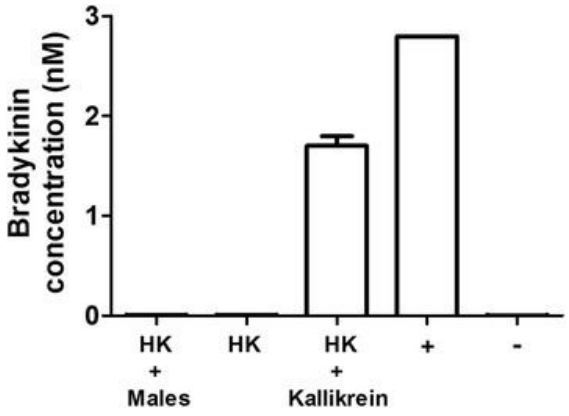


Figure 8 shows the 24 hour results. The Kallikrein group showed an abundance of easily detectable bradykinin, while the group with the male worms showed no detectable bradykinin. In fact, the bradykinin values with the male worm group showed almost no difference from the group with only HK **(*P* = 0.14)** (Wang et al.).

After these series of experiments it is observable that although the S. mansoni is effective in cleaving blood clots through producing plasmin as shown in figure 1, it does not produce the fibrinolytic byproduct of bradykinin. This in essence may allow the antithrombotic process to occur without the weakening and inflammation of blood vessels. An explanation to this behavior could be that the S. mansoni parasites *in vivo*, use this process to reduce clotting and inflammation surrounding them as a way to improve moment in vasculature - doing this they provide a healthy area surrounding them (Leontovyc et al.).

However, although this experiment produced through research on this phenomena is combined with a statistical test, the research does not provide the quantitative results and trials taken during the study, and hence it is hard to verify the source any further than average values

and statistical analysis. Yet, the statistical analysis, if valid (the researchers gathered sufficient quantities of data to allow conclusions to be drawn from the statistical tests, statistical validity), can substitute the information provided by the trials: the results are not due to chance, the trials were consistent. It is lastly important to consider that this experiment was done *ex-vivo* to ensure the isolation of the tegument, yet it is impossible to understand how the tegument will behave in terms of kallikrein activity *in vivo*.

4.3 Warfarin long term effect

Utilizing the same procedure and experiment as for figure 3, the following graph was produced that shows the opposite, the effect of discontinuation of warfarin:

Figure 9: Graphs showing the effect of warfarin discontinuation on warfarin PK, relative factor concentration, and clotting time ratio (Burghaus et al.).

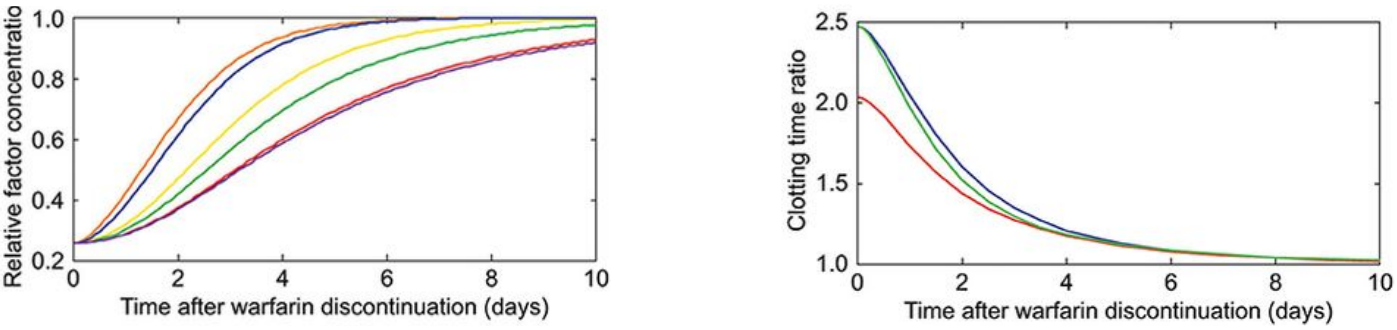


Figure 9 shows that warfarin has long lasting effects for as long as 10 days after discontinuation, meaning that stopping treatment will not completely stop the effects of warfarin. The swiftness of stopping warfarin affects is key due to its inducement of hemorrhages. The cause however, for warfarin-induced hemorrhages remains obscure (Hart), which only increases the need for a more efficient control of the drug as it is difficult to predict the risk of taking warfarin prior to starting its use. In this case, it would take days for increased bleeding to stop

due to the fact that it takes time for the body to produce enough vitamin K to induce clotting once more. Hence, often patients are given additional vitamin K orally as treatment to speed up the process, but this will still take 24 hours to have a substantial effect (Hemphill). Thus, as the dangerous connotations of warfarin are observed, they are also harder to prevent due to the difficulty to control the effects of warfarin. Fibrinolytics on the other hand, stop upon their separation with the patient, as they simply stop cleaving blood clots. In this case, it is shown that the S. mansoni is arguably a safer option than warfarin.

5. Conclusion and Discussion

5.1 Discussion

This essay aimed to compare the antithrombotic efficiency and safety of a popular anticoagulant warfarin, and the tegument of the S. mansoni. The greatest weakness of this essay is the limited research on the beneficial aspects of the parasite, leading most of the sources to be simply segments or sections of books and extensive articles. This limited research could mean that there is more to the parasite's superior efficiency and safety over warfarin (such as negative connotations of the tegument that haven't been researched). It could be beneficial to investigate more regarding the details of the argument in the future when more detailed research is available. The research that has been included in this essay has however attempted to simply shed light on the uses of the S. mansoni, and promote further investigations. Lastly, it is important that this research wasn't biased towards the S. mansoni in order to support the hypothesis. Hence, leading questions in the search engines were avoided, and if they ended up being used, the reverse

leading question will be followed soon after (ex: “benefits of warfarin” and “weaknesses of warfarin”). This attempted to bring the bias to a minimal level during the searching process.

5.2 Conclusion

The answer regarding the research question of comparing the S. mansoni and warfarin in terms of efficiency and safety has been shown in this essay in the following ways:

1. As stated by the hypothesis, this essay suggests that the S. mansoni has a more efficient pathway.
2. S. mansoni has a safer coagulation pathway compared to warfarin that causes vascular rupture and often takes days to take action.

The efficiency of the S. mansoni was established through it’s dual process as a fibrinolytic and anticoagulant, both cleaving blood clots and delaying clotting time, while warfarin was shown to take several days to take effect. The safety of the S. mansoni was shown by its lack of production of bradykinin, the hemorrhage-inducing vasodilator, in its fibrinolytic process, separating it from the danger of usual fibrinolytics. This was in contrast to the low therapeutic value of warfarin, and its high difficulty to control.

Although these results and conclusions were suggested by this essay, as stated in the 5.1 Discussion, the responses are not definitive. Research has still not been conducted on the tegument of the S. mansoni in isolation, and the investigation of why warfarin causes hemorrhages is still incomplete. Thus, the ability to definitively respond to this question is limited. On the other hand, this essay attempts to contribute to the antithrombotic industry in terms of providing new suggested thrombolytic pathways by the S. mansoni blood fluke. If

further investigation and analysis occurs in the realm of research, this could open doors for safer experiences for patients.

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Appendix i: The TEG graph

A TEG diagram can be analyzed through the following points:

Diagram 3: An annotation of a TEG graph (Morcom).