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Efficacy of intravenous aspirin on blood clotting after *Vipera russelli* injection

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ABSTRACT

Vipera russelli, more commonly known as the Russell's Pit Viper, is one of the deadliest snakes in all of South East Asia. The venom causes coagulation by activating the factor X enzyme causing the Factor XA enzyme to initiate the coagulation cascade. The antivenin used to counteract the effects of the venom is readily available in hospitals or wealthier households or businesses. However, the majority of the population in South East Asia are low income families and cannot afford the antivenin. A cheap alternative to the antivenin is intravenous aspirin, it can be easily purchased by the majority of population for a fraction of the cost. In this experiment, six trial groups were constructed, three control groups, and three different concentrations of intravenous aspirin were used test the efficacy against the coagulation effects of the *Vipera russelli* venom. The viscosity of the pig blood was measured by doing timed tilt trials which were recorded digitally. The result showed statistically that the .13 mg/ml and .26 mg/ml intravenous aspirin groups showed no significant change between the control group. However, qualitatively those two aspirin groups did have some clotting in the vials during their tilt period even though statistically they had no significant change from the control. This concludes that .13 mg/ml aspirin and .26 mg/ml aspirin can prolonging the coagulation effects of the *Vipera russelli* venom.

Keywords: *Antivenin*, *Coagulation, Non-Parametric ANOVA, Vipera Russelli, 2-Acetoxybenzoic Acid.*

INTRODUCTION

Vipera russelli has become an overwhelming problem in certain areas in South East Asia. Morbidity and mortality following envenomation are frequent in Pakistan, Sri Lanka and India (Tripathy, S., et al., 2010). *Vipera Russelli's* envenomation activates factors V, X, VIII and induces quicker clotting times inside the blood vessels (Isbister, et al., 2015). The coagulant causes prothrombin to turn into thrombin when there is a presence of factor V and a phospholipid (Tun PE et al., 1995). The venom also induces, bleeding from the gums and urine, kidney failure, and necrosis.

Vipera russelli antivenin is prepared from a horse serum and used to neutralize 0.8mg/ml venom injection in a human. It is readily available in hospitals in Asia. Side effects of the antivenin include: fever, vomiting, hypotension, vertigo, allergic reactions, and shock. Considering the severity of the allergic reactions patients may have after injection it is recommended that skin tests are performed before the administration of the antivenin (Red Cross Antivenin. 2018). Patients may develop an immune complex mediated demyelination (Tripathy, S, et al., 2010) which causes damage to be done to the myelin sheath of neurons casuing signal responses between nerves to be impaired. Antivenin is available in hospitals or for 120 euros online (243 USD or 3,331,295 Indonesian Rupiah) (Red Cross Antivenin). These are two major conflicts to the general population of an underdeveloped country.

An alternative to antivenin that could slow the progression of symptoms could increase survival is a self-induced blood thinner; aspirin. The antivenin may be readily available in hospitals or large income institutions however to the lower class population the antivenin costs are to high to have this readily available.

2-Acetoxybenzoic acid is a common blood thinner used in many applications to prevent blood clotting such as long-term prevention for heart attacks, strokes, and blood clots. It can be found over the counter at a much cheaper cost than the antivenin, which may be a more affordable choice for the lower class populations of South East Asia. The use of daily aspirin for primary prevention reduced cardiovascular events by 15%, myocardial infarctions by 30% and deaths by 6% (Sanmuganathan, et al.). Previous research dosed pigs with 2-20 times the regular dosage of ibuprofen after *Vipera Russelli* envenomation. This study concluded that ibuprofen did not effect PT time until 16 times the recommended dose (Martini, 2015) which may harm the host. Because of aspirins effectiveness at thinning blood, with the right dose, aspirin could possibly counteract the effects of the viper's venom. Aspirin tablets take a long time to get absorbed through the digestive process, therefore liquid aspirin is a better alternative. Liquid aspirin can be injected intravenously which allows the aspirin to directly inserted into the blood stream.

This experiment can help us understand the coagulation effects of the *Vipera russelli* venom on blood, and also how to quickly counteract a *Vipera russelli* bite using an over the counter blood thinner.

MATERIALS AND METHODS

This experimental design involves a quantitative analysis of the coagulation properties of pig blood when affected by *Vipera russelli* venom and aspirin. Each individual trial was performed under the same conditions to properly understand how intravenous aspirin affects the coagulation times of envenomed blood.

Six different sample groups were performed all including ten samples per group. Sample groups include: blood control (pig blood with CaCl2), venom control (Bovine blood, CaCl2, Venom), 0.0162 mg/ml aspirin group (Bovine blood, CaCl2, venom, 0.0162 mg/ml aspirin solution), 0.13 mg/ml aspirin group (Bovine blood, CaCl2, venom, 0.13 mg/ml aspirin solution) and 0.26 mg/ml aspirin group (Bovine blood, CaCl2, venom, 0.26 mg/ml aspirin solution).

It is crucial to this experiment to compare multiple experimental groups to multiple control groups to determine if the independent variable affects the dependent variable. Multiple intravenous aspirin samples were included to show the efficacy of low and high concentrations in effect to the venom and if the more concentrated aspirin will counteract the venom more successfully than the lower doses.

Whole pig blood with sodium citrate was purchased to represent human blood in this experiment. The sodium citrate additive is readily applied to blood samples because of its anticoagulant properties. Sodium citrate binds to the calcium in the blood therefore creating a non-clotting whole blood sample. Injecting a 1.5 ml .10 mM sample of calcium chloride dihydrate can reverse the effects of the sodium citrate to enable use of this blood in coagulation tests. Other products such as the *Vipera russelli* venom, non-additive 10ml blood vials were purchased from Sigma-Aldrich.

The 0.10 mM calcium chloride solution was made by dissolving 0.736 g of calcium chloride dihydrate into one liter of water (Heather Liwang et al., 2010 Each vial of blood used in this experiment will be injected with 1.5 ml of the 0.10 mM calcium chloride dihydrate solution.

Three 250 ml aspirin samples were made with different concentrations (Martini et al., 2015) by dissolving ground aspirin in isotonic saline solution (Erin Larowe et al., 2013) corresponding with amounts of intravenous aspirin that could possibly be taken by a 74.1 kg human. Concentrations include 0.0162 mg/ml (1 baby aspirin), 0.13 mg/ml (2 325mg aspirin) and 0.26 mg/ml (4 325mg aspirin).

An adult *Vipera russelii* (111 +/- 1.8 cm) averages an injection of 144.5 mg of venom per bite (Tun Pe et al., 1986). To make the solution equivalent to the normal injection amount of an adult snake *Vipera russelli* venom was dissolved into an isotonic saline solution to a concentration of 2.89e-5 g/ml. All solutions were made quantitatively using a four-place analytical balance and precise liquid measurements using pipets.

Each sample group's blood was incubated for ten minutes at 37 Celsius before adding any other solutions to simulate human body conditions. The incubation period was then followed by the addition of 1.5 ml of 0.10 mM calcium chloride dihydrate, 1.0 ml of *Vipera russelii* venom, and 1.0 ml of aspirin depending on the sample group. Each individual vial of blood was tilted twice using a viscosity apparatus made out of a pulley system and hardware parts to simulate a constant tilt speed and angle to observe the liquidity of the blood after each sample Every vial tilt was recorded in seconds from when the blood started to move until all 6 ml of it reached the bottom of the vial. Vials were tilted at two different intervals: 0 minutes which occurred directly after venom and or aspirin were injected into the sample, and 5 minutes after the additives were injected into the sample.

Analysis

I calculated and averaged the difference of the 0-minute and 5 minute intervals for each trial group.I used JASP (version 0.8.5.1) to conduct a non-parametric ANOVA test which was used to test for significant differences between the six different trial groups. This is represented by figure 1.

RESULTS

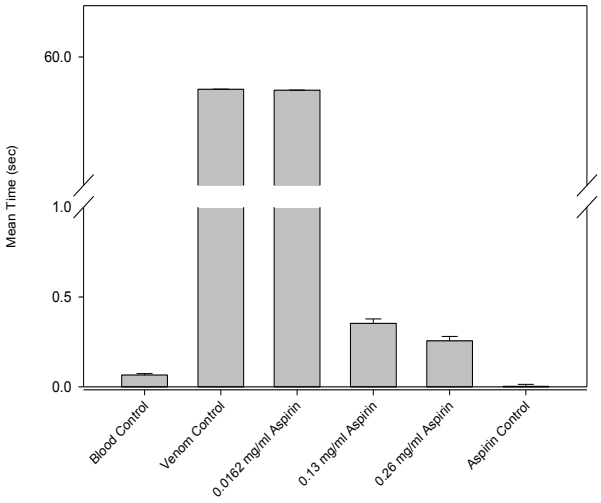

Figure 1 Showing the comparison of mean times in seconds between experimental groups with standard error bars.

In Figure 1 the columns represent the average difference of times of the ten trials for each trial group. The groups which showed significant change from the control group was the venom control (p value of 0.0) and the 0.0162 mg/ml (p value of 0.002) aspirin

sample. The 0.13 mg/ml aspirin sample (p value of 0.272), 0.26 mg/ml aspirin sample (p value of 1.000), and aspirin control (p value of 1.000) showed no significant change in their average difference times when compared to the blood control.

I did not observe a significant difference between the control group and aspirin control. This most likely occurred because the 0-minute time interval was almost exactly the same for both groups. The 5-minute interval trail of both control groups showed a 0.115 second decrease in the tilt time in the aspirin group proving that the intravenous aspirin did positively affect the viscosity of the blood in comparison to the blood control.

There was no significant change between the control group, and both the 0.13 mg/ml aspirin group and 0.26 mg/ml aspirin group. This proves that injecting both these concentrations of intravenous aspirin directly after getting envenomed will slow the clotting time of blood.

The 0.13 mg/ml aspirin group and 0.26 mg/ml aspirin group displayed slight clotting during the experiment even though the statistics proved no significant change between the trials and the blood control. The clot was not thick enough to stop the sliding of blood down the vile, which resulted in an adequate time difference in comparison to the control groups.

The statistical tests show that the efficacy of the 0.13 mg/ml aspirin group and 0.26 mg/ml aspirin group were observed to slightly reduce clotting times in effect to *Vipera russelli* venom.

DISCUSSION

After examination of the statistical analysis of the efficacy of intravenous aspirin on *Vipera russelli* venom, I observed a slight reduction in clotting times in the 0.13 mg/ml aspirin group and the 0.26 mg/ml aspirin group. These results were expected.

This experiment was conducted outside of the human body which accounts for many variables that are difficult to recreate in a laboratory setting. These variables include blood temperature, amount of oxygen to enter the blood, counteracting the sodium citrate to recreate normal clotting conditions, and expiration of the swine blood. Precautions were taken to recreate a normal human blood scenario such as refrigeration to preserve the blood and incubation to warm the blood to body temperature during the experimental period.

The venom controls showed that the venom when interacting with the blood completely clotted in the vials not allowing it to slide during the recording times. The 0.13 mg/ml and 0.26 mg/ml intravenous aspirin groups cause the blood to not combine into one congealed clot but multiple small clots. Thus, these concentrations of aspirin allowed the blood to slide down the tube but did not completely counteract the coagulation effects of the venom.

The statistical data demonstrates that the 0.13 mg/ml and 0.26 mg/ml aspirin groups successfully reduced the clotting times of the viper venom. However, qualitatively the aspirin only moderately succeeded. This may be because the aspirin did not completely counteract the activation of the factor XA enzyme which is formed by the venom and deceive the human body that a clot needs to form. The higher concentration of intravenous aspirin used may result in a more positive qualitative result.

Similar to the Martini (2015) study on ibuprofen, which recorded partial positive results at 16 times the recommended dose, we might see optimal qualitative results if we increase the concentration of intravenous aspirin to high above the recommended amounts. Increasing the concentration of aspirin can cause harmful side effects to the human body. Aspirin overdose can cause: alkalemia or acidemia, alkaluria or aciduria, hypoglycemia or hyperglycemia, water and electrolyte imbalances may occur, nausea, vomiting, tinnitus, hyperpnea, hyperpyrexia, disorientation, coma, and/or convulsions (Arch Interm Med. 1981).

Another possible replacement for the antivenin is a coagulation cascade blocker. Argatroban which is a competitive inhibitor and bivalirudin a bivalent oligopeptide that blocks the active site of protein substrates both act as thrombin inhibitors (Ustinov, N et al., 2016). This could possibly delay or reverse the coagulation cascade that is falsely brought on by the *Vipera russelli* venom.

From the statistical tests and observational data, I conclude that intravenous aspirin when injected into the blood stream right after envenomation slightly reduces coagulation effects brought on by the *Vipera russelli* venom. This is not adequate substitution to the antivenin, however it may allow the patient who has been bitten increased time to get to a hospital where the antivenin is available.

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