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Changes in the Physical and Mechanical Properties of Human Blood with Sustained Prophylactic Use of Acetylsalicylic Acid (Aspirin)—A Rheological Study

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Abstract

While the prophylactic use of regular, low dosage, acetylsalicylic acid (aspirin) has been employed due to the inhibitory effect on platelet aggregation, few if any rheological studies validate the change in the physical and mechanical properties of human blood post-aspirin administration. The viscosity and yield stress of blood from eleven donors were assessed at varied shear rates before and after daily aspirin administration (81 mg) for 14 consecutive days. The findings indicate a statistically relevant aggregated decrease with respect to viscosity and yield stress within the study population.

Keywords

Hemorheology, Rouleaux

1. Introduction

Aspirin, or acetylsalicylic acid, is one of the most commonly used drugs in the world [1] [2] and is widely utilized for the prophylaxis and treatment of atherosclerotic cardiovascular disease (ASCVD) [2] and arterial thrombotic disorders due to its ability to decrease the viscosity of whole blood and reduce clotting due to platelet aggregation [3] [4]. Recent evidence suggests aspirin may be effective in reducing COVID-19 fatalities associated with thrombosis and pulmonary embolism [5] [6]. While studies have shown that aspirin leads to decreased risk of stroke or other vascular diseases, it is also associated with increased risk of major

bleeding events [2].

The precursor to aspirin, salicylic acid, was originally isolated from the bark of the willow tree and has been utilized for its anti-pyretic, anti-inflammatory, and analgesic properties for thousands of years [1]. Felix Hoffman synthesized acetylsalicylic acid (aspirin) in 1897 to reduce the gastrointestinal distress associated with carboxylic acid moiety of salicylic acid [7]. Aspirin is rapidly absorbed post oral administration and partially hydrolyzed to salicylate on first pass through the liver [8] [9]. Following oral administration, the maximum concentration (Cmax), attained within the 30 minutes, accumulates in plasma during repeated dosing. The decrease in platelet aggregation is dose dependent [9] and the optimal dose related to the antiplatelet action of aspirin differs per indication relative to the prevention of thrombus formation in the coronary versus vascular circulation.

Aspirin has been shown to inhibit platelet thromboxane production and aggregation, which results in prolonged bleeding times [1]. Mechanistically, aspirin inhibits the synthesis of platelet thromboxane A_2 (TXA2) by irreversibly acetylating a serine reside near the active site of the enzyme cyclooxygenase-1 (COX-1) as shown in **Figure 1** [10]. The inhibition of COX-1 results in a reduction in the production of TXA2, which normally stimulates the activation of new platelets, increases platelet aggregation, and functions as a positive-feedback mediator during platelet activation [10] [11]. Since platelets do not synthesize new proteins, the resulting suppression of platelet secretion and aggregation lasts for the life of the platelet (approximately 8 - 12 days) [12]. Platelet aggregation caused by TXA2-independent pathways, such as thrombin formation, remains unchanged during aspirin administration.

While the mechanism and its effect on viscosity are well documented in the literature, few studies have used rheological models and apparatus to explore the

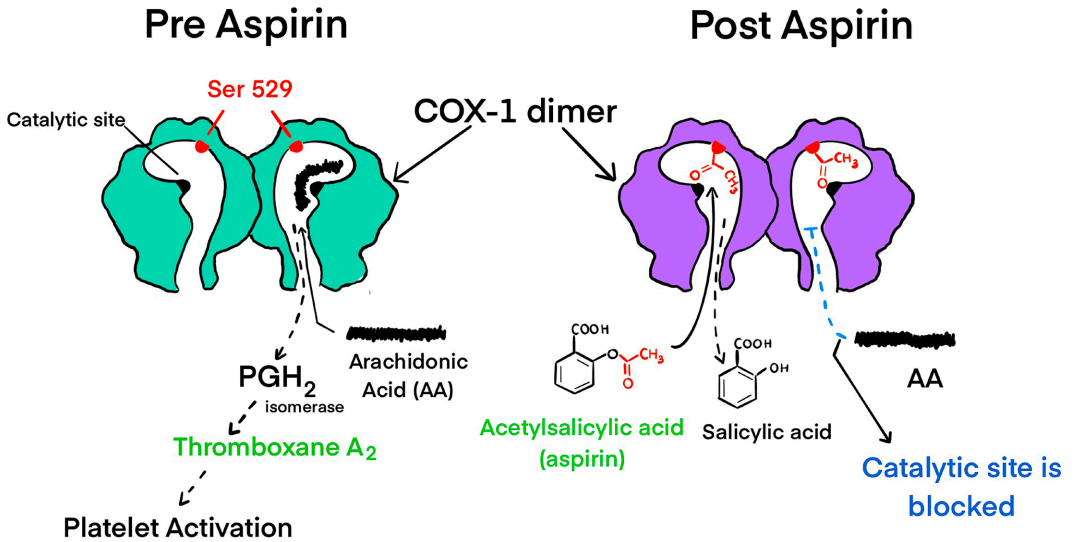

Figure 1. Mechanism of aspirin inhibition of cyclooxygenase-1 catalytic site, preventing the formation of thromboxane A2 and platelet aggregation [10] [11] [13]. Adapted from Fitzgerald & FitzGerald (2013).

viscous and elastic properties of blood and the changes in those rheological properties resulting from regular low dosage aspirin use. Hemorheology is the study of the structure, flow and deformation of blood. Human blood is a complex non-Newtonian, fluid fully described as a thixo-elasto-visco-plastic (TEVP) fluid that contains red blood cells, leukocytes, platelets, and various proteins and dissolved solutes [14]-[19]. The thixotropic property of blood means that it has an evolving microstructure that is attributed to rouleaux—the stacking of red blood cells and time-dependent shear thinning properties [14] [18] [19]. The elastic (solid-like: attributed to evolving and stretching rouleaux) and viscous (liquid-like: resistance) properties of blood also allow it to store and dissipate energy (in a nonlinear manner) and undergo deformation and return to its pre-deformed state [15] [17] [18]. The plastic property of blood means that it also has the ability to undergo permanent change in its microstructure after deformation, especially as the blood ages [17] [18]. This TEVP nature of blood is important in order to understand how the flow of blood throughout the human body is variant and dynamic [14]. That is, the physical and mechanical properties of blood and rheological parameters are related to the physiological properties.

For instance, cholesterol and triglyceride content have been shown to affect the rheological parameters of blood [14] [16] [20]. Earlier research related blood viscosity with shear rate, hematocrit levels, and the diameter of the blood vessel in which it flows [14] [21] [22]. There is a direct correlation between the hematocrit level (ratio of the volume of red blood cells to the volume of total blood) and the viscosity of the blood [23] due to the formation of rouleaux structures [24]. Rouleaux are the pseudo-microstructures that red blood cells (RBCs) form at low shear rates that appear as a stack of coins (**Figure 2**). Fibrinogen is a red blood cell binding protein, known to increase the aggregation of red blood cells into the rouleaux [17]. These factors are known to affect both the yield stress of

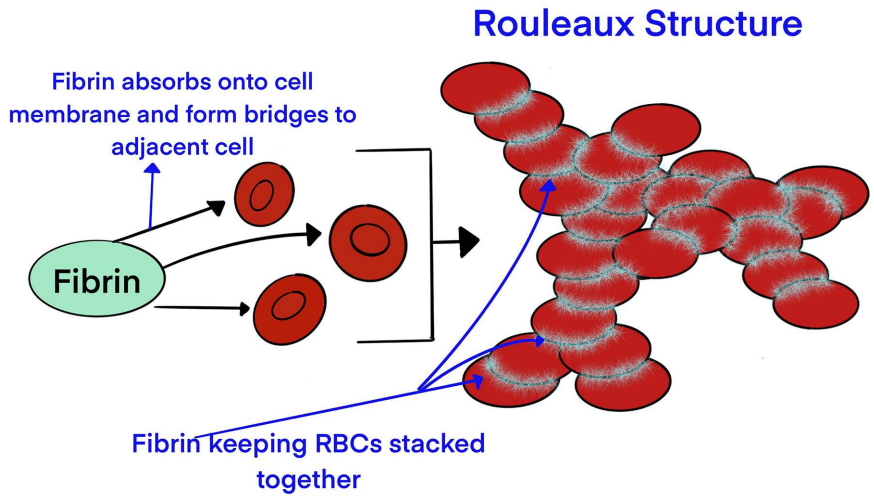

Figure 2. Mechanism by which fibrinogen interacts with red blood cells and forms the rouleaux microstructure, which is one of the major contributors to whole blood viscosity.

the blood and the absolute viscosity. Studies have shown the value of yield stress varies approximately as the cube of hematocrit and as a function of fibrinogen concentration [25]. The physiological metrics of blood can be used evaluate cardiovascular disease in humans [14] [21] [26] [27] [28]. By modeling the physiological properties, one may predict the incidence of cardiovascular disease by evaluating the various physiological parameters.

The enhanced MHAWB model is a thixo-elasto-visco-plastic model that considers both the thixotropic contributions of the rouleaux formation and the contributions of individual blood cells to shear stress [19]. It combines the White-Metzner and Cross models with linear superposition of structural effects. The end result is a model consisting of five ordinary differential equations, six algebraic equations, and ten parameters. Seven of these parameters are used in fitting the steady state rheology data, while three are used when probing the transient data.

The model represents the level of rouleaux formation with the non-dimensional structure parameter, λ, taking on values [0 1], where zero represents blood with all individual RBCs in plasma, and one represents fully "structured" rouleaux. The transient ordinary differential equations that governs rouleaux evolution is a follows

$$\frac{\mathrm{d}\lambda}{\mathrm{d}t} = \frac{1}{\tau_\lambda}\left(\left(1-\lambda\right)+\left(1-\lambda\right)tr_2\left|\dot{\gamma}_p\right|-tr_1\lambda\left|\dot{\gamma}_p\right|\right) \tag{1}$$

where τ_λ, τ_a, and τ_b are time constants representing the overall rate of structure formation, the relative rate of structure formation due to shearing, and the relative rate of structure breakdown due to shearing, respectively. Under steady shear conditions, the ODE can be simplified to

$$\lambda_{ss} = \frac{1+tr_2\left|\dot{\gamma}\right|}{1+tr_2\left|\dot{\gamma}\right|+tr_1\left|\dot{\gamma}\right|} \tag{2}$$

Equation (2) can be used to find the steady state shear rouleaux stress contribution as shown in Equation (3)

$$\sigma_{SS,R} = \sigma_y\lambda_{SS} + \mu_R\dot{\gamma}\left(\lambda_{SS}\right)^{1.5} \tag{3}$$

Additionally, the model represents the individual red blood cell shear stress contributions by a Cross model as seen below in Equation (4)

$$\sigma_{SS,C} = \left(\frac{\mu_{0,C}-\mu_{\infty,C}}{1+\tau_C\left|\dot{\gamma}\right|}+\mu_{\infty,C}\right)\dot{\gamma} \tag{4}$$

where τ_C is a time constant governing the dependence of the apparent viscosity on the shear rate. Finally, to obtain the total stress exhibited by the sample under steady shear conditions, the rouleaux contributions, Equation (3), must be added to the red blood cell contribution, Equation (4),

$$\sigma_{Tot,SS} = \sigma_{SS,R} + \sigma_{SS,c} \tag{5}$$

The resulting model has seven parameters, $tr_2, tr_1, \tau_c, \mu_R, \mu_{0,C}, \mu_{\infty,C}$ and σ_y,

which each must be fit to the collected data. To fit the model parameters we have incorporated a stochastic, global optimization algorithm, parallel simulated annealing [29] [30]. Under high shear rates, Equation (3) will go to zero, showing the decreasing contributions of rouleaux structure at high shear. Under low shear rates, the rouleaux contributions dominate the red blood cell contribution, eventually approaching the dynamic yield stress, σ_y, as $\dot{\gamma}$ approaches zero.

2. Methods

The handling and measurement protocol employed for the blood rheology results in this work follow the previously established guidelines for blood rheology [14] [18] [19] [29] [30] [31] in compliance with United States Military Academy's Institutional Review Board (RHCA19037_918567). No adverse events were reported by the study participants. The specimens collected were subsequently analyzed within a clinical laboratory (Complete Blood Count and Lipid Panel) per published procedures [18]. All rheological measurements were performed using a Discovery Hybrid Rheometer-3 (DHR-3) stress control rheometer from TA Instruments equipped with a double wall couette geometry following previously published steady state and transient protocols [14]. Venipuncture specimens were obtained at day zero (prior to aspirin dosing) and day 14 post 81 mg daily dosage of aspirin. Measurements are taken using the previously mentioned protocols. The steady state rheological experimental protocol was a log spaced in shear rate progression with a pre-shear of 300 s^{-1} for 30 s to remove historical artifacts from previous shear rate measurements; all shear rate tested at a temperature of 37˚C; and all steady states experiments were run in strain-controlled mode [14].

3. Results

In **Figures 1-4**, we show the results of the mHAWB model fitting to 11 sets of steady state data, before and after the aspirin protocol. We focus here on the 4 rheological model parameters that capture the mechanical property change with the aspirin protocol, namely: 1) zero-shear viscosity; 2) infinite-shear viscosity; 3) rouleaux viscosity; and 4) yield stress. These four parameters come directly from fitting the steady state data with a global stochastic optimization algorithm to the mHAWB TEVP model. These results are shown via box and whisker plots in **Figures 1-4**.

4. Discussion

The eleven volunteers in this study were administered 81mg daily low-dose aspirin regimen for a two-week period. **Figure 3** shows the consolidated rheological data for all samples before and after the two-week aspirin protocol. To examine the effect of the aspirin on blood rheology and mechanical properties, we constructed a set of box-and-whisker plots for three different viscosity parameters and one yield stress parameter. The plots show the change in zero shear viscosity (**Figure 3**), infinite shear viscosity (**Figure 4**), viscosity due to rouleaux (**Figure 5**),

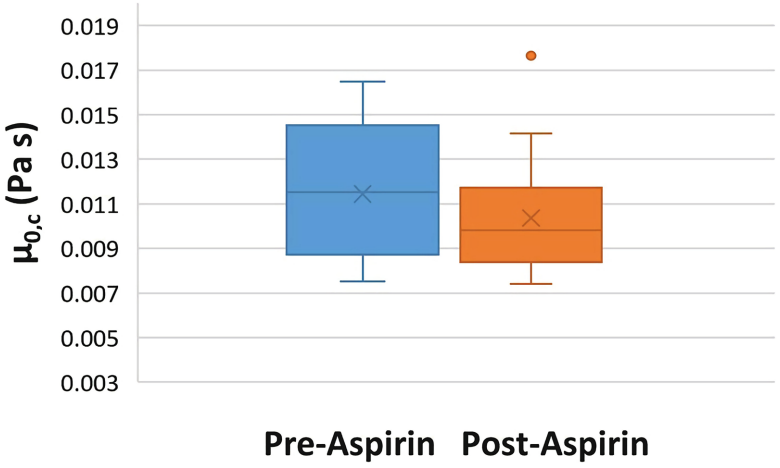

Figure 3. Box and whisker plot of zero shear viscosity before and after a two-week daily regimen of 81 mg aspirin.

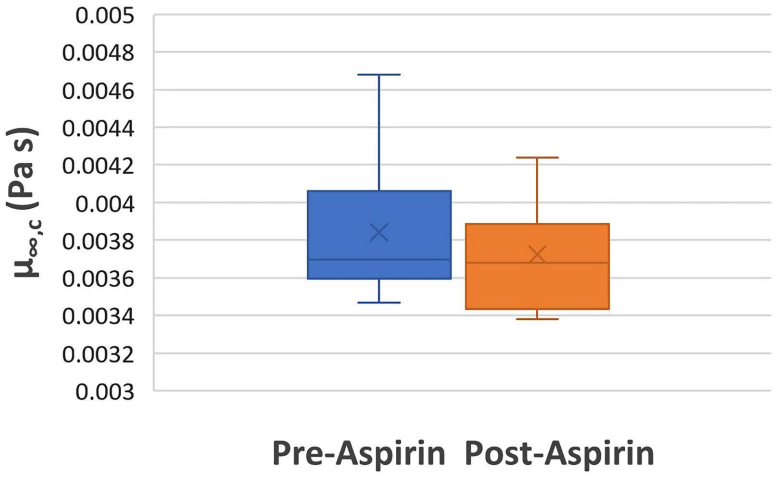

Figure 4. Box and whisker plot of infinite shear viscosity before and after a two-week daily regimen of 81 mg aspirin.

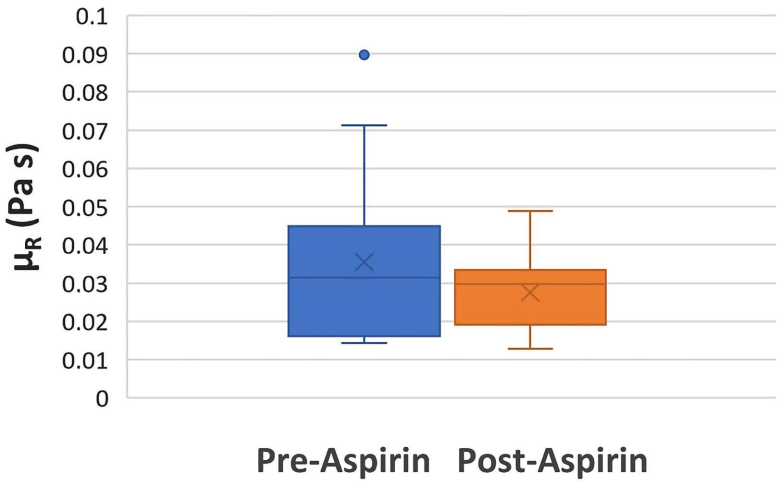

Figure 5. Box and whisker plot of viscosity due to rouleaux before and after a two-week daily regimen of 81 mg aspirin.

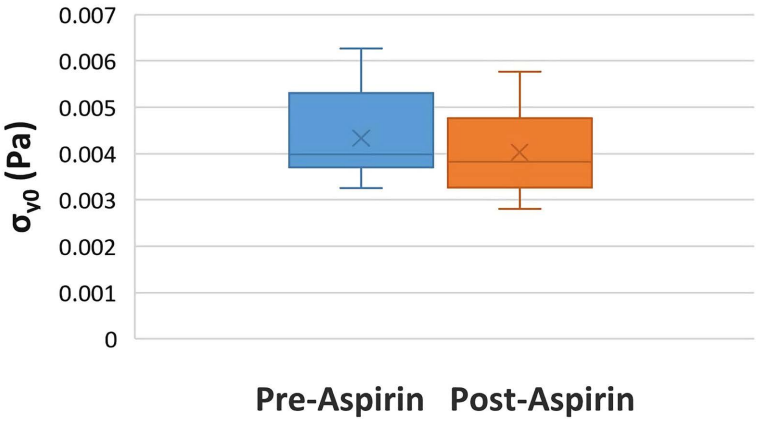

Figure 6. Box and whisker plot of yield stress before and after a two-week daily regimen of 81 mg aspirin

and yield stress (**Figure 6**) values in the blood of the donors before and after the aspirin administration. While the graphical representations indicate a decrease in blood viscosity post aspirin administration, a paired t-test was performed to determine the significance (if any) of the change in any parameter values. Of the seven parameters, only yield stress and infinite shear viscosity resulted in a p-value less than 0.05, indicating a significant change.

The data generated during this study utilized a rheometer to probe the complex mechanical properties of human blood more effectively in response to a COX-1 inhibitor (aspirin) which decreases platelet aggregation. Previous studies utilized viscometer, which itself had limited use with low viscosity materials like blood. The DHR-3 rheometer can incorporate the double wall couette geometry increasing surface area, and signal to noise ratio, thereby allowing for an increased shear rate range of experimentation with the human blood. This allowed for interrogation of viscosity at lower and higher shear rates then the viscometer could. The DHR-3 also allowed for more robust experimental control, through rheology experiments conducted at constant stress, or constant shear rate which the viscometer lacks. Thusly, the steady state curve analysis is able to probe viscosities and stresses over four logarithmic decades of shear rates. Although this study is composed of a small sample size, serves as a case study for further analyses regarding the efficacy of hemorheology in probing the more nuanced flow parameters of human blood. The pre and post physiology lab-reporting has shown a relatively constant (±10%) on average deviation in the hematocrit, total cholesterol and fibrinogen levels, know to affect the mechanical properties the most, corroborating that the blood viscosity and yield stress was affected in a statistically meaningful way by the one a day 81mg aspiring protocol for two weeks [18] [19].

5. Conclusion

We have demonstrated that regular low dosage aspirin does reduce not just total viscosity but several contributing rheological parameters, such as yield stress.

Previous studies failed to explore the effects of regular low dosage aspirin on the multiple rheological parameters of healthy adults. The results of this study will serve to fuel further expanded studies into the diagnostic potential of the field of hemorheology. The once a day, 81 mg aspirin protocol for two weeks has shown to induce a statistically significant decrease in viscosity and yield stress of human blood, with all other physiological parameters (cholesterol, fibrinogen, and HCT) remaining relatively constant ±10% of starting values, aggregated over all 11 donors, from lab pre and post lab reports [32].

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Data Availability

The data that support the findings of this study are openly available in Mendeley at Mendeley.com at: DOI: 10.17632/dttytm73pn.1.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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