The effect of salicylates on aqueous fibrinolysin

Judson P. Smith, III,* and Gerald R. Christensen

Assuming that fibrinolytic activity may play a role in cases of recurrent bleeding in traumatic hyphema, a microassay method for determining fibrinolytic activity in the aqueous humor was developed. Results indicate that the rabbit aqueous contains relatively low levels of fibrinolysin and high levels of fibrinolysin activators. Systemic administration of acetylsalicylic acid in therapeutic doses is shown to reduce the fibrinolytic activity in the aqueous of the rabbit substantially.

Key words: aqueous humor, fibrinolysin, fibrinolysin activators, acetylsalicylic acid, aspirin, salicylates, profibrinolysin, rabbit, hyphema.

While the majority of traumatic hyphemas resolve without sequelae, as many as 26 per cent of cases have been shown to develop secondary bleeding.1 This secondary hemorrhage often leads to severe glaucoma due to mechanical obstruction of the trabecular meshwork and pupil. It can be inferred from hemostatic processes elsewhere in the body that with the primary hemorrhage the ruptured vessels contract and recess, a platelet plug rapidly develops to seal off the ruptured vessels, and a firm fibrin clot develops. The factors responsible for the breakdown of these hemostatic controls leading to secondary bleeding concern certain fibrinolytic processes occurring in the plasma and aqueous humor. In particular, the enzyme fibrinolysin is assumed to play a significant role in the development of the secondary bleed. At present it is uncertain whether clot lysis occurs primarily in response to plasma fibrinolytic activity or in response to the fibrinolytic activity present in the aqueous humor. That fibrinolysin is a major factor in a secondary bleed is also an assumption, and it is quite possible that other variables in the process of hemostasis play a larger role.

In 1952, Ungar, Damgaard, and Hummel2 reported that salicylates inhibited the protease fibrinolysin (as well as many other enzymes). They attempted to correlate the anti-inflammatory action of the salicylates with their ability to inhibit fibrinolysin, a protease which is believed
Salicylates on aqueous fibrinolysin

Key:

-**+= potentiation

-\*-+= inhibition

Profibrinolysis - Fibrinolysin

Proactivator - Activator

Streptokinase

Urokinase, tissue activators, staphylokinase

Fig. 1.

to play a significant role in the inflammatory reaction. However, recent evidence indicates that in plasma, aspirin may inhibit certain inhibitors in the first stage of fibrinolysis, at least in specific concentration ranges (10^-3 M salicylic acid). That is, aspirin appears to inhibit certain inhibitors (antikinases) responsible for the conversion of profibrinolysin to fibrinolysin. This would result in an increase in fibrinolytic activity. The effect of aspirin on the second stage of fibrinolysis is being studied currently. Recent studies indicate that the second stage is not affected in the same range of concentration. Indeed, it has been well documented recently that salicylates decrease the euglobulin lysis time dramatically and may very well become one of the most important drugs in the prophylaxis of thromboembolic phenomena.

Stage I of fibrinolysis concerns the conversion of profibrinolysin to fibrinolysin (see Fig. 1). Stage II of fibrinolysis concerns the breakdown of a fibrin clot into soluble products. In the human, profibrinolysin is converted to fibrinolysin by the cleavage of a single arginyl-valine peptide bond with formation of a two-chain fibrinolysin molecule linked by a single disulfide bond.

The following experiment was devised to estimate the fibrinolytic activity of aqueous humor and to investigate the effect of therapeutic levels of pure acetylsalicylic acid on the fibrinolytic activity of the aqueous humor.

Materials and methods

Five albino rabbits weighing an average of 2.5 kilograms were used as the experimental animals; five similar rabbits served as controls. The experimental animals were given pure acetylsalicylic acid (35 mg. per kilogram) suspended in a 10 per cent acacia mucilage solution every six hours for 48 hours. The drug was administered to each animal via a plastic tube inserted through the mouth into the esophagus. Two hours after the last dose of aspirin, the aqueous humor of both eyes of each rabbit was aspirated with a No. 27 tuberculin syringe and the aqueous from both eyes was pooled and placed in a small test tube. Anesthesia during paracentesis consisted of two drops locally of a 0.25 per cent proparacaine solution (Ophthetic).

Experiment I. To estimate the fibrinolytic activity of aqueous humor, a standard lysis time determination was employed. This was conducted as follows: 1 per cent Armour bovine fibrinogen preparation (1 ml.) containing 0.9 per cent NaCl and 5 per cent imidazole buffer (pH 7.2) was added to a 0.58 per cent NaCl solution (3 ml.) This dilution produced a final fibrinogen concentration of 0.25 per cent and an NaCl concentration of 0.66 per cent. This solution (0.2 ml.) was added to aqueous humor (0.2 ml.), diluted to one half the original concentration, and placed in a small standard prothrombin time tube. To this was added a highly purified Seeger's thrombin solution (0.1 ml.) containing 300 U. per milliliter. After 30 seconds of clotting time had elapsed, a small clear plastic piston weighing 343 mg. was slid gently down the side of the tube, coming to rest on top of the clot. The tubes were then placed in a clear plastic water bath at 37° C. so that the lower level of the piston and the black mark on the tube could be matched easily at various intervals to determine the endpoint of the experiment. The endpoint was arbitrarily chosen to be the time it took the plastic piston to descend down to a black line 4 mm. from the bottom of the tube. The rate of descent of the plastic piston was thus a measure of the lysis time. The results are shown in Table I.

Experiment II. Another set of lysis time experiments was conducted as follows: 1.5 per cent profibrinolysin-free Pentex fibrinogen (2 ml.) i.e., 90 per cent clottable Pentex fibrinogen...
adsorbed with Darco-charcoal to remove the profibrinolysin adsorbed to the negatively charged fibrinogen molecules in a solution with 2 per cent NaCl and 10^{-2} M imidazole buffer (pH 7.2) was added to 2% NaCl and 10^{-2} M imidazole buffer (2 ml.) (pH 7.2). This initial dilution brought the fibrinogen concentration down to 0.75 per cent, but kept the NaCl and imidazole concentrations the same. Distilled water (2 ml.) was added to the above solution (1 ml.), bringing the NaCl concentration to 0.66 per cent and the fibrinogen concentration as in Experiment I. Highly purified Seeger's thrombin solution was added (0.2 ml. of a 300 U. per milliliter solution) to this. This lysis time was determined in Experiment I. The results are noted in Table II.

Repeated attempts were made to use the fibrin plate method of Astrup and Mullertz to estimate fibrinolytic activity in the aqueous. However, as only small changes in the lysed areas were obtained with large changes in adsorbed profibrinolysin concentration and as the plates tended to undergo spontaneous lysis (with the use of Armour fibrinogen), this method could not be used to quantitate fibrinolytic activity with any degree of accuracy of repeatability.

Results

It can be seen from Table I that the fibrinolytic activity of aqueous humor was substantially decreased in the aspirin-treated animals. The lysis time is proportional to the fibrinogen content of the sample divided by the fibrinolytic activity in the sample (i.e., lysis time = $k \frac{\text{fibrinogen concentration}}{\text{fibrinolysin activity}}$). Because the initial fibrinogen content in the experiment was the same, the conclusion is that the fibrinolytic activity of aqueous humor is decreased in the aspirin-treated animals.

The fibrinogen used in Experiment I was Armour fibrinogen, which is known to be heavily contaminated with profibrinolysin adsorbed to the negatively charged fibrinogen molecule. However, the fibrinogen used in Experiment II was highly purified and contained almost no adsorbed profibrinolysin. The lysis times with the "pure" and "impure" fibrogens in the control animals indicate that normal aqueous humor has considerable "activator" activity, i.e., "activator" being a substance capable of converting profibrinolysin to fibrinolysin. Examples are urokinase, streptokinase, and staphylokinase and, in small amounts, fibrinolysin (see Fig. 1). Thus, with the Armour fibrinogen, the lysis times were quite short for the aqueous humor of the control animals, indicating that most of the adsorbed profibrinolysin was converted to active fibrinolysin in aqueous humor.

The blood levels of aspirin in the ex-
Experimental animals ranged from 9.4 to 30 mg per cent. Aspirin may affect the first and/or second stage of fibrinolysis by inhibition or potentiation, depending on the concentration of aspirin in the aqueous humor. As aqueous humor contains predominantly "activator" and little preformed fibrinolysin, one can conclude that the major effect of aspirin must be inhibition of this "activator" in order to account for the dramatic prolongation of the lysis time in the aspirin-treated animals. Aspirin also inhibits certain vitamin K-dependent clotting factors, i.e., factors II, VII, IX, and X.

As aqueous humor contains "activator," this activity may be expressed in terms of a known activator. Lysis time data with the use of various known concentrations of urokinase was determined experimentally. The mean lysis time for 0.1 ml of aqueous in the control animals was found to be equivalent to 1.7 U of urokinase.

In the case of aspirin-treated animals, the mean lysis time per 0.1 ml of aqueous is equivalent to 0.1 U of urokinase. The results in Tables I and II were calculated to be statistically significant (p < 0.05).

Discussion

Normal aqueous humor contains considerable "activator" activity and little preformed fibrinolysin. In animals treated with aspirin, the over-all net fibrinolytic activity of aqueous humor was substantially reduced. As aqueous humor contains predominantly "activator" activity and little preformed fibrinolysin, one can conclude that the major effect of aspirin in the aqueous humor must be inhibition of this as yet unidentified "activator," in order to account for the dramatic prolongation of the lysis time with aspirin-treated animals (approximately an 89 per cent increase in duration of the lysis time). Aspirin may affect the first and/or second stage of fibrinolysis by inhibition or potentiation depending on the concentration of aspirin. Whatever the mechanism, the net result is a decrease in the fibrinolytic activity with the plasma concentration range used in this experiment. The essential question that remains to be answered with regard to the treatment of traumatic hyphema is whether fibrinolytic activity in the aqueous humor or in the plasma is the major factor leading to a secondary bleeding episode, or whether there is some in clot stability that predisposes to secondary bleeding; also, whether the clot occluding the wounded vessel is most likely to be affected by plasma factors or aqueous factors. It is also not clear whether it is necessary for salicylates to enter the anterior chamber to exert their effect. We are currently examining these problems in greater detail.

REFERENCES