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EFFECT OF ACETAMINOPHEN AND SALICYLATE ON ASPIRIN-INDUCED INHIBITION OF HUMAN PLATELET CYCLO-OXYGENASE

GHR Rao*, KR Reddy+, and JG White+*. University of Minnesota Health Sciences Center, Departments of Laboratory Medicine & Pathology* and Pediatrics+, Box 198 Mayo Memorial Building, Minneapolis, Mn 55455 USA. (reprint requests to GHRR).

ABSTRACT

Recent studies have shown that salicylic acid, a metabolite of aspirin, effectively competes for the same site on the platelet cyclo-oxygenase enzyme. In the present investigation we have evaluated the effect of salicylate and acetaminophen on aspirin induced inhibition of cyclo-oxygenase and platelet function. Results of our studies show that both drugs at equimolar concentrations had no inhibitory effect on aspirin induced blockage of cyclo-oxygenase or platelet function. Even at higher concentrations acetaminophen failed to protect cyclo-oxygenase or prevent inhibition of platelet function by aspirin. Salicylate at concentrations above 5 mM effectively blocked the inhibition of cyclo-oxygenase activity and platelet aggregation in response to arachidonate.

INTRODUCTION

Acetylsalicylic acid (aspirin, ASA) is a common antipyretic agent which inhibits the cyclo-oxygenase in platelets (1-4) and endothelial cells (5) thereby preventing conversion of arachidonic acid to endoperoxides (6). Salicylic acid is a metabolite of aspirin (7). It is a potent antiinflammatory agent, but a weak inhibitor of platelet function and prostaglandin synthesis (8-11). 4-(hydroxyphenyl) acetamide (acetaminophen) is an analgesic drug used alone or in association with aspirin. It is an effective antipyretic drug but has little influence on platelet function. Acetaminophen appears to inhibit cyclo-oxygenase to a limited degree, depending upon the tissue tested (12,13). Recent studies have shown that salicylic acid (salicylate) competes for the active site on the cyclo-oxygenase enzyme and can thereby reverse the inhibitory effect of aspirin (15-17). Aspirin is rapidly hydrolyzed to salicylic acid and its biological half-life in man is about 15 to 20 minutes. In individuals with normal renal function the various metabolites do not accumulate. However, after repeated high doses or in

pathological states such as severe liver and kidney disease, the metabolites may accumulate in high concentrations and interfere with aspirin therapy directed at blocking the cyclo-oxygenase enzyme.

In this study we have evaluated the effect of two commonly available drugs that may interfere with the effect of aspirin on cyclo-oxygenase activity. The results of our studies show that acetaminophen and salicylate at equimolar concentrations presented together with aspirin had no inhibitory effect on the aspirin induced inhibition of platelet cyclo-oxygenase activity. Salicylate significantly inhibited the action of aspirin when platelets were exposed to fifty times more of that agent than aspirin and totally blocked its effect at one hundred times the concentration.

MATERIALS AND METHODS

Materials

Arachidonic acid as the sodium salt was obtained from Nu Chek Prep, Elysian, Minnesota, and made up in 0.1 M Tris buffer of pH 7.4 [1-^{14}C]-arachidonic acid was obtained from New England Nuclear, Boston, Massachusetts. Acetaminophen, sodium salicylate and acetylsalicylic acid were purchased from Sigma Chemical Company, St. Louis, Missouri.

Methods

Blood drawn from volunteer human donors was mixed immediately with trisodium citrate-citric acid-dextrose (CCD) buffer, (Citrate 0.1 M, citric acid 7 mM, dextrose 0.14 M, pH 6.5), in a ratio of 9 parts of blood to 1 part anticoagulant. Platelet-rich plasma (PRP) was separated by centrifugation at 200 x g for 20 minutes at room temperature. Platelet-poor plasma (PPP) was separated by centrifugation at 200 x g for 20 minutes at room temperature. Platelet-poor plasma (PPP) was prepared by centrifugation of anticoagulated blood at 1,500 x g for 20 minutes. Platelet aggregation was monitored with a dual-channel Payton aggregometer at a stirring speed of 1000 rpm and calibrated using PRP and PPP (14).

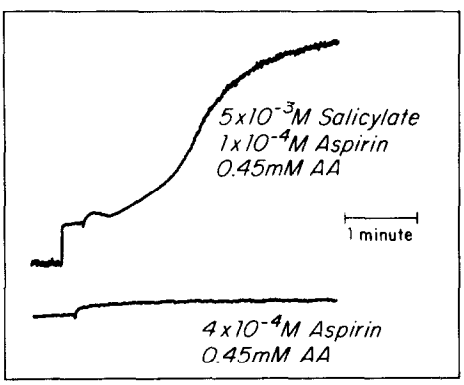
For measurement of arachidonic acid metabolism each reaction mixture (1 ml) containing 1.5 x 10^9 washed platelets was stirred on an aggregometer for 5 minutes at 37°C with 1 µg of labeled arachidonic acid (14). When inhibitors were employed, the reaction mixture was incubated with the drugs at least ten minutes before the addition of arachidonic acid. At the end of the experiment 1 ml of ethyl acetate was added to each reaction mixture and acidified with 10 µl of 0.5 M citric acid. After thorough mixing the ethyl acetate layer was separated and the reaction mixture was reextracted once more with an equal volume of ethyl acetate. Fractions of the organic phase were pooled, concentrated over nitrogen and plated on a silica gel G plate. The solvent system used for the separation of thromboxane B_2 was ether, methanol and acetic acid (135: 3:3 v/v). Radioactivity was monitored with a Berthold radiolabel scanner and quantitation was achieved by separation of the spots and scintillation counting. Standard statistical procedures were used to calculate standard deviation and Students T test of significance.

RESULTS

Effect of Salicylate and Acetaminophen on Aspirin Induced Inhibition of Platelet Response to Arachidonate

Various concentrations of salicylate (2.5, 5.0, 7.5 and 10 mM) and acetaminophen (1, 3 and 6 mM) were tested for their effect on aspirin (1×10^{-4}M) induced inhibition of platelet aggregation in response to 0.45 mM arachidonate. Platelets were incubated for 30 minutes with salicylate or acetaminophen and then aspirin was added and after 30 minutes of additional incubation they were challenged with arachidonate. Salicylate at greater than 5.0 mM concentrations effectively blocked the inhibitory effect of aspirin on platelet response to arachidonate (Fig. 1). On the other hand, acetaminophen had no influence on the aspirin induced inhibition of cyclo-oxygenase at any concentration.

Protective Effect of Salicylate on the Aspirin Induced Inhibition of Platelet Response to Arachidonate (AA)



ΔT*-change in light transition

Figure 1. Acetylsalicylic acid blocked the aggregation response of platelets to arachidonate. However, incubation of platelets with salicylate (greater than 5.0 mM) first prevented the inhibitory action of aspirin.

Effect of Acetaminophen and Salicylate on the Aspirin-Induced Inhibition of Platelet Cyclo-Oxygenase

Normal control platelets incubated with arachidonic acid generated 32% of thromboxane B_2 (Table 1). Aspirin at a concentration of 100 μM significantly inhibited the conversion of arachidonic acid to thromboxane. Acetaminophen or salicylate (100 μM), incubated simultaneously with 100 μM aspirin, offered no protection to cyclo-oxygenase against the aspirin-induced blockade of arachidonic acid conversion to thromboxane. Even when equimolar concentrations of acetaminophen and salicylate were combined and incubated simultaneously with aspirin the drugs did not

effectively compete with aspirin and prevent the inhibition of platelet cyclo-oxygenase activity by aspirin.

Table 1.

EFFECT OF ACETAMINOPHEN (AAP) AND SALICYLATE (SL) ON THE ASPIRIN (ASA) INDUCED INHIBITION OF HUMAN PLATELET CYCLO-OXYGENASE

	CPM 10^3		
	Total Counts Plated[1]	Counts Recovered as Thromboxane B_2 (TxB_2)[1]	% Conversion to TxB_2[1]
Control	131.0 ± 4.7	44.4 ± 0.3	33.9 ± 2.0
Aspirin (ASA) (1×10^{-4}M)	78.8 ± 6.2	2.1 ± 0.8	2.7 ± 0.2*
Acetaminophen + ASA (1×10^{-4}M)	108.2 ± 2.9	2.7 ± 0.4	2.5 ± 0.2*
Salicylate (SL) + ASA (1×10^{-4}M)	113.3 ± 2.7	3.9 ± 0.4	3.4 ± 0.2*
AAP + SL + ASA (1×10^{-4}M)	111.6 ± 1.9	4.2 ± 0.2	3.8 ± 0.2*

[1]Mean and the standard error (n = 4)

*P > 0.001

Table 1. Acetylsalicylic acid at a concentration of 10^{-4} M effectively blocked platelet cyclo-oxygenase activity. Equimolar concentrations of salicylate, acetaminophen, or the combination of the two drugs added along with aspirin did not prevent the inhibitory action of aspirin.

Effect of Various Concentrations of Salicylate and Acetaminophen on Inhibition of Platelet Cyclo-Oxygenase

Since initial hydrolysis of aspirin leads to the formation of salicylic acid, the effect of this metabolite on the aspirin induced inhibition of cyclo-oxygenase was followed. Normal control platelets converted 32% of arachidonic acid to thromboxane B_2 (Table 2). Aspirin at 100 μM concentration significantly inhibited the conversion of arachidonic acid. Exposure of platelets to concentrations of salicylate twenty-five times the concentration of aspirin had no influence on the inhibition of cyclo-oxygenase activity by aspirin. At concentrations fifty and seventy-five times the dose of aspirin, salicylate exerted a significant protection of cyclo-oxygenase against aspirin induced blockade of platelet cyclo-oxygenase activity. At one hundred times the dose of aspirin salicylate effectively blocked the action of aspirin on plate-

let cyclo-oxygenase activity. Because of the solubility problems acetaminophen could not be tested at higher than 6 mM final concentration. At concentrations below 6 mM, it offered no protection to cyclo-oxygenase against the inhibitory effect of aspirin.

Table 2.

EFFECT OF VARIOUS CONCENTRATIONS OF SALICYLATE ON THE ASPIRIN (100 μM) INDUCED INHIBITION OF HUMAN PLATELET CYCLO-OXYGENASE

	CPM 10^3		
	Total Counts Plated[1]	Counts Recovered as Thromboxane (TxB_2)[1]	% Conversion to TxB_2[1]
Control	129.3 ± 9.8	40.7 ± 3.3	31.5 ± 2.0
Salicylate (mM)			
2.5	131.2 ± 4.5	3.2 ± 1.2	2.5 ± 0.2*
5.0	142.4 ± 5.6	17.1 ± 1.5	12.0 ± 1.0**
7.5	134.7 ± 2.6	25.3 ± 1.6	18.8 ± 0.8**
10.0	129.4 ± 5.8	35.1 ± 1.4	27.3 ± 1.2***

* $P > 0.001$

** $P > 0.02$

*** $P < 0.05$ (not significant)

[1] Mean and the standard error (n = 4)

Table 2. Acetylsalicylic acid at a concentration of 10^{-4}M effectively blocked platelet cyclo-oxygenase activity. Salicylate at 2.5 mM concentration did not prevent the inhibitory influence of aspirin. However, greater than 5 mM salicylate effectively blocked aspirin inhibition and protected platelet cyclo-oxygenase activity.

DISCUSSION

Results of our study demonstrate that acetaminophen and salicylate, the two most common drugs present during aspirin therapy, do not interfere with the in vitro effect of aspirin on platelet cyclo-oxygenase activity when challenged simultaneously in equimolar concentrations. Acetaminophen at the concentrations tested (1, 3 and 6 mM) had no inhibitory effect on aspirin induced blockage of the platelet response to arachidonate. Similarly, when tested for its effect on platelet cyclo-oxygenase activity, none of the drug concentrations resulted in protection of cyclo-oxygenase activity from the effect of aspirin. In similar studies preincubation of platelets with salicylate at 5 mM and high concentrations prevented the aspirin induced block of platelet response

to arachidonate. At higher concentrations (greater than 5 mM) it offered significant protection of cyclo-oxygenase enzymes from aspirin.

Studies from our laboratory and that of others have shown that salicylate competes for a common site on the cyclo-oxygenase enzyme (15,17). In earlier studies using a cell free system, we have shown that salicylate competes for heme iron just like acetylsalicylic acid (17). Vargaftig arrived at a similar conclusion after his studies showed that salicylate did not affect the action of indomethacin or ETYA on cyclo-oxygenase activity, but did so preferentially on aspirin (15). However, in our studies we find that concentration of salicylate needed to block effectively the action of aspirin on cyclo-oxygenase and platelet function is too high to be considered a competative inhibitor. In addition, such levels may or may not be achieved in vivo under normal therapeutic conditions. Drug interaction and resulting inhibition of hepatic enzymes or various pathological conditions may alter the level of these compounds in various tissues. A recent study has demonstrated the augmentation of human blood aspirin levels by simultaneous administration of acetaminophen with acetylsalicylic acid (18). In special circumstances when plasma salicylate concentrations get elevated to high levels, it may pose a problem by competing for the same enzyme site as aspirin.

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