Influence of Nonsteroidal Anti-inflammatory Drugs on the Antiplatelet Effects of Aspirin in Rats

Yuuki AKAGI,* Yuta NIO, Shuji SHIMADA, and Takao AOYAMA

Faculty of Pharmaceutical Sciences, Tokyo University of Science; 2641 Yamazaki, Noda, Chiba 278–8510, Japan.

Received August 23, 2010; accepted November 16, 2010; published online November 24, 2010

Low-dose aspirin acts by irreversibly acetylating internal cyclooxygenase-1 (COX-1) on platelets, thereby suppressing platelet aggregation. Because nonsteroidal anti-inflammatory drugs (NSAIDs) also inhibit COX-1, the antiplatelet effects of aspirin may be suppressed when it is co-administered with NSAIDs. In this study, the influences of ibuprofen, loxoprofen sodium and etodolac on the antiplatelet effects of aspirin were investigated in male Sprague-Dawley (SD) rats. Aspirin and/or NSAIDs were administered orally at single or multiple daily doses. Platelet aggregation (ADP and collagen were added as stimuli) and serum thromboxane B₂ (TXB₂) concentrations were measured. The maximum inhibitions of aggregation in the aspirin before ibuprofen group were 41.0±7.8% for ADP and 38.7±5.4% for collagen at 6 h after administration; similar values were seen in the aspirin group; however, percent inhibitions in the aspirin before ibuprofen multiple administration group were lower than those in the aspirin group. Thus, the inhibitory effects of daily low-dose aspirin on platelets are competitively inhibited by the prolonged use of multiple daily doses of ibuprofen. In contrast, serum TXB₂ concentrations in all groups were lower than those in the control group (drug-free). This suggests that the relationship between the inhibition of platelet COX-1 and the suppression of platelet aggregation is nonlinear. When aspirin was administered with loxoprofen sodium, similar effects were observed; however, with etodolac, the antiplatelet effects in all groups were equal to those in the aspirin group. Accordingly, if co-administration with NSAIDs is necessary with low-dose aspirin, a selective COX-2 inhibitor, such as etodolac, should be used.

Key words aspirin; antiplatelet; nonsteroidal anti-inflammatory drug; drug interaction; rat; cyclooxygenase-1

Aspirin has long been used as an analgesic and antipyretic. Since the suppression of platelet aggregation by low-dose aspirin was noted in 1967,1) several large-scale clinical trials have demonstrated its antiplatelet efficacy.2) Aspirin was approved as a platelet aggregation inhibitor in Japan in 2000, and is now widely prescribed for the secondary prevention of cardiovascular events.3)

Low-dose aspirin inhibits cyclooxygenase-1 (COX-1) on platelets, suppresses arachidonic acid metabolism, and prevents the synthesis of thromboxane A₂ (TXA₂), a compound that causes platelet aggregation. Aspirin acts on internal COX-1 via a hydrophobic channel and irreversibly acetylates Ser529.4) Access to the COX-1 active site is then impeded for the lifetime of the platelet. After COX-1 on platelets is acetylated by aspirin, the antiplatelet effects are thought to depend on platelet turnover (approx. 7 to 10 d), and to be maintained until new platelets, unacetylated by aspirin, are produced.5)

Nonsteroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, are also used as analgesics, antipyretics and anti-inflammatory agents. Unlike aspirin, NSAIDs form a salt bridge with Arg120 of COX-1,6) and inhibit the enzymatic activity of prostaglandin H₂ synthesis. Suppression of platelet function is limited to a fixed time after taking NSAIDs, because the COX-1 inhibition is reversible.7)

The antiplatelet effects of aspirin may be decreased with co-administration of ibuprofen,4,8) and a warning is included in package inserts of both aspirin and ibuprofen. When ibuprofen binds with COX-1, it impedes aspirin acetylation of Ser529. Because numerous patients using low-dose aspirin also take NSAIDs9) to relieve pain from conditions such as arthritis and articular rheumatisms, the influence on the antiplatelet effects of aspirin is of particular interest. Loxoprofen sodium is more frequently prescribed than ibuprofen in Japan,10) but no information is available on its interaction with aspirin. Moreover, if inhibition of the antiplatelet effects of aspirin is noted, it is necessary to consider alternatives in order to avoid such interactions.

In this study, the influence of NSAIDs on the antiplatelet effects of aspirin was investigated in rats. Ibuprofen, loxoprofen sodium or etodolac (COX-2 selective inhibitor) were concurrently administered with aspirin. First, we conducted a study using a single daily dose of aspirin and/or NSAIDs, and measured platelet aggregation and serum thromboxane B₂ (TXB₂). We then undertook a study of multiple daily doses of aspirin (once a day) and/or NSAIDs (three times a day) for 3 d, as this is a clinically used regimen. Finally, we devised a method to avoid the interactions that were observed.

MATERIALS AND METHODS

Chemicals Aspirin (Lot No. TSH5399), ibuprofen (Lot No. TSG6469) and etodolac (Lot No. PEH6344) were purchased from Wako Pure Chemical Industries, and loxoprofen sodium (Lot No. 6411) was kindly provided by Daiichi-Sankyo Co. (Tokyo, Japan). Platelet aggregation was induced with ADP (Wako Pure Chemical Industries, Ltd., Osaka, Japan; Lot No. 105704) or collagen (Research Institute for the Functional Peptides, Yamagata, Japan; Lot No. 8HIA). All other chemicals were of analytical grade.

Animals Male Sprague-Dawley (SD) rats (age, 8—9 weeks; weight, 250—300 g) were used in this study.11,12) They were housed under standard conditions (23±1 °C; relative humidity, 55±5%) and maintained under a 12-h light/dark cycle. After a 12-h fast with free access to water, all rats had an indwelling cannula (Polyethylene tube, SP31; Natsume Seisakusho Co., Ltd., Tokyo, Japan) implanted in the femoral artery for blood sampling. Implantation surgery was...
was determined by measuring serum levels of TXB$_2$, the major stable metabolite of TXA$_2$, in platelets. To minimize the influence of TXB$_2$ derived from hematopoietic cells, many variables that affect platelet aggregation, we carefully standardized the conditions for blood collection and centrifugation at 2000×g for 15 min to remove platelets. Supernatants were immediately stored at −30 °C until analysis. Serum TXB$_2$ was measured by enzyme-linked immunosorbent assay (ELISA, Cayman Co., Ann Arbor, MI, U.S.A.) according to the manufacturer’s instructions, and changes were analyzed by Tukey’s t-test, with a value of p<0.05 being considered significant.

**RESULTS**

In the aspirin (Asp) group, the maximum inhibition of platelet aggregation was 44.0±3.9% for ADP (128 μM) and 37.7±4.3% for collagen (25 μg/ml) at 6 h after administration (p<0.01). In contrast, the percent inhibitions with ibuprofen (Ibu), loxoprofen sodium (Lox) and etodolac (Eto) were 0.7—18.5% (Figs. 2A, B), which were nearly identical to the values in the control group, and suppression of platelet aggregation was not observed. These inhibition rates were similar at 12 h.

In the study using a single daily dose of aspirin and NSAIDs, the maximum inhibitions of aggregation in the aspirin before ibuprofen (Asp→Ibu) group were 41.0±7.8% for ADP and 38.7±5.4% for collagen at 6 h, which was similar to values in the Asp group. Percent inhibition in the ibuprofen before aspirin (Ibu→Asp, ADP: 4.5±2.5%, collagen: 2.2±1.0%, p<0.01) group was significantly lower than that in the Asp group (Figs. 3A1, A2). These inhibition rates were similar at 12 h. For loxoprofen sodium, the maximum inhibition of platelet aggregation was equal to that of ibuprofen (Figs. 3B1, B2); however, the levels were similar to those in the Asp group, regardless of the order of co-administration with etodolac, and no suppression of antiplatelet effects was observed (Figs. 3C1, C2).

In contrast, the maximum inhibitions of aggregation in the aspirin before ibuprofen multiple administration (Asp→Ibu, multiple) group were 14.3±16.0% for ADP and −1.8±6.0% for collagen at 0 h on day 3, and these values were lower than those in the aspirin multiple administration (Asp, multiple) group (ADP: p<0.05, collagen: p<0.01). Similar values were seen at 6 and 12 h (Figs. 4A1, A2). For loxoprofen sodium (Asp→Lox, multiple), percent inhibition was equal to ibuprofen (Figs. 4B1, B2); however, almost no significant
The antiplatelet effects of low-dose aspirin are suppressed by ibuprofen, and this interaction is thought to be due to ibuprofen interfering with the inhibition of platelet COX-1.\(^4,9\) This interaction has not been reported when using COX-2 selective inhibitors, such as celecoxib and diclofenac sodium.\(^4,25\) However, the influence of other NSAIDs on the antiplatelet effects of aspirin remains unclear. We therefore conducted a study to assess whether NSAIDs interfere with aspirin-induced inhibition of platelet COX-1 in rats. In addition to ibuprofen, loxoprofen sodium (well-prescribed in Japan) and etodolac (a COX-2 selective inhibitor) were used in this experiment.

Platelet aggregation in the Asp→Ibu group was equal to that in the Asp group, thus suggesting that the influence of ibuprofen could be eliminated by administering aspirin 2 h before ibuprofen. In contrast, the percent inhibition observed in the Asp and Asp→Ibu groups was not observed in the Ibu→Asp group. These results show that the antiplatelet effects of aspirin are suppressed by ibuprofen when administered before aspirin (Figs. 3A1, A2). It has been reported that there is no decrease in antiplatelet effects when giving healthy subjects aspirin before ibuprofen; however, such effects were observed when giving ibuprofen before aspirin.\(^4\)

The results of our experiment in rats showed similar trends, and we thus considered our animal model to reflect the interaction between aspirin and NSAIDs. Co-administration of loxoprofen sodium with aspirin had a similar influence to that of ibuprofen (Figs. 3B1, B2). This interaction is consistent with the report that NSAIDs competitively inhibit the binding of aspirin at the acetylation site in platelet COX-1.\(^24\) Therefore, our findings suggest that there would be almost no interaction when aspirin is given before ibuprofen or loxoprofen sodium. By the way, ibuprofen and loxoprofen sodium significantly decreased serum TXB\(_2\) levels (i.e., COX-1 is inhibited), but these NSAIDs did not suppress platelet aggregation (Figs. 2A–C). The mechanism is unclear; however, it has been reported that antiplatelet effect of aspirin is seen when over 90% of TXA\(_2\) production is suppressed.\(^23\) It has also been suggested that the interaction between meloxicam and COX-1 may be weaker than that between aspirin and COX-1.\(^26\) Considering them, antplatelet effect might not be seen without quite a lot of and continuous suppression of TXA\(_2\) production.

The maximum inhibition of aggregation in both the aspirin before etodolac (Asp→Eto) and etodolac before aspirin groups (Eto→Asp) was equal to that in the Asp group (Figs. 3C1, C2). Serum TXB\(_2\) levels in the etodolac group at 6 h were lower than those in the control group; however, they were significantly higher than that in the ibuprofen and loxoprofen sodium groups (Fig. 2C). This suggests that etodolac slightly inhibits COX-1, but ibuprofen and loxoprofen sodium exhibit greater COX-1 inhibitory activity, and the interaction between aspirin and COX-1 may be stronger than that between NSAID and COX-1. There is also a side pocket in the hydrophobic channel of COX-2,\(^27\) to which etodolac is thought to primarily bind, but because of its weak inhibition of COX-1, it has very little influence on the antiplatelet effects of aspirin.

In our single-dose study, inhibition by NSAIDs could be avoided by administering aspirin 2 h before the single NSAID dose. However, NSAIDs taken the day before may inhibit the antiplatelet effects of aspirin\(^9\) during the actual clinical regimen. In our second study with multiple daily doses under a clinically relevant regimen (aspirin: once a day, NSAIDs: three times a day), the Asp→Ibu multiple and Asp→Lox multiple groups did not show reduced platelet aggregation (Figs. 4A1, A2, B1, B2). This was in contrast to the Asp multiple group, and showed that the inhibitory effects of daily low-dose aspirin on platelets are competi-
tively inhibited by the prolonged use of multiple daily doses of ibuprofen or loxoprofen sodium, even when aspirin was administered before the first dose of NSAIDs. Our results suggest that co-administering aspirin with these NSAIDs on a daily basis might suppress the antiplatelet effects of aspirin. Serum TXB_{2} levels in the Asp, Asp→Ibu, Ibu→Asp, and
The effects of aspirin on platelet aggregation are competitively inhibited by ibuprofen and loxoprofen sodium. Almost no influence on platelet aggregation was observed when taking a single dose of aspirin before NSAIDs; however, multiple doses did not eliminate this interaction, irrespective of when they were administered. In contrast, etodolac did not interfere with the irreversible inhibition so much. Accordingly, if co-administration of NSAIDs with low-dose aspirin is necessary, a COX-2 selective inhibitor, such as etodolac, is recommended.

Acknowledgment We would like to thank Daiichi-Sankyo Co. for kindly providing loxoprofen sodium.

REFERENCES