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Research Article

A Randomized, Quadruple Crossover Single-Blind Study on Immediate Action of Chewed and Unchewed Low-Dose Acetylsalicylic Acid Tablets in Healthy Volunteers

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Abbreviations

ADP, adenosine diphosphate; ANOVA, analysis of variance; ASA, acetylsalicylic acid; AUC, area under the concentration curve; AUMC, area under the first moment curve; COX, cyclooxygenase; ECA, enteric-coated acetylsalicylic acid tablet; HPLC, high-performance liquid chromatography; MRT, mean residence time; NBA, non-coated buffered acetylsalicylic acid tablet; TXB_2, thromboxane B_2

ABSTRACT

In the initial treatment of acute myocardial infarction, it is important to administer oral low-dose acetylsalicylic acid (ASA) within 10 min of arrival at the hospital. However, ASA is supplied as an enteric-coated tablet or buffered tablet to prevent gastric irritation. Although current guidelines recommended that patients should chew their initial dose of ASA, there is little evidence as to whether this is efficacious. Therefore, we aimed to make a direct comparison of the pharmacokinetics and pharmacodynamics of ASA after ingestion of intact and chewed non-enteric-coated buffered ASA tablet (NBA) and enteric-coated ASA tablet (ECA) in a quadruple crossover study in healthy volunteers. Chewing ECA accelerated t_{max} of ASA absorption, which became equivalent to that after ingestion of intact or chewed NBA. A significant decrease in serum thromboxane B_2 was observed 20 min after ingestion of chewed ECA, or intact or chewed NBA, and inhibition of platelet aggregation was also observed within 20 min. Thus, chewing of the ECA appears to result in a similar timing of ASA action to that in the case of chewed or unchewed NBA.

INTRODUCTION

Acetylsalicylic acid (ASA) irreversibly acetylates and inactivates platelet cyclooxygenase (COX), which catalyzes the first step in the conversion of arachidonic acid to thromboxane A_2.[1-6] Thromboxane A_2 stimulates platelet recruitment, activation, aggregation and vasoconstriction. The ASA-induced inhibition lasts for the life of the platelet, or about 7-10 days.[7-8] At the same time, inactivation of COX in vascular endothelial cells also prevents the synthesis of prostacyclin a substance that inhibits platelet aggregation. Endothelial cells can recover COX activity by biosynthesis, but platelets do not synthesize new COX.[5] Thus, though oral ASA 325-650 mg every 4-6 hr exhibits analgesic and antipyretic effects, and 3.6-5.4 g/day exhibits an anti-inflammatory effect, low-dose ASA exhibits an antiplatelet effect. Chronic inhibition of platelet activity may be achieved with daily dose of 80 mg ASA[9]. Many clinical trials have demonstrated that low-dose ASA once daily is clinically effective in prevention and treatment of myocardial infarction, acute ischemic stroke and transient cerebral ischemia, and as adjunctive therapy in revascularization procedures such as coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, carotid endarterectomy and stent implantation.[10-20]

The major side-effect of ASA is gastric irritation. So, ASA is available as sustained-release, enteric-coated tablet dosage forms or as

non-coated buffered tablets. Enteric coating prevents the release of free aspirin until after the tablet has left the stomach.[21] Buffered ASA tablets contain di-aluminate or other buffer species that protect the stomach mucosa from irritation. ASA in enteric-coated tablets is absorbed much more slowly than ASA in buffered tablets, resulting in low plasma ASA levels.[22] These dosage forms should not be crushed or chewed to prevent gastric irritation for general use.

On the other hand, the time of initiation of treatment is important in patients with acute myocardial infarction, such as ST-segment-elevation myocardial infarction. The Guidelines for the management of patients with ST-elevation myocardial infarction recommend starting MONA (morphine, oxygen, nitric oxide and aspirin) treatment within 10 min of arrival at the hospital.[23,24] As the use of morphine is difficult in some clinical situations, isosorbide dinitrate is often administered sublingually or as an oral spray and low-dose ASA is orally administered as soon as possible. Under these circumstances, however, immediate absorption and action of ASA are very important, and so it has been recommended that patients should chew the tablets. However, this recommendation does not yet have an adequate evidential basis.

The pharmacokinetic as well as pharmacological characteristics of enteric-coated, plain non-enteric-coated buffered ASA tablet and soluble ASA after normal ingestion have been clarified,[7,8,22,25-29] but there has been no

direct comparison with the characteristics after ingestion of chewed low-dose ASA tablets. Therefore, the aim of the present study was to provide clinical evidence to support the recommendation of the Guidelines for the management of patients with acute myocardial infarction. Specifically, we aimed to clarify the pharmacokinetics of ASA and the immediate effect on platelet aggregation and serum thromboxane A_2 level following ingestion of compressed non-enteric coated buffered tablet (NBA) and enteric-coated tablet (ECA) of low-dose ASA after chewing, in comparison with those after normal ingestion.

EXPERIMENTAL

Materials

Bufferin® 81 mg Tablets (non-enteric coated buffered acetylsalicylic acid tablet, NBA)) and Bayaspirin® 100 mg Enteric-Coated Aspirin Tablets (ECA) were purchased from Eisai Co., Ltd. (Tokyo, Japan) and Bayer Yakuhin, Ltd. (Osaka, Japan), respectively. Thromboxane EIA Kit was from Cayman Chemical Company (Ann Arbor, MI). 2-Methylbenzoic acid and blank human serum were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO), respectively. All other chemicals were commercial products of reagent grade or HPLC grade, and were used without further purification.

Study Design and Subjects

Volunteers were required to meet the following inclusion criteria: 1) aged 20 to 50 years, of either sex, 2) exhibited normal platelet aggregation, and 3) gave written informed consent. Exclusion criteria were: 1) a history of hypersensitivity to ingredients of Bufferin 81 mg tablet, Bayaspirin 100 mg or other salicylic acid formulations, 2) a history of aspirin asthma, 3) use of non-steroidal anti-inflammatory drugs (NSAIDs) within the previous 2 weeks, 4) administration of an anti-platelet agent within the

previous 2 weeks, 5) administration of an anti-coagulation drug within the previous 2 weeks, 6) scheduled to receive odontectomy or other invasive operation within 2 weeks, 7) diagnosed with peptic ulcer, 8) pregnant, in parturition or lactating, 9) a bleeding tendency, 10) in hypermenorrhea, 11) drinking alcohol on a regular basis, 12) any other situation that the doctor deemed inappropriate for inclusion. To determine whether or not volunteers met the above inclusion and exclusion criteria, we requested them to complete a medical interview sheet, and further medical examination was carried out by a physician when this was considered necessary. Six male and 6 female healthy volunteers aged 20–34 years participated in this study. The subjects did not ingest aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) for at least two weeks prior to the start of the study, or prior to the re-start of subsequent parts of the study, and also refrained from drinking alcohol for at least 24 hr. In addition, volunteers were screened for baseline platelet aggregation, using a blood sample taken just before ingestion of ASA, in order to confirm that none showed abnormal aggregation. We employed a randomized, single-dose, quadruple-crossover design. Each subject ingested intact or chewed (2 or 3 times within 5 seconds) NBA or ECA with a glass of water. Each subject had the same lean breakfast 1 hr prior to ASA ingestion. Thereafter, food was not permitted until the end of each part of the study. The four study periods were all separated by washout periods of at least two weeks. Water and tea were allowed throughout the study. Venous blood samples were obtained without stasis by using a 21-gauge needle or catheter

with a 3-way stopcock filled with isotonic saline. A 4 mL sample was collected in a vacuum blood collection tube. For acetylsalicylic acid, salicylic acid and thromboxane B_2 determinations, the sera were separated by centrifugation, then frozen and stored at -80°C until required for analysis. For platelet aggregation studies, a 2.7 mL sample of whole blood was collected in a vacuum blood collection tube containing 0.3 mL of 3.13% sodium citrate (9:1 vol:vol) and used immediately. The sampling was performed at 5 min before and 5, 10, 20, 30, 60, 120, 240, 360 and 480 min after tablet ingestion. All subjects provided written informed consent prior to participation. This study protocol was approved by the Institutional Review Board of Kanazawa University Hospital (approval number: 2009-028 (5584)).

Estimation of Serum ASA and SA

Serum ASA and SA concentrations were determined according to the method reported by Kees et al.[30] The HPLC system consisted of a Shimadzu LC-10ADvp pump (Shimadzu, Tokyo, Japan), SIL-10ADvp injector, CTO-10ASvp column oven, SPD-10Avp UV detector, SCL-10Avp system controller, and C-R8A Chromatopack. The analytical column was a Nova-Pak C18 3.9 x 150 mm 4 μm column from Waters (Milford, MA). The HPLC separation module was operated under the following conditions: sample temperature, 4°C; analytical column temperature, 30°C; sample injection

volume, 20 µL. The mobile phase consisted of 740 mL water, 900 µL orthophosphoric acid (85%) and 180 mL acetonitrile, and was delivered at 1.0 mL/min. The detection wavelength was set at 237 nm. The precision and accuracy were evaluated using two samples each at four (ASA) or three (SA) different concentrations for 17 days. The mean overall values of calculated accuracy and precision (percent) for each concentration over 17 days were 100 ± 10 % with a %CV of 21% and 100 ± 8.3 % with a %CV of 1.7 % for ASA and SA, respectively. The lower limit of quantitation was 80 ng/mL for ASA and 50 ng/mL for SA. All concentrations below the limits of quantitation of the assay were taken to be zero. 2-Methylbenzoic acid (5 µg/mL) was used as the internal standard.

Pharmacokinetics

The area under the serum ASA and SA concentration versus time curves up to the last sampling time $AUC_{0\text{-}8}$ (µg·hr/mL) were calculated according to the linear trapezoidal method. The mean residence time (MRT) was calculated as AUMC divided by AUC, where AUMC is the area under the fist moment curve.

Thromboxane A_2 Determination

Serum thromboxane A_2 was determined by enzyme-immunoassay of its

stable metabolite thromboxane B_2 (Thromboxane B_2 EIA Kit, Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer's protocol.

Platelet Aggregation

Blood samples were centrifuged at 1,000 rpm for 15 min to obtain platelet-rich plasma, which was transferred to a polystyrene tube with a pipette. Platelet-poor plasma was obtained by further centrifugation at 3,000 rpm for 15 min. Aggregometry was performed using a PAM-8T Aggregometer (Mebanics Inc, Tokyo, Japan). The aggregometer was adjusted before each measurement so that platelet-rich plasma gave no light transmission and platelet-poor plasma gave 100% light transmission. Collagen (Hormon Chemie, Munich, Germany) and ADP (MCM) were used as aggregating agents. The maximum platelet aggregation (%) was determined by aggregation tracing for 5 min for each blood sample obtained before and after ingestion of ASA. Inhibition of platelet aggregation following ASA ingestion was evaluated as a percentage of to the maximum platelet aggregation determined before ASA ingestion for each patient.

Statistical Analysis

Statistical analysis was performed by analysis of variance (ANOVA) with Tukey's and Dunnett's post-hoc tests, using GraphPad PRISM version 5.02 for Windows, GraphPad Software (San Diego, CA). $p < 0.05$ was considered statistically significant.

RESULTS

Serum Concentration-Time Profiles of Acetylsalicylic Acid and Salicylic Acid after Ingestion of Intact and Chewed NBA and ECA.

Twelve healthy volunteers each received four separate oral drug administrations, i.e., intact and chewed NBA and intact and chewed ECA. One volunteer withdrew in the middle of blood sampling after ingestion of intact ECA because of vascular pain owing to multiple venous punctures. Another volunteer withdrew in the middle of the intact ECA period because of feeling unwell, and also retracted her consent to participate in the period of chewed ingestion of NBA. Figures 1 and 2 illustrate the serum concentration-time profiles of acetylsalicylic acid (ASA) and salicylic acid (SA), respectively.

ASA was detectable in the serum of 8 out of 12 volunteers within 20 min after ingestion of intact NBA, although significant inter-individual variability was observed (Fig. 1). The concentration of ASA increased rapidly and peaked at 1 hr after ingestion. After ingestion of intact ECA, ASA was not detectable in serum until 4 hr. When ECA was chewed, ASA was detectable in 8 out of 12 volunteers within 20 min after ingestion. Chewing of the NBA did not significantly affect the appearance of ASA compared with that after ingestion of intact NBA.

The mean serum SA concentration peaked at 2 hr after ingestion of

intact or chewed NBA (Fig. 2). SA was not detectable until 2 hr after the ingestion of intact ECA, but when ECA was chewed, SA peaked at 2 hr after ingestion (Fig. 2). Chewing of the NBA did not significantly affect the appearance of SA compared with intact NBA.

The pharmacokinetic parameters of ASA and SA are summarized in Table 1. The MRT of ASA was 1.9 ± 0.9 hr (mean ± S.D., n = 12) and 6.8 ± 1.3 hr (n = 10) after ingestion of NBA and ECA, respectively. Chewing ECA greatly shortened the MRT to 2.4 ± 1.2 hr (n = 12).

Effect of Ingestion of Intact and Chewed NBA and ECA on Serum Thromboxane B_2 Level

Table 2 summarizes the effects of ingestion of intact and chewed NBA and ECA on serum thromboxane B_2 levels (TXB_2). As baseline serum TXB_2 levels varied greatly among volunteers, the effect of ASA on TXB_2 was expressed as a percentage of the baseline TXB_2 value measured before ingestion for each volunteer. A significant decrease in TXB_2 was observed at 20 min after ingestion of intact NBA (Table 2), but not until 8 hr after ingestion of intact ECA, although average TXB_2 was decreased to less than 50% after 4 hr (Table 2). When ECA was chewed, the decrease in TXB_2 after 20 min was comparable to that in the case of intact NBA. When NBA was chewed, the average TXB_2 was decreased to less than 60% and the decrease reached statistical significance after 1 hr.

Effect of Ingestion of Intact and Chewed NBA and ECA on Platelet Aggregation

We employed two different aggregating agents, collagen and ADP. ADP at 2 μM induces primary and secondary aggregation while 1 μg/mL collagen induces secondary aggregation of platelets. ASA is expected to have a much stronger inhibitory effect on secondary aggregation.

Table 3 summarizes the effect of ingestion of intact and chewed NBA and ECA on platelet aggregation induced with 1 μg/mL collagen. The aggregation was inhibited significantly within 20 min after ingestion of intact NBA (Table 3), but no effect was observed until 4 hr after ingestion of intact ECA. When ECA was chewed, inhibition of platelet aggregation was observed after 20 min, as in the case of intact NBA. Chewing NBA had little effect on inhibition of platelet aggregation, compared with the intact tablet. The effect of ASA on platelet aggregation was also evaluated using 2 μM ADP as the aggregating agent (Table 4). In this case, platelet aggregation was slightly inhibited by intact and chewed NBA and by chewed ECA.

DISCUSSION

The FDA approved general ASA formulations as an antiplatelet drug in 1998. In Japan, “Baby Bufferin”, which contained 81 mg ASA at that time, and Bayaspirin 100 mg Tablet were used as off-label pharmaceuticals before 1999, when Ministry of Health, Labour and Welfare, Japan approved their use. These approvals were not based on a new clinical trial, but were based on literature data. Indeed, the ACC/AHA (the American College of Cardiology and the American Heart Association) and Japanese Guidelines for the Management of Patients with Acute Myocardial Infarction[23,24] were largely based on results from the ISIS-2 study (Second International Study of Infarct Survival),[13] where the patients ingested 162.5 mg enteric-coated tablets that were crushed, sucked or chewed to achieve a rapid effect. However, there has been no comprehensive clinical study showing that crushing, sucking or chewing ASA tablet actually accelerates the absorption and antiplatelet effect compared with ingestion of the intact preparations. Nonetheless, patients with myocardial infarction are customarily instructed to chew their initial dose of ASA, and current guidelines have encouraged this practice. In order to obtain evidence for the efficacy of this practice, we designed a quadruple-crossover study in 12 healthy volunteers to directly compare the pharmacokinetics and pharmacodynamics of NBA and ECA ingested intact or after chewing.

Chewing of the ECA greatly accelerated t_{max} and MRT of ASA (Fig. 1 and Table 1). The t_{max} (1.4 hr) and C_{max} (198 ng/mL) were comparable to those after ingestion of intact NBA (0.9 hr and 174 ng/mL, respectively). Significant decrease of TXB_2 and inhibition of platelet aggregation were observed at 20 min after ingestion of chewed ECA, or intact or chewed NBA. The results indicate that platelet COX was inhibited within 20 min after ingestion of chewed ECA as was also the case after ingestion of intact or chewed NBA (Tables 3 and 4). Thus, inhibition of COX was achieved before the serum ASA level peaked. Schwertner et al have suggested that a plasma salicylate concentration of 2.46 μg/mL is required to inhibit platelet aggregation.[29] But, as the anti-platelet effect of ASA results from irreversible acetylation of COX and inhibition of thromboxane A_2 production, the acetylsalicylic acid concentration rather than the salicylic acid concentration would be important for anti-platelet effect. In this study, we measured serum acetylsalicylic acid as well as salicylic acid (Figs 1 and 2), and tried to identify the serum concentration required to inhibit COX activity and platelet aggregation. Although there was significant inter-individual variation, the inhibition of platelet aggregation, as well as the decrease of serum TXB_2 level, in the early phase took place at a serum ASA concentration below the detection limit of <30 ng/mL. These results are broadly consistent with previous reports.[7,22,31] Slow release, enteric-coated formulations may deliver ASA to the portal circulation at a slow enough rate

to allow substantial deacetylation of aspirin during its first pass through the liver. Ali et al have hypothesized that plasma ASA level, at least in the portal circulation, attains a bolus threshold required for maximum acetylation.[7] Our study supports this hypothesis.

Many of the previous studies were performed in the fasting state, but this may not always reflect the clinical situation. Patients may be admitted to hospital after a meal. The rate of gastrointestinal absorption of ASA is determined by the rate of dissolution into solution, luminal pH, and gastric emptying. Although most soluble ASA (pKa =3.5) is present in the non-ionized form at the stomach pH (pH 1.5 to 3.0), the solubility of ASA is low at the stomach pH. The pH shift in the upper small intestine decreases and increases the non-ionized form and solubility, respectively, and the net effect is to increase the absorption. So, delayed gastric emptying would reduce the rate of ASA absorption and consequently the effect on platelet aggregation. Therefore, this study was performed in volunteers who had the same lean breakfast 1 hr prior to ASA ingestion. Indeed, it is known that the t_{max} of enteric-coated ASA formulation is delayed from 3-4 hr to 9 hr.[32] In this study, blood sampling was performed through 8 hr. The serum ASA concentrations in most volunteers were still increasing (Fig. 1), so t_{max} of ASA after ingestion of ECA might be greater than 8 hr. The t_{max} of ASA after ingestion of intact or chewed NBA (0.9-1.0 hr, Fig. 1 and Table 1) was also delayed compared with that observed in the fasting state (0.3-0.5 hr).[22,28]

Decrease of serum TXB_2 and onset of platelet inhibition were delayed to 20 min (Tables 3,4) compared with those in the fasting state (5-12 min).[28] Chewing NBA had little effect in accelerating ASA absorption or onset of action (Fig. 1, Tables 2,3). These observations may be ascribed to the effect of the fed state.

In conclusion, our quadruple-crossover clinical trial provides pharmacokinetic and pharmacodynamic evidence to support the recommendation of the Clinical Guidelines for Acute Myocardial Infarction that the initial dose of aspirin following admission to hospital should be chewed. Chewing ECA accelerated ASA absorption and onset of antiplatelet action even in the nonfasting state. Ingestion of either intact or chewed NBA provided outcomes comparable to that after ingestion of chewed ECA.

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LEGENDS

Figure 1

Serum Concentration-Time Profiles of Acetylsalicylic Acid after Ingestion of Intact and Chewed NBA and ECA

Serum concentration of acetylsalicylic acid after ingestion of intact (open) and chewed (closed) NBA (circle) and ECA (triangle) were measured for 8 hrs. Each point represents the mean ± S.E.M. of 10-12 patients.

Figure 2

Serum Concentration-Time Profiles of Salicylic Acid after Ingestion of Intact and Chewed NBA and ECA

Serum concentration of salicylic acid after ingestion of intact (open) and chewed (closed) NBA (circle) and ECA (triangle) were measured for 8 hrs. Each point represents the mean ± S.E.M. of 10-12 patients.

Table 1

Pharmacokinetic Parameters of Acetylsalicylic Acid and Salicylic Acid after Ingestion of Intact and Chewed Non-coated Buffered ASA (NBA) and Enteric Coated ASA (ECA)

Ingestion	NBA		ECA	
	intact	chewed	intact	chewed
Acetylsalicylic acid				
C_{max} (ng/mL)	174 ± 156	223 ± 112	104 ± 291	198 ± 137
t_{max} (hr)	0.9 ± 0.6	1.0 ± 0.6	≥8	1.4 ± 1.0
$AUC_{0\text{-}8}$ (ng·hr/mL)	379 ± 310	491 ± 251	240 ± 326	480 ± 359
MRT (hr)	1.9 ± 0.9	2.3 ± 0.5	6.8 ± 1.3*	2.4 ± 1.2
Salicylic acid				
C_{max} (μg/mL)	4.26 ± 1.30	4.21 ±1.18	2.77 ± 2.53	4.55 ± 1.55
t_{max} (hr)	1.8 ± 0.8	2.1 ± 0.7	≥8	2.6 ± 1.1
$AUC_{0\text{-}8}$ (μg·hr/mL)	19.2 ± 5.9	19.7 ± 6.7	6.9 ± 6.9*	19.7 ± 7.5
MRT (hr)	4.2 ± 0.5	4.3 ± 0.4	6.8 ± 1.4*	4.4 ± 0.6

Pharmacokinetic parameters were calculated by moment analysis as described in Materials and Methods. Data represent the mean ± S.D. of 10-12 patients. Significant differences ($p < 0.05$) from other three groups are indicated by an asterisk (*); One-way ANOVA with Tukey's *post hoc* test.

Table 2

Effect of Ingestion of Intact and Chewed NBA and ECA on Serum Thromboxane B_2 Level

Ingestion	NBA		ECA	
	intact	chewed	intact	chewed
Time	*% of baseline*	*% of baseline*	*% of baseline*	*% of baseline*
5 min	104.7 ± 59.0	142.6 ± 92.9	142.1 ± 71.7	126.3 ± 71.6
10	91.3 ± 56.7	118.5 ± 72.4	101.9 ± 64.1	120.2 ± 50.8
20	48.7 ± 28.0 *	57.6 ± 59.5	69.1 ± 47.4	47.3 ± 27.7 *
30	86.4 ± 72.8	68.0 ± 57.1	150.0 ± 120.5	54.9 ± 43.7 *
1 hr	27.0 ± 37.5 *	19.0 ± 15.7 *	77.7 ± 43.6	14.6 ± 16.0 *
2	16.5 ± 21.5 *	12.8 ± 12.0 *	92.2 ± 65.1	10.9 ± 13.1 *
4	5.0 ± 7.7 *	6.2 ± 7.0 *	49.8 ± 19.9	5.2 ± 6.0 *
6	3.7 ± 5.3 *	4.5 ± 4.4 *	37.3 ± 36.8	3.3 ± 4.3 *
8	3.4 ± 4.1 *	3.8 ± 3.6 *	22.1 ± 37.0 *	2.8 ± 3.9 *

The maximum platelet aggregation was determined by aggregation tracing for 5 min for each blood sample obtained after ingestion of ASA. Inhibition of platelet aggregation by ASA was expressed as a percent of the maximum platelet aggregation determined before ASA ingestion (baseline) for each patient. Each value represents the mean ± S.D. of 10-12 patients. A significant difference ($p < 0.05$) from the baseline is indicated by an asterisk (*); One-way ANOVA with Dunnett's *post hoc* test.

Table 3

Effect of Ingestion of Intact and Chewed NBA and ECA on Platelet Aggregation Induced with 1 µg/mL Collagen

Ingestion	NBA		ECA	
	intact	chewed	intact	chewed
Time	*% of baseline*	*% of baseline*	*% of baseline*	*% of baseline*
5 min	79.2 ± 20.3	87.0 ± 36.6	67.4 ± 26.0	68.8 ± 31.4
10	77.6 ± 38.3	94.6 ± 43.6	88.5 ± 32.2	88.7 ± 40.4
20	47.6 ± 43.9 *	60.4 ± 36.8 *	74.0 ± 24.1	56.3 ± 51.0 *
30	53.2 ± 45.9 *	51.9 ± 47.8 *	85.0 ± 27.8	47.2 ± 43.3 *
1 hr	26.5 ± 28.6 *	35.1 ± 33.2 *	66.5 ± 33.3	24.7 ± 36.9 *
2	14.5 ± 19.9 *	19.4 ± 12.2 *	73.9 ± 28.7	29.8 ± 37.7 *
4	12.4 ± 17.8 *	12.5 ± 12.6 *	46.8 ± 42.3 *	16.7 ± 22.4 *
6	7.7 ± 10.6 *	7.7 ± 11.5 *	31.2 ± 44.5 *	11.2 ± 18.2 *
8	7.4 ± 13.1 *	5.2 ± 6.9 *	15.8 ± 23.5 *	9.4 ± 17.5 *

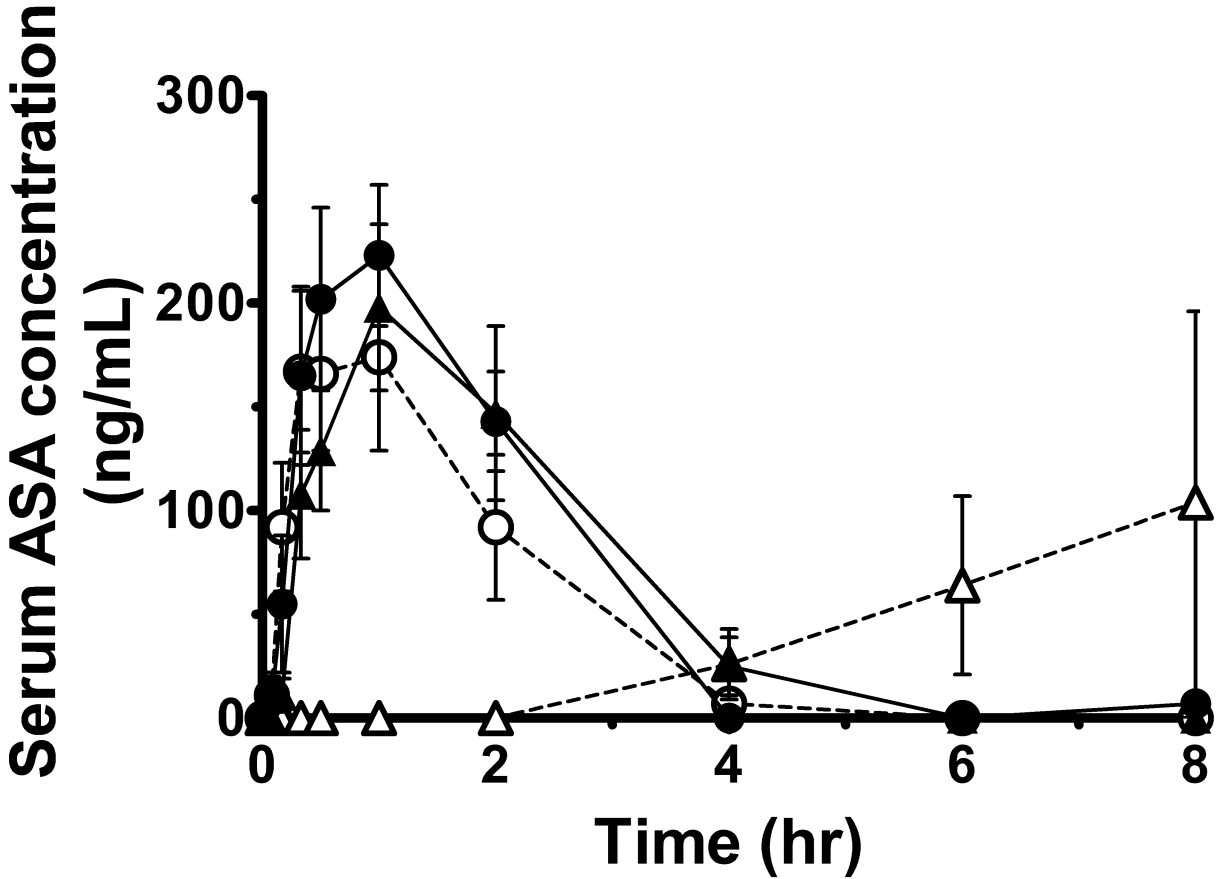
The maximum platelet aggregation induced with 1 µg/mL collagen was determined by aggregation tracing for 5 min for each blood sample obtained after ingestion of ASA. Inhibition of platelet aggregation by ASA was expressed as a percentage of the maximum platelet aggregation in the baseline condition for each patient. Each value represents the mean ± S.D. of 8-12 patients. A significant difference ($p < 0.05$) from the baseline is indicated by an asterisk (*); One-way ANOVA with Dunnett's *post hoc* test.

Table 4

Effect of Ingestion of Intact and Chewed NBA and ECA on Platelet Aggregation Induced with 2 μM ADP

Ingestion	NBA		ECA	
	intact	chewed	intact	chewed
Time	*% of baseline*	*% of baseline*	*% of baseline*	*% of baseline*
5 min	95.4 ± 26.1	103.5 ± 36.6	94.7 ± 18.1	95.6 ± 18.7
10	73.9 ± 19.3	86.6 ± 31.6	86.9 ± 17.8	90.8 ± 21.1
20	85.5 ± 28.7	84.7 ± 39.7	77.9 ± 21.0	83.1 ± 26.0
30	82.0 ± 15.9	86.0 ± 25.1	79.3 ± 22.8	81.8 ± 26.2
1 hr	86.3 ± 21.0	82.0 ± 25.9	95.0 ± 27.9	81.8 ± 27.0
2	64.4 ± 23.0 *	76.1 ± 12.3	79.8 ± 30.9	79.7 ± 20.0
4	69.5 ± 24.0 *	76.3 ± 23.9	91.9 ± 23.0	80.3 ± 15.1
6	75.6 ± 17.7 *	67.9 ± 24.2 *	91.8 ± 20.4	73.4 ± 25.5 *
8	76.6 ± 17.4	73.8 ± 23.7	79.6 ± 20.3	68.1 ± 28.3 *

The maximum platelet aggregation induced with 2 μM ADP was determined as described in the legend of Table 3. Each value represents the mean ± S.D. of 8-12 patients. A significant difference ($p < 0.05$) from the baseline is indicated by an asterisk (*); One-way ANOVA with Dunnett's *post hoc* test.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(ng/mL)
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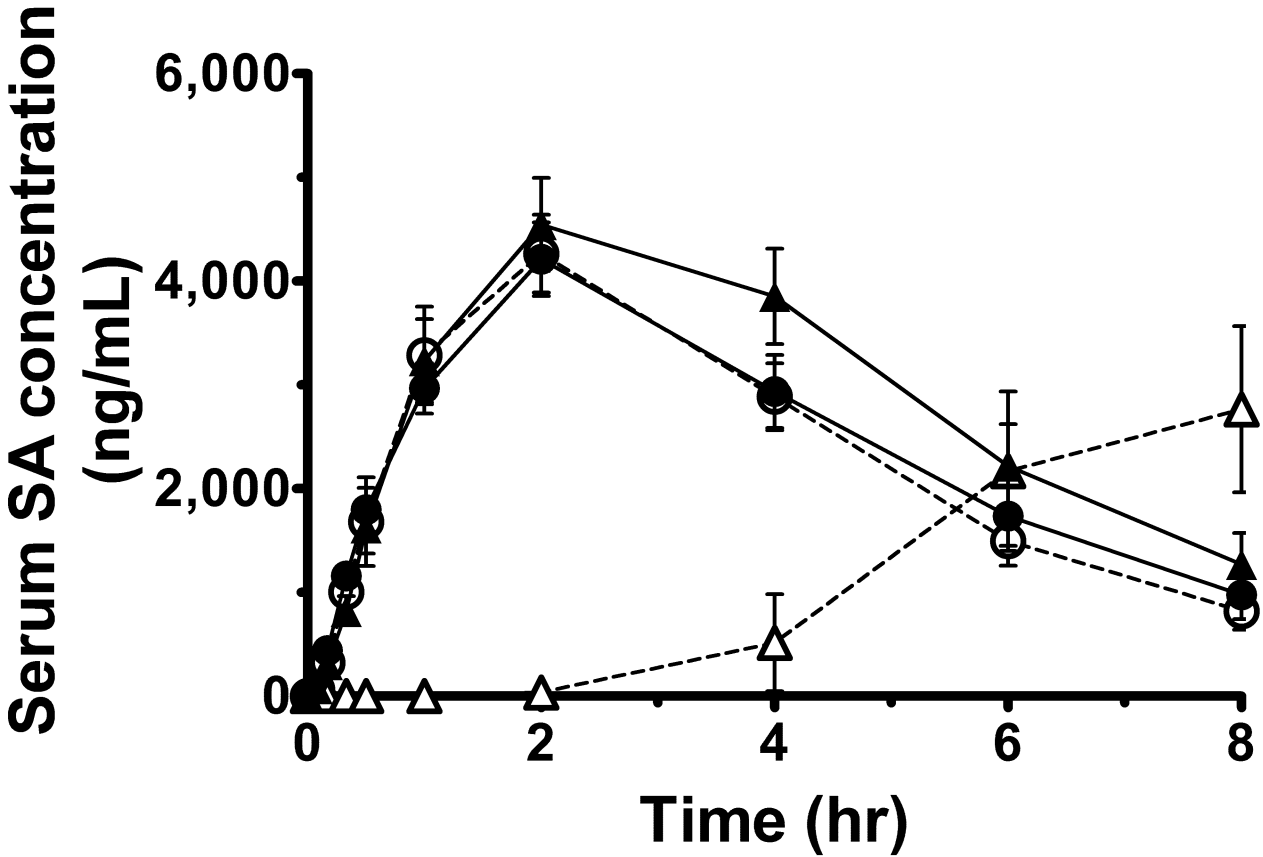
200
100
0
0
2
4
6
8
Time (hr)


Serum SA concentration (ng/mL)
6,000
4,000
2,000
0
0
2
4
6
8
Time (hr)