Antagonistic Action of AA-2414 on Thromboxane A2/Prostaglandin Endoperoxide Receptor in Platelets and Blood Vessels

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Abstract—AA-2414, (±)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid, inhibited the aggregation of guinea pig platelets induced by a prostaglandin endoperoxide (PGH2) analogue, U-44069 and the specific binding of another analogue, [3H]U-46619 to washed guinea pig platelets with IC50 values of 3.1 × 10⁻⁷ and 8.2 × 10⁻⁸ M, respectively. AA-2414 competitively inhibited the contraction of rabbit aorta and pig coronary arteries induced by U-44069 with pA2 values of 8.3 and 9.0, respectively. AA-2414 also inhibited the contraction of rabbit aorta induced by PGF2α (pA2: 7.8) and the contraction of pig coronary arteries induced by PGF2α, PGD2 and 9α,11β-PGF2α with pA2 values of 7.8, 8.6 and 7.8, respectively. But, AA-2414 had no effect on the antiaggregatory effect of PGD2 on the aggregation of guinea pig platelets. In experiments with guinea pigs ex vivo, AA-2414 (0.1–1 mg/kg, p.o.) dose-dependently inhibited the platelet aggregation induced by U-44069; the inhibition at a dose of 1 mg/kg was 100% at 1 hr and was 89% even at 24 hr after the administration. The thromboxane (TX) A2/PGH2 receptor antagonistic action of AA-2414 was stereospecific. These results show that AA-2414 is a potent, orally active and long acting TXA2/PGH2 receptor antagonist. In addition, AA-2414 has PGF2α, PGD2 and 9α,11β-PGF2α antagonistic effects.

Among the metabolic products of arachidonic acid, thromboxane (TX) A2 and prostacyclin (PGI2), which are derived from the same precursor, prostaglandin endoperoxide (PGH2), exert potent but opposite biological effects on platelets and smooth muscle; TXA2 induces platelet aggregation and contracts vascular smooth muscle, while PGI2 inhibits platelet aggregation and relaxes smooth muscle (1–3). An imbalance between TXA2 and PGI2 has been suggested to be involved in various diseases including ischemic heart disease, thrombotic disorder, asthma etc. (4). Two types of drugs, TXA2 synthetase inhibitors and TXA2/PGH2 receptor antagonists, are being developed for treatment of these diseases. Specific TXA2 synthetase inhibitors can block the biosynthesis of TXA2 and simultaneously enhance the production of PGI2 (4). However, PGH2, accumulating after TXA2 synthetase inhibition, may act as an agonist on TXA2/PGH2 receptor (5). Though TXA2/PGH2 antagonists inhibit the effects of TXA2 and PGH2, they do not enhance the production of PGI2. At present, it is not convincing which type of drugs, TXA2 synthetase inhibitors or TXA2/PGH2 receptor antagonists, are more beneficial for clinical use than the other.

We have already reported that CV-4151 [(E)-7-phenyl-7-(3-pyridyl)-6-heptenoic acid], a TXA2 synthetase inhibitor, has a TXA2/PGH2 antagonistic action (6, 7), and shows an antithrombotic effect and protective effect on the experimental myocardial infarction (8, 9). Recently, we synthesized a novel

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TXA$_2$/PGH$_2$ receptor antagonist, AA-2412, (±)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenyl-heptanoic acid (10). The present paper describes the antagonistic action of AA-2414 against TXA$_2$/PGH$_2$ receptor and other eicosanoid receptors in platelets and blood vessel preparations in vitro and in the guinea pig ex vivo.

Materials and Methods

1. Platelet aggregation: Platelet aggregation studies were done as described before (11). Blood was collected in 3.15% sodium citrate (1 ml for 9 ml of blood) by cardiac puncture from conscious male guinea pigs (Shizuoka Laboratory Animal Center, 300–500 g). Platelet rich plasma (PRP) and platelet poor plasma (PPP) were obtained from the blood by centrifugation at 1000 g for 5 sec and at 1000 g for 10 min at room temperature, respectively. The platelet density of PRP was adjusted to 450,000 platelets/µl with PPP. Platelet aggregation was measured with a photometer (Rikadenki, Platelet Aggregometor, Japan) according to the method described by Born (12). The PRP (250 µl) was preincubated at 37°C for 2 min and then incubated for 2 min with AA-2414, its isomers, BM-13505, or vehicle (25 µl) followed by stimulation with U-44069, arachidonic acid (AA), collagen, ADP or platelet activating factor (PAF) (25 µl). The concentration of these inducers for aggregation was used to obtain the submaximal aggregation (U-44069: 0.3–1 µM, AA: 0.3–0.6 mM, collagen: 1–7 µg/ml, ADP: 0.5–3 µM and PAF: 1–3 nM). To examine the effect of AA-2414 on the anti-aggregatory action of PGD$_2$ and PGI$_2$, PRP (250 µl) treated with aspirin (final concentration of 10$^{-4}$ M) was preincubated at 37°C for 2 min with AA-2414 or vehicle (25 µl) and then incubated for 2 min with PGD$_2$ or PGI$_2$ (25 µl) followed by stimulation with PAF (25 µl). In experiments ex vivo, drugs suspended in a gum arabic solution were given orally to guinea pigs (300–400 g) 1, 12 or 24 hr before the sampling. The blood was adopted to prepare PRP as described above.

2. Binding of [³H]U-46619 to platelets: The experiments were done according to the method described by Kattelman et al. (13) with slight modifications (7). Blood was collected in 3.15% sodium citrate containing 10 mM aspirin (1 ml for 9 ml of blood) by cardiac puncture from conscious guinea pigs. The PRP fraction was obtained from the blood by centrifugation at 1000 g for 5 sec at room temperature. The PRP was treated with 1 µM prostaglandin E$_1$ (PGE$_1$) and then centrifuged at 1000 g for 5 min to obtain a platelet pellet. The platelet density of washed platelet suspension was adjusted to 600,000 platelets/µl with a modified 25 mM Tris-HCl buffer (pH 7.4) containing 138 mM NaCl, 5 mM KCl, 5 mM MgCl$_2$ and 5.5 mM glucose. Platelets in 450 µl of the buffer were preincubated with AA-2414, its isomers, BM-13505 or U-46619 (20 µl) at 25°C for 6 min and [³H]U-46619 (20 µl, 3.3 nM) was then added. The reaction mixtures were incubated for 6 min and the binding reaction was stopped by adding 3 ml of ice-cold buffer. Platelets were isolated by vacuum filtration on glass filters (Whatman®, GF/C filter). The reaction tube and filter were washed twice with 3 ml of ice-cold buffer. The radioactivity on the glass filter was counted with a liquid scintillation counter (Aloka, LSC-900, Japan, scintillator consisting of 12 I toluene, 12 g p-bis-(O-methylstyryl) benzene, 180 g 2,5-diphenyloxazole and 5.16 I nonion). Non-specific binding of [³H]U-46619 to the platelet was estimated in the presence of 10$^{-6}$ M unlabeled U-46619, and non-specific binding was 14.1±1.9% (n=8) of total binding.

3. Contraction of rabbit aorta and pig coronary artery: New Zealand white rabbits (Rabiton Institute Incorporation, male, 2–3 kg) were sacrificed and the thoracic aorta was excised and placed in the Krebs-Henseleit solution of the following composition: 118 mM NaCl, 4.75 mM KCl, 2.54 mM CaCl$_2$, 1.19 mM KH$_2$PO$_4$, 1.19 mM MgSO$_4$, 12.5 mM NaHCO$_3$ and 10 mM glucose. Adhering fat and connective tissues were removed and spiral strips of the aorta were prepared. Each strip (2–3 mm in width, 3 cm in length) was mounted in an organ bath containing 20 ml of the Krebs-Henseleit solution, bubbled with 95% O$_2$–5% CO$_2$ gas 37°C. A resting tension of 2 g was applied. After equilibration for 1 hr, the tension developed was isometrically recorded on an ink-writing polygraph (San-ei,RECTI-HORIZ-8k, Japan) via a force dis-
placement transducer (Nihon Kohden, Model SB-1T, Japan). The aortic strip was treated with AA-2414 or BM-13505 for 10 min before the agonists were added. The contractile responses of the strip to U-44069 (10^-9-3x10^-7 M) and PGF_2 alpha (10^-7-10^-5 M) were examined in the presence or absence of AA-2414 or BM-13505. The left anterior descending coronary arteries were excised from pig hearts, which were obtained freshly from a slaughter house, and placed in the Krebs-Henseleit solution. Adhering fat and connective tissues were removed and spiral strips (2 mm in width and 3 cm in length) were prepared. The experiments were done according to the methods described above. The contractile responses of the strip to U-44069 (10^-9-10^-6 M), PGF_2 alpha (10^-7-3x10^-5 M), PGD_2 (10^-7-3x10^-5 M) and 9 alpha,11 beta-PGF_2 (10^-7-10^-5 M) were examined in the presence or absence of AA-2414. The concentration of KCl and norepinephrine that induced a submaximal contraction was used (KCl: 60 mM, norepinephrine: 10^-6 M).

4. Statistics: Data were shown as the mean±S.E.M. Student's t-test was used for evaluating the effects of AA-2414 on anti-aggregatory effect of PGD_2 and PGI_2. Williams Wilcoxon test was used in the experiment with guinea pigs ex vivo. The pA_2 values were calculated by the method described by Arunlakshana and Schild (14) and van Rossum (15). Fifty percent inhibiting concentration (IC_50) and 50% inhibiting dose (ID_50) values were calculated from the concentration (dose)-% inhibition relations by the method of least squares. Ninety five percent confidence limits of the IC_50 and ID_50 values were calculated according to Fieller's theorem (16).

5. Agents: AA-2414, its optically active R-(+)- and S-(-)-isomers, BM-13505, 4-[2-(4-chlorobenzene sulphonamide) ethyl] phenylacetic acid and PAF (1-O-octadecyl-2-acetyl-sn-glycerol-3-phosphorylcholine) were synthesized in Chemistry Research Laboratories, Takeda Chemical Industries. U-44069 (15(S)-hydroxy-9 alpha,11 alpha-(epoxymethano) prosta-5Z,13E-dienoic acid) was a kind gift from Upjohn Company (U.S.A.). [3H]U-46619 (15(S)-hydroxy-11 alpha,9 alpha-(epoxymethano) prosta-5Z,13E-dienoic acid) (24.5 mCi/mmol, New England Nuclear, U.S.A.), collagen (Holm, W-Germany), norepinephrine, arachidonic acid, aspirin, PGD_2, PGE_1, PGF_2 alpha and 9 alpha,11 beta-PGF_2 (Sigma, U.S.A.), PGI_2 (Funakoshi Chem., Japan), EDTA (Dojin Reagents, Japan), and other reagents (Wako Pure Chemicals, Japan) were commercial products. U-44069 was dissolved in 100 mM Na_2CO_3 solution. AA, collagen, ADP, PGE_1, PGF_2 alpha and 9 alpha,11 beta-PGF_2 were dissolved in 0.9% saline. PGI_2 was dissolved in 50 mM Tris-HCl (pH 10.0) and stored on ice. PAF was dissolved in 0.9% saline containing 0.25% bovine serum albumin. AA-2414 and BM-13505 were dissolved in dimethyl sulfoxide and then diluted with 0.9% saline.

BM-13505 was synthesized in Chemistry Research Laboratories, Takeda Chemical Industries, which was confirmed to be identical chemically (IR, NMR) and biologically (inhibition of guinea pig platelet aggregation and rabbit aortic contraction induced by U-44069) with BM-13505 kindly supplied by Boehringer Mannheim (W-Germany).

Results

1. Inhibitory effect on platelet aggregation:

![Graph](Fig. 1. Inhibitory effects of AA-2414 and BM-13505 on the aggregation of guinea pig platelets induced by U-44069. The experiments were done with platelet-rich plasma (PRP) obtained from 2-5 guinea pigs. The concentrations of U-44069 were 3x10^-7-10^-6 M and % aggregation with respect to the control was 80±3% (n=5). ( ): number of experiments.)
Figure 1 shows the inhibitory effect of AA-2414 and BM-13505 on the aggregation of guinea pig platelets induced by U-44069. AA-2414 and BM-13505 inhibited the aggregation in a concentration dependent manner with IC50 values of $3.1 \times 10^{-7}$ and $1.9 \times 10^{-6}$ M respectively.

AA-2414 also inhibited AA- and collagen-induced platelet aggregation with IC50 values of $4.9 \times 10^{-7}$ and $2.3 \times 10^{-7}$ M, respectively, and BM-13505 did with IC50 values of $9.2 \times 10^{-7}$ and $1.0 \times 10^{-6}$ M, respectively (Table 1). AA-2414 (3.0 $\times 10^{-7}$ M) did not inhibit the primary aggregation induced by ADP, but did almost completely the secondary one; no further inhibitory effect was observed even at a higher concentration of $3.0 \times 10^{-5}$ M (Table 1). BM-13505 (3.0 $\times 10^{-6}$ M) behaved as AA-2414 (3.0 $\times 10^{-7}$ M) on the ADP aggregation. Both agents (3.0 $\times 10^{-6}$ M) had no effect on the PAF-induced platelet aggregation (Table 1). AA-2414 itself did not induce shape change and aggregation even at a high concentration of $9 \times 10^{-4}$ M (data not shown).

2. Inhibitory effect on the specific binding of $[^3H]$U-46619 to washed platelets: As shown in Fig. 2, AA-2414 inhibited the specific binding of $[^3H]$U-46619 to washed guinea pig platelet with an IC50 value of $8.2 \times 10^{-9}$ M (95% confidence limits: 5.9–11.4 $\times 10^{-9}$ M, n=12). AA-2414 inhibited the specific binding of $[^3H]$U-46619 more effectively than unlabelled U-46619 (IC50: $3.7 \times 10^{-8}$ M, 95% confidence limits: 2.7–5.0 $\times 10^{-8}$ M, n=24). BM-13505 inhibited the binding with an IC50 value of $4.2 \times 10^{-8}$ M (95% confidence limits: 2.2–7.1 $\times 10^{-8}$ M, n=12). AA-2414 (10$^{-6}$ M) had no effect on the nonspecific binding of $[^3H]$U-46619 to the platelets (data not shown).

3. Inhibitory effect on U-44069- and PGF$_{2\alpha}$-induced contraction of rabbit aorta: Figure 3a shows the inhibitory effect of AA-2414 on the contraction of the rabbit aorta induced by U-44069. AA-2414 competitively inhibited the contraction with a pA$_2$ value of 8.3 (Table 2). BM-13505 also inhibited the...

Table 1. Inhibitory effects of AA-2414 and BM-13505 on the aggregation of guinea pig platelets induced by U-44069, arachidonic acid (AA), collagen, ADP and platelet activating factor (PAF)

| Inducer          | Concentration | Drug  | IC50 (M) | 95% Confidence limits |
|------------------|--------------|-------|----------|-----------------------|
| U-44069          | 0.3–1 μM     | AA-2414 | $3.1 \times 10^{-7}$ | 1.2–6.8 $\times 10^{-7}$ |
|                  |              | BM-13505 | $1.9 \times 10^{-6}$ | 0.7–5.1 $\times 10^{-6}$ |
| AA               | 0.3–0.6 mM   | AA-2414 | $4.9 \times 10^{-7}$ | 0.6–9.5 $\times 10^{-7}$ |
|                  |              | BM-13505 | $9.2 \times 10^{-7}$ | 4.1–18.9 $\times 10^{-7}$ |
| Collagen         | 1–7 μg/mL    | AA-2414 | $2.3 \times 10^{-7}$ | 0.4–7.9 $\times 10^{-7}$ |
|                  |              | BM-13505 | $1.0 \times 10^{-6}$ | 0.4–2.3 $\times 10^{-6}$ |
| ADP              | 0.5–3 μM     | AA-2414 | $3 \times 10^{-6}$ | (% inh. 31±2)## |
|                  |              | BM-13505 | $3 \times 10^{-6}$ | (% inh. 38±2)## |
| PAF              | 1–3 nM       | AA-2414 | $3 \times 10^{-6}$ | (% inh. 1±0)## |
|                  |              | BM-13505 | $3 \times 10^{-6}$ | (% inh. 3±2)## |

Number of experiments were 12–15, 10–11, 10, 3–4 and 4 for U-44069, AA, collagen, ADP and PAF, respectively. *: 95% confidence limits of the IC50 values were calculated according to Fiellers et al. (16). ##: Percent inhibition at a concentration of $3 \times 10^{-5}$ M.
of 9.0 and 7.8, respectively (Fig. 4a and b). AA-2414 also inhibited the PGD₂- and 9α,11β-PGF₂α, a metabolite of PGD₂-induced contraction with pA₂ values of 8.6 and 7.8, respectively (Fig. 4c and d).

5. Effect on the anti-aggregatory effect of PGD₂ and PGI₂: As shown in Fig. 5, PGD₂ inhibited the PAF induced aggregation of guinea pig platelets with an IC₅₀ value of 3.4 × 10⁻⁸ M (95% confidence limits: 2.5–4.6 × 10⁻⁸ M, n=18), while AA-2414 (3 × 10⁻⁵ M) had no effects on the anti-aggregatory effects of PGD₂; an IC₅₀ value of PGD₂ in the presence of AA-2414 was 2.6 × 10⁻⁸ M (95% confidence limits: 1.9–3.5 × 10⁻⁸ M, n=18). AA-2414 (3 × 10⁻⁵ M) also did not affect the inhibitory effect of PGI₂ on the platelet aggregation (data not shown).

6. Effect of optically active isomers of AA-2414: As shown in Table 2, the effect of AA-2414 was stereospecific. An R-(+)-isomer inhibited the contraction of rabbit aorta (pA₂: 8.6) and the aggregation of guinea pig platelets (IC₅₀: 1.0 × 10⁻⁷ M) induced by U-44069 more potently than AA-2414 (pA₂: 8.3, IC₅₀: 3.1 × 10⁻⁷ M) and an S-(−)-isomer was much less potent (IC₅₀: 9.0 × 10⁻⁶ M) than AA-2414. An R-(+)-isomer also inhibited the specific binding of [³H]U-46619 to washed guinea pig platelets with an IC₅₀ value of 1.9 × 10⁻⁹ M more potently than AA-2414 (IC₅₀: 8.2 × 10⁻⁹ M). An S-(−)-isomer (IC₅₀: 1.5 × 10⁻⁷ M) was much less potent than AA-2414.

7. Inhibitory effect of platelet aggregation in guinea pigs ex vivo: Oral administrations of AA-2414 (0.01–1 mg/kg) and BM-13505 (0.1–30 mg/kg) to guinea pigs inhibited the U-44069-induced platelet aggregation in a dose-dependent manner, and ID₅₀ values were 0.1 mg/kg (95% confidence limits: 0.05–0.2 mg/kg, n=18) and 2.5 mg/kg (95% confidence limits: 1.4–4.4 mg/kg, n=24), respectively (Fig. 6a). The inhibitory effect of AA-2414 (1 mg/kg) lasted more than 24 hr; the inhibition was 100% at 1 hr and was 89% even at 24 hr after the dosing (Fig. 6b). On the other hand, BM-13505 (30 mg/kg) had little effect at 24 hr after the dosing (Fig. 6b).

Discussion
TXA₂/PGH₂ antagonists so far reported can
Fig. 4. Inhibitory effect of AA-2414 on U-44069 (a), PGF$_{2\alpha}$ (b), PGD$_2$ (c) and 9x,11-PGF$_2$ (d)-induced contraction of pig coronary artery. The contractile responses of the pig coronary artery to U-44069 (10$^{-9}$-10$^{-6}$ M), PGF$_{2\alpha}$ (10$^{-7}$-3×10$^{-6}$ M), PGD$_2$ (10$^{-7}$-3×10$^{-6}$ M) and 9x,11-PGF$_2$ (10$^{-7}$-10$^{-5}$ M) were examined in the absence (control) or presence of AA-2414 (3×10$^{-9}$-10$^{-7}$ M). Results shown are the means±S.E.M. of 3-6 experiments. ( ): number of experiments.

Table 2. Inhibitory effects of optically active isomers of AA-2414 on the contraction of rabbit aorta and platelet aggregation of guinea pig induced by U-44069 and the binding of [3H]U-46619 to washed guinea pig platelets

|                  | Contraction of aorta | Aggregation of platelet | Specific binding of [3H]U-46619 to washed platelets |
|------------------|----------------------|-------------------------|---------------------------------------------------|
|                  | pA$_2$ | IC$_{50}$ (M) | IC$_{50}$ (M) | IC$_{50}$ (M) |
| AA-2414         | 8.3    | 3.1×10$^{-7}$ | 8.2×10$^{-9}$ |
| R-(+) Isomer    | 8.6    | (1.2-6.8×10$^{-7}$) | (5.9-11.4×10$^{-9}$) |
| S-(−) Isomer    | not tested | 1.0×10$^{-7}$ | 1.9×10$^{-9}$ |
|                  |         | (0.7-1.4×10$^{-7}$) | (0.5-4.1×10$^{-9}$) |
| 9x,11-PGF$_2$   | 9.0×10$^{-6}$ | (4.7-16.6×10$^{-9}$) | (1.0-2.5×10$^{-7}$) |
| BM-13505        | 8.5    | 1.9×10$^{-8}$ | 4.2×10$^{-8}$ |
|                  |         | (0.7-5.1×10$^{-9}$) | (2.2-7.1×10$^{-8}$) |

See legends for Fig. 4a, Fig. 1 and Fig. 3. Numbers in parentheses are 95% confidence limits. Numbers of experiments were 9-15.
Fig. 5. Inhibitory effect of PGD₂ on the PAF-induced aggregation of guinea pig platelets in the absence or presence of AA-2414. Aspirin-treated PRP was preincubated with AA-2414 (3x10⁻⁵ M) or vehicle (control, 25 μl) for 2 min at 37°C and then incubated with PGD₂ (10⁻⁸–10⁻⁷ M) for 2 min followed by stimulation with PAF (25 μl). Results are shown as the mean±S.E.M. of 5 experiments.

Fig. 6. Inhibitory effect of AA-2414 and BM-13505 on the aggregation of guinea pig platelets induced by U-44069 in the ex vivo experiments. a) Dose-response curve: Blood was collected one hr after the p.o.-administration of various doses of AA-2414, BM-13505 or vehicle (control). b) Time course study: Blood was collected 1, 12 and 24 hr after the p.o.-administration of vehicle (control) or drugs. Results are shown as the mean±S.E.M. (*): number of animals. *P<0.05, **P<0.01 (Williams Wilcoxon test) vs. control.

TXA₂/PGH₂ Receptor Antagonist, AA-2414

be classified into two groups: TXA₂ or PGH₂ analogs such as PTA₂ (pinane TXA₂), 13-APA (13-azaprostanic acid) and SQ 29548 {1S-[1,2(5Z),3,4]-7-[3-[[2-[(phenyl amino) carbonyl] hydrazino] methyl]-7-oxabicycle [2.2.1] hept-2-yl]-5-heptenoic acid} and non-prostanoid compounds such as L-636499 {3-carboxyl-dibenzo (b,f) thiepin 5,5-dioxide} and BM-13177 {4-[(2-benzene sulfonamide)-ethyl] phenoxyacetic acid} (5).

In this study, we have shown that AA-2414, a quinone derivative, is a potent and orally active TXA₂/PGH₂ antagonist. AA-2414 inhibited the aggregation of guinea pig platelets and the contraction of rabbit aorta and pig coronary artery induced by U-44069 (Figs. 1, 3 and 4). The inhibitory effect of AA-2414 (IC₅₀: 3.1x10⁻⁷ M) on the platelet aggregation of guinea pigs induced by U-44069 was approximately 10 times more potent than that of BM-13505 (IC₅₀: 1.9x10⁻⁶ M), one of the potent TXA₂/PGH₂ antagonists so far reported (17). AA-2414 potently inhibited the specific binding of [³H]U-46619 to washed guinea pig platelets (Fig. 2). AA-2414 competitively inhibited the U-44069-induced rabbit aorta contraction as potent as BM-13505 (Fig. 3) and it also did the contraction of pig coronary artery induced by U-44069 (Fig. 4). Thus, AA-2414 appears to be a potent TXA₂/PGH₂ antagonist at both the receptor sites of platelets and vascular beds. The TXA₂/PGH₂ antagonistic action of AA-2414 was stereospecific: the rank order of inhibitory effects of optically active isomers were R-(+)-isomer,
AA-2414 and S-(-)-isomer. The inhibitory effect of AA-2414 and S-(-)-isomer can be explained by R-(+)-isomer; contents of R-(+)-isomer are 50% for the former and 2% for the latter, respectively.

AA-2414 has no agonistic action, since AA-2414 at a concentration of 9.1×10^{-4} M did not induce shape change and platelet aggregation. Furthermore, AA-2414 at a concentration of 3×10^{-5} M did not influence the basal tone of rabbit aorta and pig coronary arteries.

AA-2414 also inhibited the contraction of rabbit aorta and pig coronary artery induced by contractile prostaglandins, PGF_{2α}, PGD_{2}, and 9α,11β-PGF_{2} as well as by U-44069 (Figs. 3 and 4). These inhibitory effects of AA-2414 may be beneficial for its clinical use, since possible involvement of these contractile prostaglandins in asthma and cardiovascular diseases has been suggested (18, 19). Interestingly, AA-2414 did not affect the inhibitory effect of PGD_{2} on the platelet aggregation as well as PGI_{2}. Two subtypes of PGD_{2} receptor have been reported (20): one mediates the contraction of the trachea and the blood vessel including coronary artery, and the other mediates the inhibition of platelet aggregation. Thus, AA-2414 appears to inhibit selectively the PGD_{2} receptor for the contraction of the blood vessels. As both PGD_{2} and PGI_{2} are endogenous potent inhibitors of platelet aggregation (21), the character that AA-2414 has no effect on the anti-aggregatory activity of PGD_{2} and PGI_{2} may be favorable for the clinical use. The inhibitory effect of AA-2414 on the contraction of blood vessels may be specific to contracting eicosanoids, since AA-2414 at a concentration of 3×10^{-5} M did not inhibit the contraction of rabbit aorta induced by KCl and norepinephrine.

In experiments with guinea pigs ex vivo AA-2414 inhibited the U-44069-induced platelet aggregation about 25 time more potently than BM-13505 (Fig. 6a). Even 24 hr after the administration, AA-2414 at an oral dose of 1 mg/kg inhibited the aggregation by 89% (Fig. 6b). On the other hand, the inhibitory effect of BM-13505 at a higher dose of 30 mg/kg (p.o.) was not significant 24 hr after the administration. These results show that AA-2414 exerts more potent and longer acting TXA_{2}/PGH_{2} antagonistic action than BM-13505.

As native TXA_{2} is highly unstable (1), we used stable analogs of PGH_{2}, U-44069 and U-46619, which have been reported to act as the TXA_{2} agonist (5). Further studies are needed to show that AA-2414 antagonizes the effects of native TXA_{2}. However, AA-2414 inhibited the platelet aggregation of guinea pig induced by collagen and AA as well as by U-44069 (Table 1). The platelet aggregation induced by collagen and AA has been reported to be mediated mainly by TXA_{2} (22). Furthermore, AA-2414 from a lower concentration of 3×10^{-7} M inhibited the secondary phase of platelet aggregation induced by ADP, which is also believed to be mediated by TXA_{2} (22). AA-2414 (3×10^{-5} M) did not inhibit cyclooxygenase (T. Matsumoto, unpublished data). Thus, inhibitory effect of AA-2414 on native TXA_{2} can be expected. Contribution of TXA_{2}/PGH_{2} in PAF-induced platelet aggregation is controversial. As AA-2414 and BM-13505 even at a concentration of 3×10^{-5} M showed no inhibitory effect on the aggregation of guinea pig platelets induced by PAF (Table 1), it appears that TXA_{2} does not mediate the platelet aggregation induced by PAF in guinea pigs.

AA-2414 has been confirmed to inhibit potently the broncho-constriction in guinea pigs induced by a variety of spasmogenic prostanoids, including U-46619 (23).

In conclusion, AA-2414 is a non-prostanoid and long acting TXA_{2}/PGH_{2} antagonist, and may be useful for the treatment of ischemic heart diseases, thrombotic disorders and asthma.

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