The Effect of a Cystamine Derivative, Bis[2-(*E*-2-hexenoylamino)ethyl] Disulfide, on Rat Platelet Aggregation

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Bis[2-(*E*-2-alkenoylamino)ethyl] disulfides (compds. I), synthesized from cystamine and 2-*trans* fatty acids, inhibited collagen-induced rat and rabbit platelet aggregation. The most potent compound was bis[2-(*E*-2-hexenoylamino)ethyl] disulfide (compd. I-1), and this compound suppressed thromboxane B_2 formation from arachidonic acid in rat platelets. The results suggested that compd. I-1 has an inhibitory effect on cyclooxygenase.

Keywords cystamine; bis[2-(*E*-2-hexenoylamino)ethyl] disulfide; platelet aggregation; thromboxane B_2 formation

Various sulfur-containing compounds, such as allicin[1] and gliotoxin,[2] have platelet aggregation-inhibitory activity. Cysteamine, which is a sulfur containing amine, also has this activity. Consequently, we thought that cystamine, a metabolite of cysteamine might also have the activity; in fact it has much stronger activity than cysteamine.[3] We have also screened several compounds synthesized from cystamine and free fatty acids for anti-inflammatory[4] and anti-ulcer activities.[5] In the present study, we examined compounds synthesized from cystamine and 2-*trans* fatty acids for platelet aggregation-inhibitory activity.

Experimental

Materials Collagen (type I from bovine achilles tendon) and arachidonic acid (AA) (approx. 99%, from porcine liver) were obtained from Sigma. Adenosine 5′-diphosphate 2Na (ADP) was obtained from Kojin Co., Ltd. The thromboxane B_2 (TXB_2) ^{125}I assay kit was a product of Amersham. The series of compds. I were synthesized in our laboratory as described previously.[5] We also synthesized one new compd. I (I-10) for this experiment.

Synthesis of Bis[2-(*E*-2-pentenoylamino)ethyl] Disulfide (I-10) The procedures used for I-1 were repeated with 2-*trans*-pentenoic acid (15 g) to obtain I-10,[5] which was purified by recrystallization from ethyl acetate–diethylether (4.7 g, 54%). mp 119—120.5 °C (pale white crystals). IR ν_{max}^{KBr} cm^{-1}: 3290, 1665, 1620, 1540, 975. ^1H-NMR ($CDCl_3$) δ: 1.07 (t, 3H, J=7.0 Hz), 1.90—2.50 (m, 2H), 2.85 (t, 2H, J=6.5 Hz), 3.62 (dt, 2H, J=6.5, 6.5 Hz), 5.89 (dt, 1H, J=1.5, 15.0 Hz), 6.88 (dt, 1H, J=6.0, 15.0 Hz), 6.95 (br, 1H).

Preparation of Test Samples and Platelet-Rich Plasma (PRP) Samples were dissolved in methanol and diluted with saline to yield 1.0% methanol in the assay system. Rat PRP were prepared from the citrated blood of male Wistar rats, and the platelet concentration of PRP was adjusted to 10^9/ml, as previously described.[6] Rabbit PRP were also prepared from citrated blood.

Platelet Aggregation Assay Platelet aggregation studies were performed according to the turbidimetric method of Born and Cross[7] in an NKK Hema Tracer 1 aggregometer. In collagen-induced aggregation, 25 μl of test sample was added to 225 μl of PRP 3 min prior to the addition of the inducer. The extent of aggregation was expressed in terms of the maximum change of light transmission expressed as a percentage, taking the difference between light transmission for PRP and platelet-poor plasma as 100%. The percent inhibition of aggregation by test samples were calculated by dividing the percent aggregation by that observed in the control run, and then multiplying by 100. Fifty percent inhibitory concentration (IC_{50}) values were estimated previously.[3]

Assay for TXB_2 Formation Rat PRP (470 μl) and AA (0.6 mM) were incubated with or without test samples (30 μl) at 37 °C for 5 min. The reaction was terminated by adding 118 μl of 1 mM indomethacin solution containing 77 mM ethylenediaminetetraacetic acid (EDTA). After centrifugation for 10 min at 1200 *g*, TXB_2 in the supernatant was measured using the TXB_2 radioimmunoassay kit.

Results and Discussion

The compounds all inhibited collagen-induced rat and rabbit platelet aggregation. The most potent inhibitor was compd. I-1 and its IC_{50} values were found to be 4.0 μM on rat platelet aggregation and 85 μM on rabbit platelet aggregation. Compound I-1 also inhibited AA-induced platelet aggregation but not ADP-induced platelet aggregation (Table I). The effect of compd. I-1 resembled that of aspirin. We next examined the effect on TXA_2 formation. Compound I-1 strongly inhibited the formation of TXB_2, a stable metabolite of TXA_2, in the AA-treated platelets (Table II). From this result, it was assumed that compd. I-1 inhibits cyclooxygenase.

Using sheep seminal vesicle microsomes, the inhibitory activity of compd. I-1 on prostaglandin E_2 (PGE_2) formation was demonstrated. Compound I-1 inhibited the

TABLE I. Effects of Cystamine and Compds. I on Collagen-, ADP- and Arachidonic Acid-Induced Rat Platelet Aggregation and Collagen-Induced Rabbit Platelet Aggregation

Sample	IC_{50} (μM)			
	Rat platelet			Rabbit platelet
	Collagen (10 μg/ml)[a]	ADP (4 μM)[a]	Arachidonic acid (0.6 mM)[a]	Collagen (10 μg/ml)[a]
Compd. I-10	13	>500	13	180
Compd. I-1	4	>500	7	85
Compd. I-2	26	>500	17	310
Cystamine	25	80	44	50
Aspirin	65	>1000	100	54

a) Final concentrations of stimuli are given in parenthesis. IC_{50} values were estimated from the slope of the log dose–response relationship for each drug, determined from the mean values of 3—4 experiments.

TABLE II. Effect of Compd. I-1 on Arachidonic Acid-Induced TXB_2 Formation in Rat PRP

Sample	Concentration (μM)	TXB_2 (ng/2 × 10^8 platelets)	Inhibition (%)
Control[a]	—	44.0 ± 2.0	—
Compd. I-1	0.5	40.1 ± 3.9	8.9
	1.0	34.5 ± 1.1[b]	21.6
	5.0	8.4 ± 0.8[c]	80.9
	10.0	3.2 ± 1.8[c]	92.7
Control[a]	—	53.5 ± 3.1	—
Aspirin	100	19.9 ± 1.3[c]	62.8

a) 1% methanol. All values represent mean ± S.E. ($n=3$). Significantly different from the control group: *b*) $p<0.01$, *c*) $p<0.001$.

conversion of AA to PGE_2 and unconverted AA did not change to other metabolites (PGG_2 or PGH_2). Thus, compd. I-1 inhibits cyclooxygenase (data not shown).

It is noteworthy that compd. I-1 has activity similar to that of aspirin, although the chemical structure is completely different from that of non-steroidal anti-inflammatory drugs.

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References

1) S. F. Mohammad and S. C. Woodward, *Thromb. Res.*, **44**, 793 (1986).
2) M. Sakai and M. Watanuki, *Agric. Biol. Chem.*, **51**, 2167 (1987).
3) T. Mimura, Y. Kohama, S. Kuwahara, K. Yamamoto, Y. Komiyama, M. Satake, Y. Chiba, K. Miyashita, T. Tanaka, T. Imanishi and C. Iwata, *Chem. Pharm. Bull.*, **36**, 1110 (1988).
4) T. Mimura, H. Nakajima, Y. Kohama, K. Tsujikawa, S. Itoh, T. Ohmura, M. Iwai and K. Yokoyama, *Chem. Pharm. Bull.*, **35**, 4579 (1987).
5) M. Iwai, I. Kohda, C. Fukaya, Y. Naito, K. Yokoyama, H. Nakajima, K. Tsujikawa, M. Okabe and T. Mimura, *Chem. Pharm. Bull.*, **35**, 4616 (1987).
6) Y. Kohama, K. Iwabuchi, M. Okabe and T. Mimura, *J. Pharmacobio-Dyn.*, **9**, 182 (1986).
7) G. V. R. Born and M. J. Cross, *J. Physiol.*, **168**, 178 (1968).