Potential Effectiveness of Minidose Aspirin Combined with Dilazep in Anti-Platelet Therapy

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(Received on October 29, 1987) (Express)

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The anti-platelet aggregating action of minidose aspirin and its combined effect with dilazep were examined in healthy male volunteers. A quantitative and dose-dependent decrease in platelet aggregability was observed on a single oral administration of 30 mg, 100 mg and 300 mg of aspirin and also on the repeated oral administration of 10 mg and 30 mg three times a day for 5 days. Administration of 100 mg of dilazep in combination with 30 mg or 100 mg of aspirin produced an anti-platelet effect equivalent to a 3-fold dose of aspirin alone. In the repeated administration test, the t.i.d. administration of aspirin 10 mg + dilazep 100 mg was confirmed to be close to the ideal in the balance between anti-platelet action and the reduced production of PGI₂.

Key words: anti-platelet therapy, minidose aspirin, dilazep, platelet aggregability, PGI₂

Introduction

In treatment or prophylaxis of thrombotic diseases the usefulness of aspirin as an anti-platelet agent has been recognized.¹⁻³⁾ However, there are associated problems such as the development of gastrointestinal disturbances and inhibition of PGI₂ production in the vascular wall.^{4,5)} Accordingly, a selective minidose of aspirin seems significant for the anti-platelet therapy with this drug.⁶⁻⁸⁾

On the other hand, it is known that dilazep dihydrochloride (Comelian[®], 1, 4-bis-[3-(3, 4, 5-trimethoxybenzoyloxy) propyl] perhydro-1, 4-diazepine dihydrochloride monohydrate), a coronary vasodilator⁹⁾ and cerebral vasodilator,¹⁰⁾ acts not only to increase peripheral flow but also to potentiate the abilities of erythrocyte oxygen dissociation and blood fibrinolysis.¹¹⁻¹³⁾ Moreover, its anti-platelet action, based on its membrane stabilizing

action and Ca²⁺ antagonism,¹⁴⁻¹⁶⁾ suggests that this drug may be applicable in the combination with aspirin for treatment of thrombotic disease.

From this viewpoint we previously performed *ex vivo* experiments using experimental animals, and the results showed that the combined administration of aspirin and dilazep potentiated an anti-platelet action, without influencing PGI₂ production in the vascular wall.¹⁷⁾ Therefore, we conducted a crossover study using healthy male volunteers and confirmed the usefulness of the combination of both drugs from the viewpoint of clinical pharmacology.

Materials and Methods

Drugs

Aspirin (the standards of the Japanese Pharmacopoeia) was purchased from Eisai Co. It was diluted 20-fold with lactose to prepare a powder for ingestion. Dilazep was presented from Kowa Co. (Comelian[®] 50 mg Tablets).

Single administration test

A total of 12 healthy male volunteers (age, 27.8

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Tab. 1 Schedule for single-dose crossover study of combined oral administration of aspirin and dilazep in 12 healthy male volunteers

Group	Volunteer	Age (yr)	Height (cm)	B. W. (kg)	Dosage (mg)		
					Exp. 1	Exp. 2	Exp. 3
I	A	32	161	62	A 30 + D100	A 30	A 300
	B	29	165	64	A 30 + D100	A 30	A 300
II	C	28	167	70	A100 + D100	A30 + D100	A 30
	D	26	174	76	A100 + D100	A30 + D100	A 30
III	E	27	169	58	A 30	A100	A 30 + D100
	F	26	172	66	A 30	A100	A 30 + D100
IV	G	25	170	68	A100	A300	A100 + D100
	H	27	178	80	A100	A300	A100 + D100
V	I	28	170	74	A300		A100
	J	30	172	72	A300	A100 + D100	A100
	K	28	178	72			A 30
	L	28	178	72			A100 + D100
mean $\pm$ SD		27.8 $\pm$ 1.9	171.2 $\pm$ 5.4	69.5 $\pm$ 6.2			

After each experiment, all volunteers had a washing-out period of two weeks during which the anti-platelet effect of high-dose aspirin disappeared completely. A ; aspirin, D ; dilazep

± 1.9 yr) were divided into 5 groups which participated in three crossover tests following the schedule in Tab. 1. The drugs were taken at 9 a. m. during fasting. Blood was collected from the elbow vein before administration, and 0.5, 1, 3, 6, and 24 hr after administration using a plastic syringe (containing 1/10 volume of 3.8% sodium citrate solution). Meals were taken twice a day, i.e., immediately after blood sampling at 1 p. m. and at 6 p. m. Platelet aggregability was determined by the method of Born.¹⁸⁾ Collected blood was centrifugated at $200 \times g$ for 7 min to prepare platelet rich plasma (PRP) then the residue was centrifugated at $1,500 \times g$ for 10 min to give platelet poor plasma (PPP). A glass tube containing 250 μ l of PRP was set up in an aggregometer (NKK, Hematracer 1), and after preincubation for 1 min, 10 μ l of collagen suspension (Horm Co.) was added to give final levels of 0.5, 1, 2, and 4 μ g/ml PRP and aggregation curves were recorded. Plasma salicylic acid levels and blood dilazep levels were determined respectively by high-performance liquid chromatography and GC/MS (selective ion monitoring).

Repeated t.i.d. administration test

A total of 16 healthy male volunteers (age, 22.4 ± 1.5 yr) were divided into 5 groups which participated in two crossover tests following the schedule in Tab. 2. The drugs were administered in designed doses three times a day (9 a. m., noon,

5 p. m.) repeatedly for 5 days. Meals were taken 30 min after each drug intake. Blood was collected under the same conditions as in the single-administration test, three times a day (immediately before drug intake at 9 a. m., noon, and 3 p. m.) on the first 3 days after the start of administration, and once a day (immediately before drug intake at 9 a. m.) on day 4 and day 5. After completion of the repeated administration for 5 days, a period of non-administration began during which blood was collected at 9 a. m. on day 3 and day 7. The collected blood was treated similarly to that in the single-administration test and used for the determination of platelet aggregability. In addition, the blood collected 6, 24 and 72 hr after the start of administration and at 9 a. m. on day 7 during the period of non-administration was used for the assay of plasma TXB₂ and 6-keto-PGF_{1 α} levels which were estimated by radioimmunoassay method (RIA kit. New England Nuclear).

Analysis of platelet aggregability

Platelet aggregability was calculated as reported previously.¹⁷⁾ The maximal aggregation velocity (maximal decrease rate of optical density : %/min) was calculated from aggregation curves at each level of added collagen as shown in Fig. 1 (A), and the dose-response curve was prepared as shown in Fig. 1 (B). Similar dose-response curves were prepared from the aggregation curves obtained after drug administration. In this case, the stronger the

Tab. 2 Schedule for repeated-dose crossover study of combined oral administration of aspirin and dilazep in 16 healthy male volunteers

Group	Volunteer	Age (yr)	Height (cm)	B. W. (kg)	Dosage (mg, 3 times a day for 5 days)	
					Exp. 1	Exp. 2
I	a	25	170	72	A10 + D100	A10
	b	22	180	62.5	A10 + D100	A10
	c	22	170	55.5	A10 + D100	A10
	d	23	175	78	A30 + D100	A30
II	e	24	175	58	A30 + D100	D100
	f	26	160	53	A30 + D100	D100
	g	22	170	66	A10	A10 + D100
III	h	23	173	63	A10	A10 + D100
	i	21	171	57.5	A10	A10 + D100
	j	22	171	73	A30	A30 + D100
IV	k	22	176	60	A30	A30 + D100
	l	21	175	56	A30	D100
	m	21	173	63	D100	A30
V	n	20	172	58	D100	A30 + D100
	o	21	179	71		D100
	p	22	187	76		A30
mean $\pm$ SD		22.4 $\pm$ 1.5	173.7 $\pm$ 5.6	64.1 $\pm$ 7.7		

A four-week interval was left between experiment 1 and experiment 2 to completely wash out the anti-platelet effect of aspirin. A; aspirin, D; dilazep.

Fig. 1 Analytical method for the estimation of collagen-induced platelet aggregability. (A): Calculation of maximal slope (%/min) of collagen-induced platelet aggregation curves. (B): Calculation of platelet aggregability (%) using the curve relating maximal slope of platelet aggregation and concentration of collagen added. ○-○, before medication; ●-●, after medication.

anti-platelet action of the drug is, the longer will be distance between the dose-response curves before and after administration. Figure 1 (B) implies that a 2-fold dose of collagen is needed for the med-

icated blood platelets to give the same magnitude of platelet aggregation as the non-medicated blood platelets where the medicated platelet aggregability was expressed as having decreased to 1/2 or 50%.

Fig. 2 Time course of platelet aggregability induced by collagen after single oral administration of aspirin and dilazep in healthy male volunteers. Each value represents the mean of 6 volunteers □-□, 30 mg of aspirin; ○-○, 100 mg of aspirin; △-△, 300 mg of aspirin; ■-■, 30 mg of aspirin and 100 mg of dilazep; ●-●, 100 mg of aspirin and 100 mg of dilazep. Significantly different from the corresponding aggregability with aspirin alone (*, $P < 0.05$; **, $P < 0.01$).

Results

Single administration test

Changes in platelet aggregability of the various drug administration groups are shown in Fig. 2. In the aspirin 30 mg group, aggregability decreased slightly to $84.3 \pm 5.6\%$ after 3 hr and returned to the initial value 24 hr after drug administration. In the aspirin 100 mg group, platelet aggregability decreased to $49.5 \pm 8.7\%$ 1 hr, to about 50% 3 and 6 hr, and remained at $59.2 \pm 4.2\%$ 24 hr. In the aspirin 300 mg group, it decreased to $36.0 \pm 5.3\%$ 1 hr and remained to almost the same extent up to 24 hr. In the combination group of aspirin 30 mg and dilazep 100 mg, platelet aggregability showed significant decreases to 75.0 ± 9.4 , 63.3 ± 3.4 , and $66.7 \pm 4.8\%$ respectively 1, 3 and 6 hr compared with the group given aspirin 30 mg alone, but it returned to $97.2 \pm 3.0\%$ of the initial value 24 hr after drug administration. In the combination group of aspirin 100 mg and dilazep 100 mg, it showed significantly lower values of 30.5 ± 3.9 , 29.7 ± 3.2 , and $32.0 \pm 3.6\%$ respectively 1, 3, and 6 hr in comparison with the group given aspirin 100 mg alone. In addition, the inhibitory action persisted at $44.7 \pm 6.3\%$ 24 hr later. Changes in

plasma salicylic acid levels and blood dilazep levels are shown in Fig. 3. Salicylic acid levels peaked 30 min after administration, the peak heights depending on the dose of aspirin. No difference in plasma salicylic acid levels was observed between the aspirin-alone group and the dilazep combination group (Fig. 3 A). Also, plasma dilazep levels showed no difference when dilazep was given in combination with 30 mg or 100 mg of aspirin, in both cases reaching the maximal blood level of 90 ng/ml blood (Fig. 3 B).

Repeated t.i.d. administration test

Changes in platelet aggregability before and during drug administration (5 days), and 7 days after cessation are shown in Fig. 4. In the aspirin 10 mg (daily 30 mg) administration group, the inhibitory action developed from day 3 of administration, and aggregability showed $39.4 \pm 5.7\%$ on day 4 and $36.6 \pm 4.6\%$ on day 5. After cessation, aggregability began to recover and returned to $94.8 \pm 10.2\%$ of the initial value on day 7. In the aspirin 30 mg (daily 90 mg) administration group, the onset of an obvious inhibitory action was observed on day 2 after the start of administration, and aggregability fell to about 20% during the adminis-

Fig. 3 Plasma concentration of salicylic acid (A) and concentration of dilazep (B) after single oral administration of aspirin and dilazep. (A): □-□, 30 mg of aspirin ($n=5$); ■-■, 30 mg of aspirin and 100 mg of dilazep ($n=4$); ○-○, 100 mg of aspirin ($n=4$); ●-●, 100 mg of aspirin and 100 mg of dilazep ($n=5$); △-△, 300 mg of aspirin ($n=4$) (B): ■-■, 30 mg of aspirin and 100 mg of dilazep ($n=6$); ●-●, 100 mg of aspirin and 100 mg of dilazep ($n=7$). Each value represents the mean $\pm$ SE.

tration period. On day 3 after cessation it recovered to $66.5 \pm 9.5\%$, but even on day 7 it remained depressed at $72.3 \pm 4.8\%$. In the dilazep 100 mg (daily 300 mg) administration group, a slight inhibitory action appeared immediately after the start of administration, and, although aggregability returned to the initial value 24 hr later on the first 3 days, persistent changes in the inhibitory action were observed thereafter. After cessation it gradually recovered and returned to $94.6 \pm 4.8\%$ of the initial value on day 7. In the combined administration group of aspirin 10 mg and dilazep 100 mg, platelet aggregability fell to about 60% immedi-

ately after the start of administration (3 or 6 hr later), a significant decrease compared with the aspirin 10 mg alone group. In the combined administration group of aspirin 30 mg and dilazep 100 mg, platelet aggregability decreased to about 10% from day 2 after the start of administration, a significant decrease in comparison with the aspirin 30 mg alone group. Platelet function recovered to about 80% on day 7 after cessation. Plasma levels of TXB_2 and $6\text{-keto-PGF}_{1\alpha}$ before, during drug administration, and on day 7 after cessation are shown in Table 3. The pretreatment values of TXB_2 varied in each group at the range of 179–235 pg/ml. The single administration groups of aspirin 10 mg, aspirin 30 mg, and dilazep 100 mg showed a slight decrease in TXB_2 6 hr after the start of administration. TXB_2 values remained slightly low during the administration period but returned almost to the initial values on day 7 after cessation. In the combined administration groups of aspirin and dilazep, TXB_2 values decreased to a similar extent and no significant difference was found from the non-combined groups. The initial levels of $6\text{-keto-PGF}_{1\alpha}$ also varied in each group at the range of 30.7–40.7 pg/ml. These values showed a slight ups and downs during the administration period in all of the treatment groups, but no significant difference was observed from the initial values.

Discussion

The clinical dose of aspirin as an antipyretic analgesic is generally 600–1,500 mg (t.i.d)/day. These doses have been adopted when aspirin was used as an anti-thrombotic agent. A dilemma arose, however, when it was found, by elucidation of the anti-platelet mechanism of aspirin, that aspirin inhibited the production of PGI_2 in the vascular wall.^{4–6} Since then, the low-dose usage of aspirin and the utility of aspirin in combination with other drugs have been discussed,^{3,6–8,19} but few clinico-pharmacological analyses of the anti-platelet action of aspirin have been reported. One reason may be problems in the determination and the analysis of platelet aggregability. Previously,¹⁷ we administered aspirin and dilazep in combination to experimental animals and presented a pharmacological analysis of their anti-platelet actions. In the present clinical trial using human volunteers, a similar method for quantitative analysis of platelet aggreg-

Fig. 4 Time course of platelet aggregability induced by collagen after the repeated oral administration of aspirin and dilazep for 5 days in healthy male volunteers. Each value represents the mean of six volunteers. $\square$ - $\square$, 100 mg of dilazep; $\circ$ - $\circ$, 10 mg of aspirin; $\triangle$ - $\triangle$, 30 mg of aspirin; $\bullet$ - $\bullet$, 10 mg of aspirin and 100 mg of dilazep; $\blacktriangle$ - $\blacktriangle$, 30 mg of aspirin and 100 mg of dilazep. Medication: t. i. d.

Tab. 3 Time course of plasma levels of TXB₂ and 6-keto-PGF_{1 α} before and after the administration of aspirin and dilazep

Dosage		Before	During drug medication			After 7 days
(mg, 3 times a day for 5 days)	factors		6 hr	24 hr	3 days	
ASA 10+DZP 100	TXB 2	219. 3±13	125. 3±13*	168. 7±24	164. 7±22	189. 0±17
	6-Keto-PGF1α	33. 7±7. 1	38. 0±2. 8	44. 2±3. 2	42. 5±8. 4	38. 5±7. 4
ASA 30+DZP 100	TXB 2	235. 0±30	134. 0±15*	154. 2±19*	148. 5±25	182. 3±14
	6-Keto-PGF1α	31. 7±5. 7	40. 8±5. 3	41. 7±3. 8	39. 3±6. 9	46. 7±7. 0
ASA 10	TXB 2	179. 2±13	122. 8±12*	148. 2±9. 0	166. 4±19	173. 6±14
	6-Keto-PGF1α	30. 7±3. 9	44. 3±4. 2	57. 5±10	61. 0±21	32. 6±9. 5
ASA 30	TXB 2	205. 5±22	141. 2±20	147. 8±15	153. 8±21	222. 8±25
	6-Keto-PGF1α	40. 7±11	65. 8±6. 8	49. 3±3. 2	55. 0±13	38. 3±9. 0
DZP 100	TXB 2	198. 0±24	156. 0±11	176. 0±13	161. 8±10	194. 5±35
	6-Keto-PGF1α	35. 3±5. 0	36. 4±6. 4	50. 0±9. 2	35. 2±12	31. 0±8. 0

Values of TXB₂ (pg/ml) and 6-keto-PGF_{1 α} (pg/ml) represent mean $\pm$ SE (n=6). Significantly different from those before the administration by t-test (*, P<0.05).

ability was adopted. Thus a dose-response curve was drawn by plotting the maximal slope (%/min) of platelet aggregation against the concentration of collagen added to the PRP, and aggregability was calculated from the shift of the curve as a percentage of the pretreatment value. The second fea-

ture of the clinicopharmacological test is the use of a crossover study with a definite number of healthy adult male volunteers. The selected volunteers were prohibited from taking non-steroidal anti-inflammatory drugs, especially aspirin, for 2 weeks before and after the experiment. Regrettably, a

complete crossover study was rendered impossible by a number of dropouts. In the single-administration test, volunteer I, who had no problem in Exp. 1, dropped out in Exp. 2, and accordingly volunteers K and L had to be newly adopted in Exp. 3 to balance the overall subject numbers in the various groups. In the repeated administration test, there was one dropout during the relatively long (12-day) term of observation (this case is excluded from the results). Therefore, 2 new subjects (volunteers O and P) were added to balance the subject numbers of the various groups. On administration of aspirin alone, dose-dependent anti-platelet actions were detected following the intakes of 30, 100 and 300 mg; and when aspirin was combined with 100 mg dilazep, the results suggested that the dose of aspirin could be reduced to one-third. Simultaneous measurement of changes in plasma salicylic acid levels and blood dilazep levels revealed that the combination of drugs had no influence on the pharmacokinetics of the individual drugs, suggesting that the potentiation of the anti-platelet action on combined use of the two drugs is due to a synergistic effect between them. In the repeated administration (5-day) test, the results of the single-administration test were taken as reference, and the customary t.i.d. drug administration was adopted. The t.i.d. administration of dilazep 100 mg occasioned a slight decrease in platelet aggregability during the administration period, followed by rapid recovery after cessation. On the t.i.d. administration of aspirin 10 mg, aggregability gradually decreased from day 1, reaching 40% on day 5, then recovered almost to the pretreatment value on day 3 after cessation. On combined administration of the two drugs in these doses, aggregability fell to 40% as early as day 2 after the start of administration. This effect was maintained throughout the administration period, following which aggregability showed a similar recovery to that in the respective drug alone groups. In the aspirin 30 mg t.i.d. administration group, platelet aggregability decreased to 20% after 2 days but showed no further decrease on continual administration. In the t.i.d. combined administration group of aspirin 30 mg and dilazep 100 mg, a stronger anti-platelet action was demonstrated, and aggregability of about 10% lasted from days 2 to 5 after the start of administration. The recovery of aggregability after withdrawal of medication was similar in both

groups, with aggregability returning to about 60% of the pretreatment value after 3 days and 80% after 7 days. Thus, the administration of either aspirin alone or aspirin plus dilazep resulted in a quantitative, dose-dependent decrease in platelet aggregability. On this occasion the plasma levels of TXB₂ and 6-keto-PGF_{1α} (i.e. those produced in the normal circulating blood) were determined rather than their serum levels (i.e. those produced during the process of platelet aggregation). A slight decrease was observed only in TXB₂ levels during the administration period, and no change was observed in the level of 6-keto-PGF_{1α}. This suggests that the aspirin 30mg t.i.d. oral administration for 5 days does not cause fatal inhibition of systemic arachidonic acid metabolism.²⁰⁾ In the present study we neither assayed prostaglandins in the urine nor examined PGI₂ producibility using the isolated vein. However, in the light of the results reported by P. Patrignani et al.,²⁰⁾ S.P. Hanley et al.,^{18,21)} G. Masotti et al.,²²⁾ K.K. Wu et al.,²³⁾ G. Davi et al.,²⁴⁾ and F.E. Preston et al.,⁵⁾ the dose of aspirin which inhibits only the TXB₂ producibility of the platelet without influencing PGI₂ production in the vascular wall is 30–40 mg/day. Consequently, the administration of aspirin 10 mg + dilazep 100 mg (aspirin 30 mg, dilazep 300 mg daily) is close to the ideal combination for therapy from the clinico-pharmacological standpoint. However, its evaluation as a true antithrombotic therapy may need long-term observation in various thrombotic diseases.

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