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ANTIPLATELET THERAPY USING A COMBINATION OF DILAZEP AND ASPIRIN IN PATIENTS WITH OCCLUSIVE CEREBROVASCULAR DISEASES

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INTRODUCTION

Aspirin (acetylsalicylic acid: ASA) has been used as an agent for the prophylaxis and for the treatment of various thrombotic diseases. ASA in a large dose inhibits not only platelet function but also the production of prostacyclin (PGI_2) in the vascular walls. However, recently a small dose was shown to be desirable in order to obtain a selective antiplatelet action of ASA (1-4).

In recent studies, the authors administered a small dose of ASA concurrently with dilazep dihydrochloride (DZP, Comelian, Kowa) to the patients with occlusive cerebrovascular disorders and confirmed that this therapy could sustain a satisfactory antiplatelet effect.

SUBJECTS

The subjects were selected healthy volunteers and patients hospitalized in the Department of Neurosurgery, Kuwana Hospital from February, 1983 to January, 1984. Occlusive cerebrovascular disorders were diagnosed by CT scanning and cerebral angiography.

(a) Combined administration of 100 mg ASA and 150 mg DZP was performed on three patients with occlusive cerebrovascular disorders: N. F. (51-year-old male with multiple cerebral infarction), S. Y. (66-year-old male, cerebral infarction with hemorrhagic infarction) and K. K. (74-year-old male with cerebral infarction).

(b) Combined administration of 30 mg ASA and 100 mg DZP, 3 times a day, was performed on two patients with occlusive cerebrovascular disorders: I. M (71-year-old male with right cerebral infarction), K. W. (68-year-old male with suspected right cerebral infarction), and to two healthy voluntees Y. K. and A. S. (25-year-old male and 27-year-old female).

METHODS

[1] Test methods:

(1) Using the cross over method, the sole administration of 100 mg ASA, or the combined administration of 100 mg ASA and 150 mg DZP per day was performed on three patients with cerebral infarction, and the assays for coagulation, fibrinolysis, and platelet aggregation were exmained at 0, 3, 6, 24, 48 and 72 hours after the administration.

(2) To the two patients with cerebral infarction and to the healthy volunteers, 30 mg ASA and 100 mg DZP were administered three times a day, and the assays for coagulation, fibrinolysis, platelet aggregation, plasma thromboxane B_2 (TXB_2) level, and 6-keto-prostaglandin $F_1\alpha$ (6-keto-$PGF_1\alpha$) level were performed at 0, 3 and 24 hours after the administration.

[2] Assay methods:

For coagulation: activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen (Fbg), antithrombin III (AT-III). For fibrinolysis: euglobulin lysis time (ELT), α_2-plasmin inhibitor (α_2-PI). For platelets: platelet count, platelet factor 4, platelet aggregation induced by collagen and ADP. Preparing platelet rich plasma (PRP), the platelet aggregation induced by 0.5, 1, 2 and 4 μg/ml of collagen, and by 0.5, 1, 2 and 4 μM ADP were determined by aggregometry. The maximal aggregation rate (%/min) in the collagen-induced aggregation and the aggregation area (area/5 min) in the ADP-induced aggregation were investigated. Then after drawing the aggregation reaction curve as shown in Fig. 1, the concentration of collagen which induced the maximal aggregation rate were read on the curves before (a) and after (b) administrating the antiplatelet drug. The platelet aggregation rate was calculated from the following formula: $a/b \times 100$ (%).

Miscellaneous: RBC and WBC were counted. Immediately after blood collection in an ice cooled tube including EDTA, centrifugation was perfomed at 0 °C, 3,000 rpm for 30 min, and these supernatants were used for the determination of plasma TXB_2 and 6-keto-$PGF_1\alpha$, by the radioimmunoassay method.

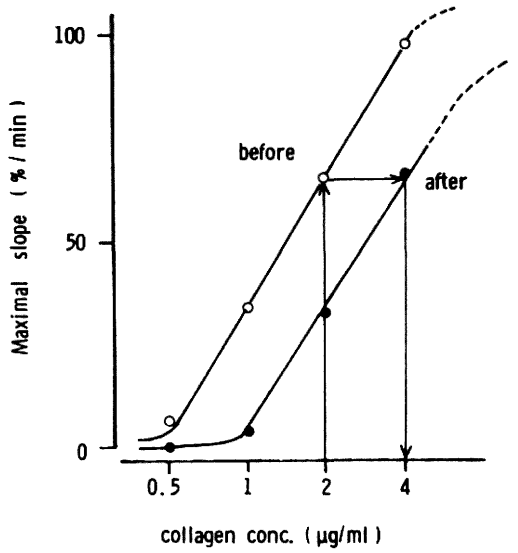

Fig. 1. Platelet aggregation reaction curves.
Platelet aggregation rate: (a)/(b) ×100 %
(a) is the concentration of collagen before administration.
(b) is the concentration of collagen after administration which induces the same maximal slope (%/min) as (a).

RESULTS

(1) The comparison of the daily administration of 100 mg ASA with the combined administration of 100 mg ASA and 150 mg DZP:

The results are shown in Table 1. When both 100 mg ASA and 150 mg DZP were administered, some shortenings were observed in aPTT at 72 hours and PT at 24 and 72 hours compared with those before the administration, but no obvious changes were observed in the others (Fig. 2).

Although no prominent changes were observed in the levels of plasminogen and α_2-PI before and after administration in both groups, significant shortenings were observed in ELT at 3 hours after the sole administration of 100 mg ASA and at 6 and 48 hours after the combined administration of 100 mg ASA and 150 mg DZP, showing an increase of fibrinolysis (Fig. 3).

Although no prominent changes were shown in the platelet count of both groups, inhibition of the platelet aggregation and a decrease of platelet factor 4 were clearly observed.

Both the sole therapy and the combined therapy showed the lowest platelet aggregation rates at 3 hours after administration. Collagen-induced aggregation was 36 % by

Table 1. Hematological findings in the cases administered with 100 mg of ASA daily, 100 mg of ASA with 150 mg of DZP daily, respectively.

	ASA 100mg					ASA 100mg + DZP 150mg					
	0	3	24	48	72	0	3	6	24	48	72hrs
a PTT sec	30.2 1.8	30.1 1.7	29.9 1.9	29.5 1.4	30.2 1.2	32.1 2.1	31.4 1.6	32.7 1.7	29.6 1.7	29.3 1.8	30.3* 1.9
PT sec	11.4 0.2	11.5 0.2	10.9** 0.2	10.9* 0.3	11.0 0.2	10.8 0.2	11.0 0.1	10.9 0.3	10.8 0.2	10.8 0.1	11.2 0.3
Fbg mg/dl	327 79	321 94	313 86	310 92	299 71	305 20	309 28	293 7	310 28	312 47	318 46
PLT $\times 10^4/mm^3$	22.4 2.4	19.0 3.6	21.4 1.4	21.4 2.7	22.6 1.5	22.6 8.6	21.2 8.7	21.3 8.3	24.9 6.5	29.7 2.0	21.9 5.6
Aggreg. (Collagen)	100	35.6** 2.5	43.5** 2.1	53.3* 5.6	72.2 2.3	100	15.7*** 2.3	26.4* 7.9	28.1* 11.1	32.0** 6.1	27.4** 4.8
〃 (ADP)	100	58.6* 7.2	86.8 11.6	95.7 14.0	99.0 15.8	100	52.3* 6.7	49.5* 6.3	61.2* 6.9	59.9* 7.1	79.1* 4.3
ELT min	446 115	155 29	428 149	403 173	378 166	453 61	340 49	341* 60	398 130	363 78	378 101
Plasminogen activator	0.55 0.02	0.58 0.02	0.57 0.09	0.56 0.08	0.54 0.04	0.31 0.02	0.31 0.10	0.23 0.04	0.28 0.03	0.30 0.06	0.30 0.02
Plasminogen mg/dl	10.3 1.2	10.1 1.3	10.7 1.0	10.5 1.1	10.5 1.1	8.9 0.5	9.0 1.2	8.5 0.7	9.3 1.1	9.9 0.7	9.7 1.2
α_2-Pl %	93.3 9.3	85.0 7.9	91.0 7.2	91.0 6.0	88.0 6.1	90.0 15.3	93.3 11.0	90.0 7.5	93.0 6.0	94.7 8.5	95.3 10.5
AT-III mg/dl	22.0 1.0	22.0 1.5	23.0 0.6	24.0 0.6	22.7 0.7	23.0 1.7	22.0 0.6	22.0 1.2	23.0 0.0	23.0 0.0	22.3 0.9
〃 %	102.0 4.5	96.0 4.9	98.7 0.9	99.7 3.2	96.3 5.2	97.7 5.8	92.7 1.8	90.7 3.2	97.0 1.5	96.0 1.0	94.0 4.0
Prekallik U/ml	0.42 0.06	0.41 0.05	0.43 0.05	0.43 0.05	0.44 0.06	0.41 0.06	0.43 0.05	0.39 0.04	0.41 0.06	0.40 0.06	0.42 0.06
WBC $\times 10^3/mm^3$	5.70 0.67	5.97 0.62	5.83 0.73	5.67 0.70	5.73 0.47	7.17 0.38	8.40 0.91	6.87 0.32	6.60 0.46	6.73 0.42	6.77 1.28
RBC $\times 10^6/mm^3$	3.89 0.38	3.71* 0.41	3.68 0.33	3.64** 0.35	3.66 0.27	3.97 0.49	4.06 0.51	3.85 0.39	4.02 0.39	3.79 0.43	3.90 0.38

Upper line: Mean value,
Lower line: Standard error
t Test comparing with values before administration: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

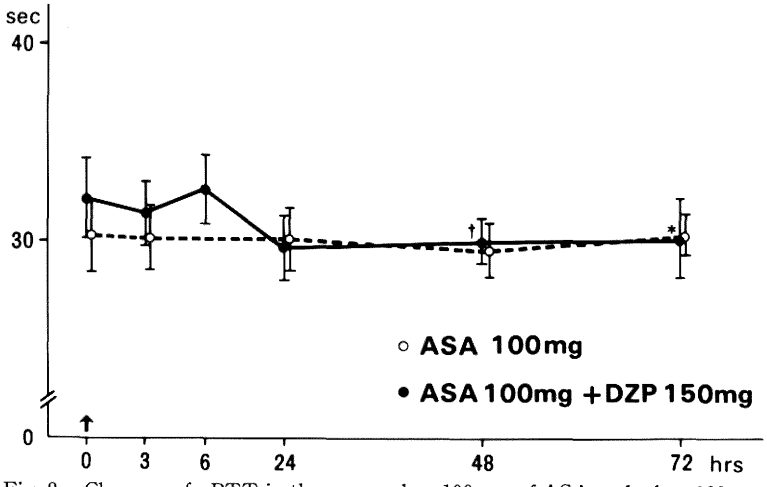

Fig. 2. Changes of aPTT in the cases when 100 mg of ASA and when 100 mg of ASA and 150 mg of DZP were adminstered, respectively.
†: $p<0.10$, *: $p<0.05$

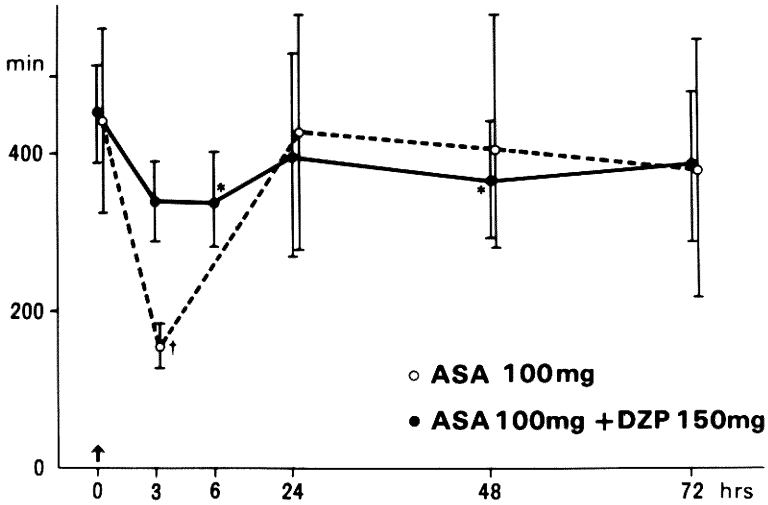

Fig. 3. Changes in euglobulin lysis time in the cases when 100 mg of ASA and when 100 mg of ASA and 150 mg of DZP were administered, respectively.
†: $p<0.10$, *: $p<0.05$

sole therapy and 16 % by combined therapy, and ADP induced a state of gradual recovery at 6, 24, 48 and 72 hours after administration by sole therapy, but the figures by the combined therapy remained close to the lowest rate, indicating a delayed recovery. From these results, it was found that the combined therapy produced a stronger and longer lasting antiplatelet effect than the sole therapy and the aggregation induced by collagen was more inhibited than that by ADP in both groups (Fig. 4).

Fig. 5 shows a case in which the influences of platelet factor 4 were examined. The decrease of platelet factor 4 was observed at 3 hours after the administration, the level of decrease was more remarkable in the combined therapy than in the sole therapy. From these results, it was suggested that the inhibition of the secondary platelet aggregation would be occurred in these experiments. Simultaneously TXB_2 values clearly decreased at 3 and 5 hours after administration in both groups, and 24 hours later they tended to recover (Fig. 6).

(2) Study on the administration of 30 mg ASA and 100 mg DZP, 3 times a day:

From the results of (1), it was found that the antiplatelet effect of ASA with DZP was evident, and the dose of ASA might be reduced in the cases when it was combined with DZP. Therefore, as shown below, the antiplatelet effect after the administration of 30 mg ASA with 100 mg DZP 3 times a day was examined in 4 cases; 2 patients with cerebral infarction and 2 healthy volunteers, at 24 hours after the administration.

Table 2 shows the findings of these cases. No changes were found in the coagulation studies after the administration, but in regard to fibrinolysis, a shortening of ELT at 3 hours after administration was found. Platelet aggregation rates were reduced at 3 and

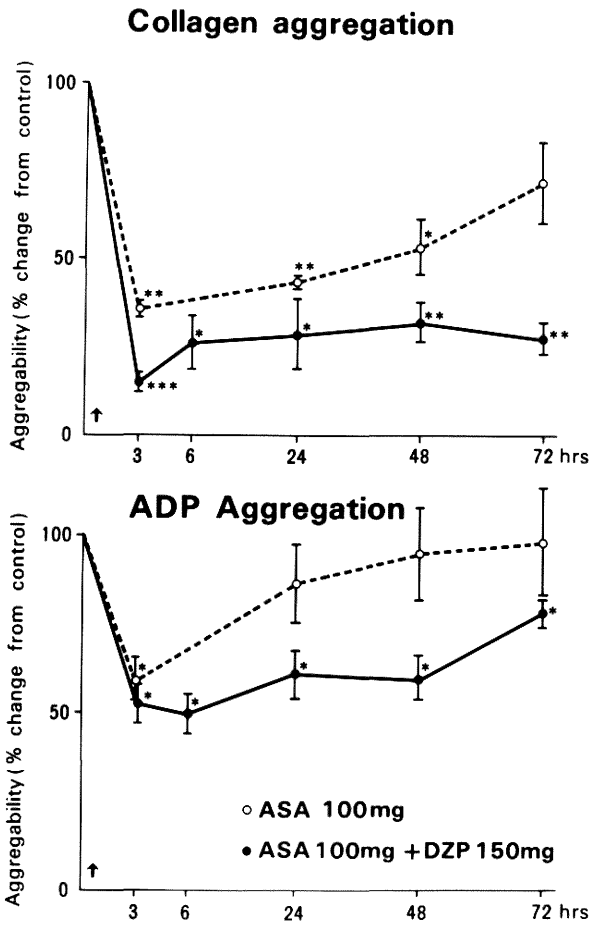

Fig. 4. Changes in platelet aggregation using collagen and ADP when 100 mg of ASA, or 100 mg of ASA and 150 mg of DZP was administered.
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

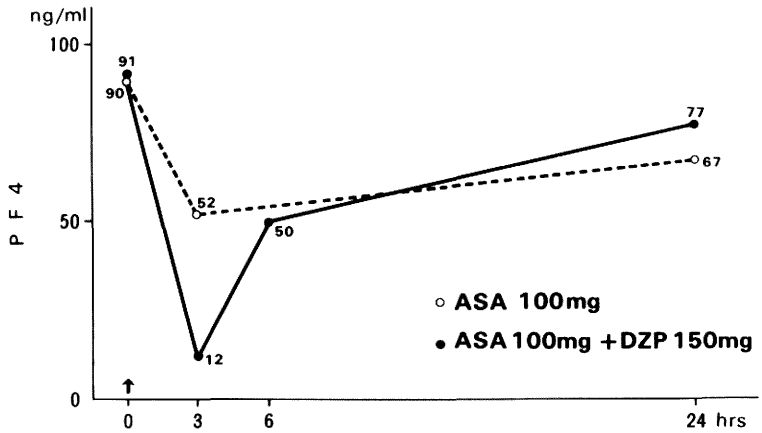

Fig. 5. Changes of platelet factor 4 when 100 mg of ASA, or 100 mg of ASA and 150 was administered to the patient with cerebral infarction, 74-year-old male.

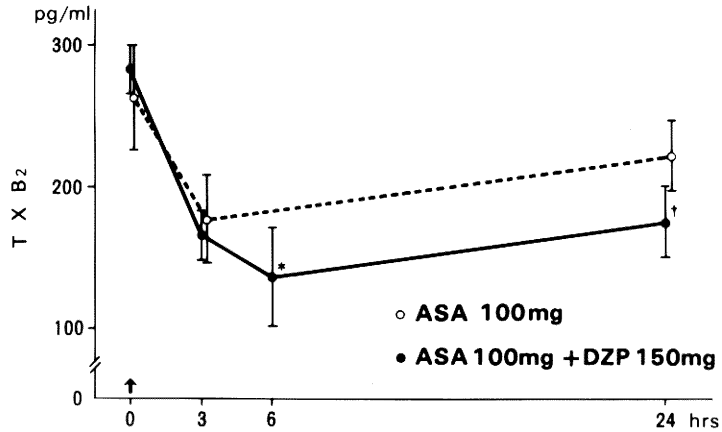

Fig. 6. Changes of thromboxane B_2 when 100 mg of ASA, or 100 mg ASA and 150 mg of DZP was administered to the patient with cerebral infarction, 74-year-old male.
†: $p<0.10$, *: $p<0.05$

Table 2. Hematological findings in the cases administered with 30 mg of ASA with 100 mg of DZP 3 times a day.

		N	ASA 30mg + DZP 100mg × 3/day		
			0	3	24 hrs
a-PTT	sec	4	35.3 ± 2.1※	34.6 ± 2.2※	35.6 ± 2.4※
PT	%	4	104 ± 7	103 ± 8	97 ± 14
Fbg	mg/dl	4	235 ± 38	224 ± 34	207 ± 26
PLT	$\times 10^4/mm^3$	4	26.1 ± 11.3	25.9 ± 13.1	27.3 ± 9.6
Aggreg.	(Collagen)	4	100	73† ± 7	37† ± 16
〃	(ADP)	4	100	81† ± 6	63† ± 9
TXB_2	pg/ml	4	237 ± 82	251 ± 35	180 ± 40
6-keto $PGF_{1\alpha}$	pg/ml	4	41 ± 7	35 ± 2	43 ± 2
ELT	min	4	443 ± 102	258 ± 30	448 ± 74
Plasminogen	mg/dl	4	10.5 ± 0.5	10.3 ± 0.5	10.0 ± 0.6
α_2-PI	%	4	96.8 ± 8.1	93.0 ± 7.7	96.0 ± 5.4
AT-III	mg/dl	4	28.8 ± 1.4	28.5 ± 0.9	27.8 ± 1.1
〃	%	4	113.3 ± 3.0	112.5 ± 2.7	108.3 ± 3.9

t Test comparing with values before administration:
†: $p<0.10$, ※mean±SD

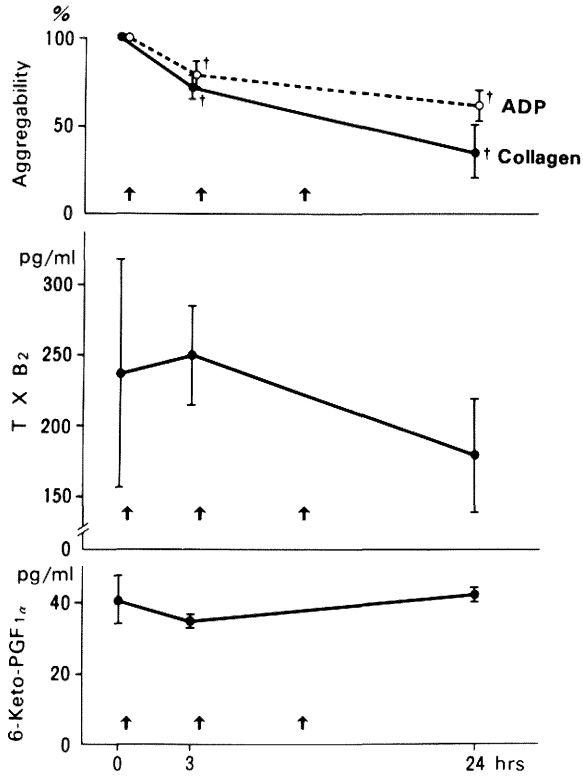

Fig. 7. Changes of platelet aggregability, thromboxane B_2, and 6-keto-$PGF_1\alpha$ in the case of combined administration of 30 mg ASA with 100 mg DZP, three times a day to the two patients with cerebral infarction and two healthy volunteers.
†: $p<0.10$

24 hours after the administration in the cases of platelet aggregation induced by collagen (73 and 37 %) and ADP (81 and 63 %) (Fig. 7). The plasma TXB_2 value was reduced at 24 hours, but no siginificant change was observed in 6-keto-$PGF_1\alpha$, compared with its value before administration.

Discussion

When using ASA in a variety of thromboses, the high dosage not only produces antiplatelet effects by inhibition of cyclooxygenase but inhibits the production of PGI_2 in the vessel walls. Lately, a low dosage of ASA which produces an antiplatelet effect without influencing the production of PGI_2 has been recommended in order to obtain a suitable antiplatelet effect. Frequent gastrointestinal disorders have been reported when using 600 mg ASA a day. Thus, from the viewpoint of gastrointestinal disturbance, a lower dosage of ASA is recommended. In recent years, a daily 300 mg dosage or the intermittent administration of low dose has been recommended (5, 6). DZP, which is

used in treating cerebrovascular disorders and angina pectoris, has a vasodilating action due to an adenosine potentiating action (7) and a moderate Ca^{2+} antagonistic action (8). The authors already reported on the antiplatelet and fibrinolytic action of DZP in vitro and in vivo (9, 10). These results have also been confirmed by various reseachers (11-16).

The mechanism of this antiplatelet effect of DZP is due to a platelet membrane stabilizing action, differing from that of ASA, and has been already clarified through animal experiments by Nakajima et al (17, 18). They reported in that the combined use of ASA and DZP revealed a strong antiplatelet action on healthy volunteers after oral administration. On the patients with occlusive cerebrovascular disorders, we performed a study clinical pharmacologically on the combined effects of both drugs. As a result in this study, the combined therapy of ASA and DZP produced stronger antiplatelet effects than the sole ASA therapy. In addition to these results, fibrinolysis was observed without influencing coagulation significantly.

When the dose of ASA was further reduced to 30 mg with 100 mg DZP, the TXB_2 value was decreased, but no change was observed in the value of plasma 6-keto $PGF_1\alpha$. It is necessary to make further studies in order to determine the optimum dose.

SUMMARY

The authors investigated the antiplatelet effects of the therapy combining DZP and ASA in five patients with occlusive cerebrovascular disorders and two normal subjects. The results were as follows:

(1) The combination therapy of 100 mg ASA and 150 mg DZP showed stronger antiplatelet effects as compared with the sole therapy of 100 mg ASA, and when 30 mg ASA with 100 mg DZP were used 3 times per day, thoroughly satisfactory antiplatelet effects were also obtained.

(2) An effective antithrombotic therapy using the combination of 30 mg ASA and 100 mg DZP, 3 times per day for patients with cerebral infarction and transient ischemic attack can be expected.

(3) By reducing the ASA dose, we can avoid gastrointestinal disorders.

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