ROLE OF PLATELETS AS A FACTOR AGGRAVATING CEREBRAL ISCHEMIA

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In order to clarify the role of platelets as a factor aggravating cerebral ischemia, an experimental model of ischemia was investigated using thrombocytopenic rats. In addition, the prostacyclin derivative (OP-41483) and the thromboxane A_2 synthetase inhibitor (OKY-046), both of which inhibit platelet aggregation, were tested for possible beneficial effects on cerebral ischemia.

Cerebral ischemia was produced in spontaneously hypertensive male rats using bilateral common carotid artery ligation (BLCL). Thrombocytopenia was produced with an antiplatelet antiserum which reduced the platelet count to less than $6 \times 10^4/\mu\text{l}$ by 24 h. OP-41483 was administered four times hourly ($500\text{ng/kg} \times 4$, i.p.), beginning 1h prior to BLCL. Similarly, OKY-046 was injected four times hourly ($10\text{mg/kg} \times 4$, i.p.). Brain metabolites such as ATP, lactate and pyruvate and water content were determined after 3h of cerebral ischemia.

Brain levels of ATP in the ischemic rats with thrombocytopenia were higher than those of the ischemic rats without thrombocytopenia. In addition, thrombocytopenia reduced the increase of lactate and water content in the ischemic brain. Animals treated with OP-41483 also maintained higher levels of ATP and lower levels of lactate and water compared to animals given a vehicle.

OKY-046 significantly reduced brain water content, but had no effect on the ischemic alteration of brain metabolite levels.

These results indicate that platelets play an important role in the progression of metabolic change during ischemia.

IT is well known that unsaturated fatty acids, especially arachidonic acid (AA), are liberated from ischemic brain tissue^{1,2} and that specific prostaglandins are synthesized in different organs³. Thromboxane A_2 (TXA₂), a prostaglandin which is mainly synthesized in platelets, has potent

platelet aggregatory and vasoconstrictive actions^{4,5}. Therefore, platelets might play an important role in aggravating cerebral ischemia in the acute stage.

In order to clarify the role of platelets as a factor in aggravating cerebral ischemia, we produced thrombocytopenia in a model of cerebral ischemia and measured the resulting effects on brain metabolites and water content. In addition, a prostacyclin derivative (OP-41483) and a TXA₂ synthetase inhibitor (OKY-046), both of which inhibit platelet aggregation, were tested for

Key words:

Thrombocytopenia, Cerebral ischemia
PGI₂ derivative (OP-41483)
Thromboxane A_2 synthetase inhibitor
(OKY-046)
Brain metabolites

(Received July 3, 1989; accepted February 19, 1990)

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TABLE 1 THROMBOCYTE COUNT 24h AFTER INJECTION OF ANTISERUM TO PLATELETS

<i>Antiserum-treated rats</i>	<i>Saline-treated rats</i>
$58 \times 10^3 / \mu\text{l}$	$474 \times 10^3 / \mu\text{l}$
53	577
44	540
51	550
46	578
53	427
40	529
$49.3 \pm 5.7 \times 10^3 / \mu\text{l}$	$525.0 \pm 51.5 \times 10^3 / \mu\text{l}$

possible beneficial effects on metabolite levels and water content during cerebral ischemia.

MATERIALS AND METHODS

Sixteen-week-old spontaneously hypertensive male rats (SHR), having a blood pressure of approximately 180mmHg, were anesthetized with 2% fluothane, and the body temperature kept at about 37°C using a heating pad. Both common carotid arteries were exposed through a neck incision and occluded with commercial cotton thread to induce cerebral ischemia.

1. Preparation of cerebral ischemic model associated with thrombocytopenia

An antiserum to rat platelets was prepared in rabbits according to the method of Ebbe et al with modification^{6,7}. A suspension of rat platelets in saline ($5-10 \times 10^9$ platelets) was intravenously injected into rabbits twice a week for 2 weeks. Sixteen days after the final injection, the rabbits were sacrificed and the rabbit serum was collected. The serum was heated at 56°C for 45min then absorbed three times with an equal volume of red blood cells. The antiserum was stored at -20°C until use. Rats were injected intravenously with antiserum (0.07ml/100g body weight), which produced severe thrombocytopenia by 24h. Blood was collected from the femoral artery and the platelets counted with a commercial counter (Sysmex PL 100, Toua-Iyodenshi KK). Injection of the antiserum did not affect the levels of red blood cells and leukocytes. Cerebral ischemia was produced in thrombocytopenic animals 24h after

injection of the antiserum and in control animals given the same volume of saline. There were 7 animals in each group.

2. Administration of prostacyclin derivative (OP-41483) and TX A₂ synthetase inhibitor (OKY-046)

OP-41483 (5 (E) 6, 9-Deoxa-6, 9-methylene-15-cyclopentyl-16, 17, 18, 19, 20-pentano-PGI₂· α -cyclodextrin clathrate, Dainippon Seiyaku/Ono Yakuhin) was administered intraperitoneally four times hourly (500ng/kg) beginning 1h prior to occlusion^{8,9}. Control animals were given the same amount of saline in the same manner. Six animals were used for the OP-41483 treated group and 7 for controls. OKY-046 (Sodium (E)-3 [4-(1-imidazolylmethyl)phenyl]-2-propionate, Ono Yakuhin/Kittsei Yakuhin) is an imidazole derivative which inhibits thromboxane A₂ synthetase^{10,11}. OKY-046 was dissolved in saline and administered intraperitoneally four times hourly (10mg/kg) beginning 1h prior to occlusion. Control animals were given saline instead of OKY-046. There were 6 animals in each group.

3. Measurement of brain water content and metabolites

After 3h of ischemia, animals were sacrificed by microwave irradiation (Toshiba Microwave Applicator, 5kw output for 1.5 sec). The forebrain was used for measurement of water content, and the hindbrain was analyzed for ATP, lactate and pyruvate.

Water content was measured using the vacuum freeze-dry method. ATP was analyzed with luciferin-luciferase reaction using Fire Lantern Extract (Sigma Chemical), while lactate and pyruvate were mea-

TABLE II EFFECT OF THROMBOCYTOPENIA, OP-41483 AND OKY-046 ON WATER CONTENTS AND METABOLITES IN ISCHEMIC BRAIN

	Water Content	Brain Metabolites		
		ATP	lactate	pyruvate
Sham-operated controls	78.09 ± 0.31	2.64 ± 0.11	1.38 ± 0.28	0.36 ± 0.05
Thrombocytopenic rats	78.04 ± 0.52	0.75 ± 0.39	8.9 ± 4.9	0.20 ± 0.10
	$p < 0.05$	$p < 0.05$	$p < 0.001$	NS
ischemic controls	78.77 ± 0.58	0.33 ± 0.16	27.3 ± 10.1	0.22 ± 0.11
OP-41483	78.87 ± 0.67	0.51 ± 0.24	14.9 ± 9.1	0.34 ± 0.08
	$p < 0.05$	$p < 0.05$	$p < 0.05$	NS
ischemic controls	79.72 ± 0.69	0.19 ± 0.12	26.3 ± 6.9	0.29 ± 0.11
OKY-046	78.57 ± 0.85	0.41 ± 0.27	20.2 ± 12.5	0.30 ± 0.15
	$p < 0.05$	NS	NS	NS
ischemic controls	79.60 ± 0.41	0.29 ± 0.12	22.3 ± 6.6	0.24 ± 0.05

Values are mean ± S.D.. Water content is indicated as percent.

ATP, lactate and pyruvate are indicated as $\mu\text{mol/g}$. NS: no significance.

sured spectrophotometrically using enzymatic methods.

Six sham-operated SHR were also sacrificed in the same manner and brain water content and metabolites were determined.

RESULTS

1. Ischemic thrombocytopenic rats

The rats administered antiserum became severely thrombocytopenic. The platelet count in thrombocytopenic rats was less than 10% that of the controls (Table I).

After 3h of ischemia, thrombocytopenic rats maintained higher levels of ATP and lower levels of lactate compared to ischemic controls (Table II). While there was little difference in the levels of pyruvate between the groups, the ratio of lactate/pyruvate in the thrombocytopenic rats was significantly lower than the ischemic controls (62 ± 50 vs 155 ± 82 , $p < 0.05$). The water content of the brain in thrombocytopenic rats was also significantly lower than the ischemic controls (Table II).

2. Administration of OP-41483 and OKY-046

Treatment with OP-41483 maintained ATP at significantly higher levels and reduced the accumulation of lactate, relative to the ischemic controls (Table II). Again,

there was little difference in the pyruvate levels between groups. The lactate/pyruvate ratio was lower in the OP-41483 treated group, but the difference was not statistically significant (50 ± 39 vs 107 ± 60). Brain water content was significantly reduced in OP-41483 treated animals compared to that of the ischemic controls (Table II). Administration of OKY-046 had no effect on the levels of ATP, lactate, or pyruvate (Table II). The ratio of lactate/pyruvate also did not differ significantly between groups. However, the brain water content in animals treated with OKY-046 was significantly lower than that in the ischemic controls (Table II).

DISCUSSION

SHR were employed to induce cerebral ischemia in this study. It is well known that SHR are suitable to induce global incomplete cerebral ischemia without lowering blood pressure of the rats^{12,13}. After a 3h occlusion of the bilateral common carotid arteries, cerebral blood flow reduction, metabolic disruption and edema occur in the rats. In this study, in the sham-operated SHR, the brain water content was $78.09 \pm 0.31\%$ (mean ± S.D.), the concentration of ATP was $2.64 \pm 0.11 \mu\text{mol/g}$, lactate $1.38 \pm 0.28 \mu\text{mol/g}$ and pyruvate 0.36 ± 0.05

μ mol/g. In comparison, the ligated SHR controls which were given saline showed a marked increase of water content and lactate levels. They also revealed a significant reduction of ATP and pyruvate levels as the values in Table II show.

There are several indications that platelets play an important role in aggravating cerebral ischemia¹⁴⁻¹⁷ as well as in precipitating cerebral ischemia^{18,19}. It has also been reported that injection of platelet-aggregating substances such as arachidonic acid and ADP into the internal carotid artery causes cerebral ischemia and increases permeability of the vessels^{16,17}. There is also evidence that platelets are implicated in development of cerebral ischemia. The activated form of platelets was reported to increase during the acute phase of cerebral ischemia¹⁵. Platelet aggregation during ischemia has been observed²⁰ and a reduction of platelets in the blood during ischemia has also been reported²¹. These findings suggest that substances released from aggregated platelets might aggravate cerebral ischemia in its acute phase.

Our initial objective was to determine whether thrombocytopenia affected the degree of ischemic alteration. Thrombocytopenia reduced the decrease in ATP and the increase of lactate, lactate/pyruvate ratio, and the water content during the 3h of ischemia. Thus, the degree of ischemic alteration was significantly reduced in thrombocytopenic rats. These results suggest that platelets may adhere to the vessels and disturb the blood circulation. In addition, aggregated platelets may discharge vasoactive substances²²⁻²⁴ which further compromise the cerebral microcirculation and enhance brain edema. These events should be markedly diminished in thrombocytopenic rats.

Secondly, two different kinds of antiaggregatory agents were administered in order to prevent aggregation of platelets during ischemia. OP-41483, a PG-I₂ derivative, showed remarkable amelioration of metabolic derangement and edema. On the other hand, OKY-046, a TXA₂ synthetase inhibitor, reduced brain edema, but had no effect on metabolites. These results indicate that antiplatelet therapy in the acute phase of ischemia may reduce the

impairment of cerebral metabolism and edema. Thus, prevention of platelet aggregation and suppression of the release of various vasoactive substances, such as serotonin, prostaglandin endoperoxides and TXA₂ from aggregated platelets might be beneficial during cerebral ischemia^{4,5,24}. Serotonin is known to increase vascular permeability²⁵. Prostaglandin endoperoxides are known to be vasoconstrictors. TXA₂, which is synthesized in the platelets, is also a vasoconstrictor and causes platelet aggregation. Cerebral ischemia liberates free fatty acids, including AA, which is the substrate for the synthesis of prostaglandins^{1,2}. Disproportionate synthesis of TXA₂ relative to PG I₂ has been reported during and after ischemia^{26,27}.

Therefore, it is possible that the ischemic increase of AA might further enhance platelet aggregation in the vessels. Vasoactive substances that injure the vascular endothelium may also aggravate ischemia^{17,19}. Damaged endothelium may promote platelet adhesion to the vessels, thus impairing the microcirculation. Brain edema may also be aggravated by damaged endothelium.

The difference between the effectiveness of OP-41483 and OKY-046 in this study may be due either to a difference in their mechanisms of action or in the dosages used. OP-41483, a PG I₂ derivative, has potent activities, one-third the antiaggregatory action and vasodilatory activity of PG I₂^{8,9}. In contrast, OKY-046, the TXA₂ synthetase inhibitor, also has potent antiaggregatory activity, but for the dose used in the present study, no vasodilatory activity was produced^{28,29}. The choice of the dosages of these agents was based on the papers that showed inhibition of platelet aggregation^{8-11,28,29}.

In summary, these experiments demonstrate that platelets play an important role in aggravating cerebral ischemia and that antiplatelet treatment with OP-41483 and OKY-046 are beneficial during cerebral ischemia.

Acknowledgment

We thank F.A. Welsh, Ph.D., Division of Neurosurgery, University of Pennsylvania for support of this paper. This work was supported by 14th

KANAEIYAKU Grant. The agents tested were supplied by ONO Pharmaceutical Company.

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