A Study on the Changes of Platelet Aggregability Due to Ischemia*

Takemichi KANAZAWA**, Masahiro IZAWA**, Hirohiko KANEKO**,
Tsugumichi UEMURA**, Yoshiyuki KONTA**, Kogo ONODERA**,
Hirobumi METOKI***, Chusei NIHEI***, Osamu KOMATSU***
and Yasaburo OIKE***

Platelet aggregability in ischemic diseases such as myocardial infarction and cerebrovascular diseases has been reported¹⁻³⁾ numerously in the past. According to these reports, the platelet aggregability is higher in patients with arteriosclerotic diseases than in healthy persons. We also had previously published^{4,5)} that platelet aggregabilities increase in patients with those diseases.

To clarify the mechanisms of the increasing platelet aggregabilities in ischemic diseases, we had investigated whether ischemia, namely, lack of oxygen, increased or decreased platelet aggregability.

Experimental Method

1) Platelet aggregability

Platelet aggregabilities induced by ADP (2 μ M/l as final concentration), collagen (1 μ g/ml), and epinephrine (1 μ g/ml) were measured by the formal method which has been presented in our previous reports.^{4,5)}

The aggregability was expressed in maximum platelet aggregabilities (MPA) calculated from aggregation profiles.

2) MPA before and after oxygen (O₂) inhalation

MPAs before and after O₂ inhalation were estimated in 6 patients with 2 strokes, 2 old myocardial infarctions, 1 cor pulmonale and 1 hypertensive. They received O₂ of 2 l/min via nasal canal for dyspnea. Their blood was drawn twice before O₂ inhalation, and 60 minutes after an inhalation.

3) MPA of venous and arterial blood

Nine ml of blood from the cubital vein and the femoral artery of 6 patients with 3 cerebral thrombosis, 2 ischemic heart diseases, and 1 hypertensive was drawn simultaneously into two syringes with 1 ml of 3.8% sodium citrate. The platelet rich plasmas (PRP)⁶⁾ of venous and arterial blood were separated and MPAs were estimated.

4) Ischemic state by ligation

The upper arms in 10 healthy subjects were ligated with systolic blood pressure plus a pressure of another 20 mmHg for 5 minutes. Blood was drawn twice before the ligations and 5 minutes after ligations from their cubital veins in the side of their ligated arms.

5) Oxygen (O₂), nitrogen (N₂), and carbon monoxide (CO) bubblings

The above gases were bubbled into the PRP⁶⁾ at the rate of two bubbles per second. The bubbling condition was crucial here because the rate over 2 bubbles per second created many bubbles in the tubes which disturbed the estimation of the platelet aggregability. The subjects were 8 healthy persons.

Therefore, a specific gas controller was used to maintain this rate. The gases were purchased from Seikagaku Industrial Company for the gas chromatography.

Experimental Results

1) The effect of O₂ inhalation on MPA

Compared with MPAs before O₂ inhalation of 2 l/min for 60 minutes, the MPAs induced by ADP and epinephrine were after O₂ inhalation suppressed (as shown in Fig. 1). A decreasing trend was recognized after the O₂ inhalation in collagen induced MPA.

2) Comparison of MPAs between venous and arterial blood

MPAs showed a higher trend in the platelet

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** Second Department of Internal Medicine, Hiro-saki University School of Medicine, Hiro-saki, Japan

*** Reimeikyo Rehabilitation Hospital

Fig. 1 The effect of O₂ inhalation (2 l/min 60 min) on the platelet aggregation.

Fig. 2 Comparison of the platelet aggregation between arterial PRP and venous PRP.

aggregability in the venous blood than in the arterial blood (in the aggregants of ADP and epinephrine). But in collagen induced MPA, there were no differences (as shown in Fig. 2).

3) The influence of anoxia to MPA by ligation

As shown in Fig. 3 and Table 1; ADP, collagen, and epinephrine induced MPA before ligation were clearly suppressed in post ligation. The profiles of the platelet aggregations were mentioned in Fig. 3.

4) The effects of ADP induced MPA by O₂, N₂, and CO bubblings

a) O₂

As shown in Figs. 4 and 5, the MPA increased after the 30 sec and 60 sec O₂ bubblings. But the MPA did not show an increase after the O₂ bubbl-

Table 1 Comparison of platelet aggregability between before and after ligation on arm

Healthy Persons		
Aggregants	Before (%)	After (%)
ADP 1 μM/l	28.4 ± 10.9	16.5 ± 11.1 *
	69.3 ± 18.9	53.3 ± 30.9
Collagen 0.5 μg/ml	29.8 ± 10.9	18.7 ± 7.4 *
	70.6 ± 13.0	60.8 ± 19.1
Epinephrine 1 μg/ml	28.4 ± 10.9	16.5 ± 11.1 *
	69.3 ± 18.9	53.3 ± 15.6

N = 10, *p < 0.05, Mean ± SD.

Left arm was ligated by the pressure with maximum blood pressure plus 20 mmHg for 5 minutes.

Fig. 3 Profiles of platelet aggregation by each aggregants before and after ligation on arm.

Fig. 4 O₂-bubbling.

ing of over 2 minutes to PRP.

b) N₂

As shown in Figs. 6 and 7, the MPA did not show any clear change until after 5 minutes.

c) CO

As shown in Figs. 8 and 9, the MPA (pre-CO bubblings) decreased tremendously after the bubblings. The MPA after the 30 sec CO bubblings were the most suppressed.

Discussion

It has been often reported⁷⁻¹⁰⁾ that the MPA in patients with ischemic diseases such as myocardial infarction, cerebral thrombosis, and arteriosclerosis obliterance, increases more compared to the MPA of healthy persons. We have observed^{4,5)} that the MPA also increases in ischemic heart diseases and cerebrovascular diseases. Although the mechanism

Fig. 5 The effect on ADP ($2 \mu M/l$) induced platelet aggregability by O_2 gas bubbling.

Fig. 6 N_2 -bubbling.

is unclear, from these results, it was presumed that the deficiency of O_2 in the tissue would make the MPA increase.

Parts of the results of this experiment supports

the above speculation. Namely, the MPAs in the venous blood showed a higher trend compared the MPAs in the arterial blood, and the MPAs after the O_2 inhalation were suppressed compared to the

Fig. 7 The effect on ADP ($2\text{ }\mu\text{M/l}$) induced platelet aggregability by N_2 gas bubbling.

Fig. 8 CO -bubbling.

MPAs before the O_2 inhalation.

On the other hand, the ischemia caused by arm ligations decreased the MPAs compared to the MPAs of preligation. These results contradict the previous explanations. But the following explanations will allow us to make a reasonable interpretation of the results.

In the chronic ischemic state, the MPA increased is caused by the movements of the platelets through the sclerotic vessel or by the deficiency of O_2 for a long time. But in the acute ischemic state, the MPA is suppressed because by inhibiting platelet aggregability the homeostasis is kept. We were not able to find reports concerning those experiments.

Fig. 9 The effect on ADP ($2 \mu\text{M/l}$) induced platelet aggregability by CO gas bubbling.

In vitro, although if the ischemic state is kept for the PRP by passing the N_2 gas, the MPA does not significantly change. Thus, it suggests that the ischemic state in vivo causes various pathophysiology to the platelet aggregation.

In our other experiment, the O_2 gas bubblings increased the MPA markedly. Although we cannot explain this mechanism, a possible explanation is that the O_2 has radical electrons¹¹⁻¹³⁾ which might excite the aggregation of the PRP. Furthermore, O_2 also various unstable electrons such as the superoxide anion¹⁴⁻¹⁶⁾. These unstable electrons react with various lipids in the body, and they change the lipids to peroxides.¹⁷⁾ Consequently, these peroxides (for example, various prostanoids) might be the cause of the MPA increase.

The CO suppressed the MPA tremendously. As seen in other articles it is known that the CO combines with the P450 proteins¹⁸⁾ and together inhibit the electron transfer system. Therefore, it is speculated that the electron transfer system plays an important role in the development of the MPA. (Cinti¹⁹⁾ also reports that the CO inhibits collagen induced MPA.)

From these experiments we conclude that: if the electron transfer system is not kept in a normal condition for the chronic anoxia, the aggregation of PRP will be enhanced, but in an acute ischemic state, its normal electron transfer system will react for the regulation of platelet aggregation, namely as a suppression of the MPA. Finally, the complete disturbance of the electron transfer system will inhibit the platelet aggregation because of the lack of energy.

These results are very important in understanding the mechanisms of the platelet aggregation in ischemic diseases.

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Summary

To clarify the mechanism of increasing platelet aggregation in ischemic diseases, the following experiments were carried out.

1) Maximum platelet aggregability (MPA) before and after O₂ inhalation were estimated in 6 patients (2 a victim of strokes, 2 a victim of old myocardial infarctions, and 1 a victim of cor pulmonale, and 1 a victim of hypertensive) who were given O₂ of 2 liter/min via nasal canal for dyspnea. After the O₂ inhalation of 60 minutes, the ADP and epinephrine induced MPAs were suppressed compared to the MPAs before the inhalation. In the collagen induced MPAs of the same above condition, a decreasing trend was recognized.

2) The ADP induced and the epinephrine induced MPAs in the venous blood showed higher trends compared to the same two kinds of MPAs in the arterial blood, but in the collagen induced

MPA, there were no differences found (between the venous and the arterial blood). MPAs were measured in 6 patients; 3 of whom were victims of cerebral thrombosis, 2 of whom were victims of ischemic heart diseases, and 1 who suffered from hypertensive.

3) The MPAs in the ischemic blood (by arm ligation) were defined in 10 healthy persons. After the 5 minute ligations, the MPAs were suppressed.

4) After the O₂ bubblings, the MPAs in 8 healthy persons increased.

After the N₂ bubblings the MPAs did not change significantly, but after the CO bubblings, the MPAs decreased markedly.

Key words: Platelet aggregation in venous and arterial blood, O₂, N₂ and CO bubbling to PRP, arm ligation (ischemia) and platelet aggregation, O₂ inhalation and platelet aggregation.