◆原 著◆

Thrombotic Tendency of Spontaneously Hypertensive Rats Measured by the He-Ne Laser-induced Thrombus Formation Method

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He-Ne レーザー惹起血栓形成法で測定した
自然発症性高血圧ラットの血栓形成傾向

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自然発症性高血圧ラット (SHR) とその対照ラット Wistar Kyoto (WKY) の血栓形成傾向と血小板凝集能を、それぞれ He-Ne レーザー惹起血栓形成法と全血凝集能測定法で測定した。閉塞性血栓形成に要する照射回数は SHR の方が WKY より小さく、全血凝集能は SHR の方が WKY より亢進を示した。以上の結果から、SHR の易血栓形成傾向の原因の一つとして血小板凝集能亢進の関与が考えられた。

Abstract

This experiment measured the thrombotic tendency and platelet aggregability of a spontaneously hypertensive rat (SHR) and its control, Wistar Kyoto (WKY), using the He-Ne laser-induced thrombus formation method and the whole blood aggregation method, respectively. The two findings of this

experiment were the number of irradiations required to induce occlusive thrombus formation was smaller in SHR than in WKY and platelet aggregability in SHR was higher than in WKY. These results suggest that higher thrombotic tendency may be partly due to the higher platelet aggregability in SHR.

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Introduction

Thrombus formation has been well known to play an important role in the initiation and development of cerebral and cardiovascular diseases. It is therefore necessary to develop a more practical and useful method of detecting the thrombotic tendency to aid in the prevention and medical treatment of these diseases, although various tests for platelet aggregability and activities of the coagulation and fibrinolytic systems are currently in use¹⁾ and various animal models inducing thrombosis experimentally also have been proposed^{2)~15)}.

Though there are many factors contributing to thrombosis formation, the platelet thrombus formation is probably the initial event. Therefore, measurement of the tendency to develop platelet thrombus formations is a reasonable approach for determining the thrombotic tendency. The laser-induced thrombus formation method which induces thrombus formation in the microvessels is suitable for the purpose of determining the thrombotic tendency.

It is known that the thrombotic tendency is higher in patients with hypertension as well as in SHR developed by Okamoto and Aoki¹⁶⁾ and widely used as an animal model of essential hypertension. The laser-induced thrombus formation method has shown that the thrombotic tendency is higher in SHR than in WKY¹⁷⁾¹⁸⁾. On the other hand, it is also reported that the thrombotic tendency and platelet aggregability are lower in SHR than in WKY^{19)~21)}. The aim of the present study is to gain evidence indicating relationship between the thrombotic tendency and platelet aggregability of SHR.

Materials and Methods

The male SHR and its control rat (WKY)

at 10 weeks old were obtained from the Charles River Co. Ltd. (Japan). They were fed with a commercial solid diet (MF, Oriental Yeast Co. Ltd., Japan) and tap water ad libitum.

Experimental microthrombus formation by He-Ne laser irradiation

The experiment was performed when the rats were at 25 weeks old. The microthrombus was formed using the method described by Kovacs et al.⁴⁾⁵⁾, with slight modifications. The details are described elsewhere¹⁵⁾.

Platelet aggregation

Platelet aggregation in whole blood was measured using the impedance method with a Chronolog C-500 aggregometer. Blood was collected from the abdominal aorta into 3.2% sodium citrate (blood vol.: citrate vol.=9:1) immediately after the microthrombus formation experiment, and kept at room temperature for 30 min until it was used. To 0.5ml of Locke solution containing 2mM CaCl_2 and prewarmed to 37°C, 0.5ml of citrated blood was added. The mixture was allowed to stand until the temperature rose to 37°C (approximately 4 min). Twenty microliters of agonists, i.e. collagen (1mg/ml, Horm), thrombin (100u/ml in 50 mM Tris-HCl containing 100 mM NaCl, pH 7.3, Mochida Pharmaceutical Co. Ltd., Japan) or ADP (10mM in 50 mM Tris-HCl pH 6.8, stored at -80°C, Sigma) were added to the mixture and aggregation was recorded for 6 min. The reaction was observed in a plastic cuvette at 1,000 rpm. Collagen-induced platelet aggregation was started 30 min after the blood was collected, then thrombin- and ADP-induced platelet aggregation tests followed sequentially.

The blood cell count was done using an automatic cell counter (Sysmex-microcellcounter CC-180A, Toa Medical Electronics Co. Ltd., Japan).

Blood pressure

The maximal and mean blood pressure were measured by the tail cuff method with a hemodynamometer (UR-1000, Ueda Seisakusho Co. Ltd., Japan).

Statistics

Wilcoxon's two-sample test was employed in the analysis of the number of exposures to the laser and platelet aggregation level, unpaired Student's *t* test for body weight, blood pressure, blood parameters and laser power.

Results

The growth curve and blood pressure are shown in **Figs. 1** and **2**, respectively. Though the difference in body weight was not significant at 25 weeks old, the SHR was slightly bigger and its blood pressure was significantly higher than that of WKY.

The number of laser irradiations necessary to induce occlusive thrombus formation in the arterioles and venules of SHR and WKY are shown in **Table 1**. Significant differences were observed between SHR and WKY showing that SHR has a higher thrombotic tendency than WKY.

Platelet aggregability and blood parameters are shown in **Table 2** and **3**, respectively.

Fig. 1 Growth curve of SHR and WKY

Values are expressed in mean $\pm$ SE ($n=20$ in each group).

a: SHR, b: WKY. **: $P < 0.01$

SHR showed a significantly higher platelet aggregability as well as platelet and erythrocyte counts which were higher than those found in WKY, but differences in leukocyte count and hematocrit were not observed.

Discussion

Since thrombus formation is induced by the interaction of many factors such as coagulo-fibrinolytic factors, platelets, erythrocytes, leukocytes, vessel walls and blood flow, it is safe to speculate that a minute and singularly undetectable change in each factor might be detected using the method in which the process of thrombus formation can be observed. The laser-induced thrombus formation method is suitable for this purpose. This ex vivo method can detect a minor change in the thrombotic tendency of physically trained rats at rest, although other tests cannot¹⁵⁾.

He-Ne laser irradiation induces platelet thrombus formation. Fibrin can be rarely seen in this type of thrombus. He-Ne laser irradiation injures and denudes endothelial

Fig. 2 Change of blood pressure with age

Blood pressure of the same subjects shown in Fig. 1 was measured and expressed in mean $\pm$ SE.

a: maximal blood pressure of SHR, b: mean blood pressure of SHR, c: maximal blood pressure of WKY, d: mean blood pressure of WKY. **: $p < 0.01$

Table 1 Laser-induced thrombus formation in SHR and WKY

Subject no	Body weight (g)	Arteriole	Venule
SHR			
1	390	10.7	5.0
2	380	5.5	5.0
3	380	8.0	5.0
4	380	7.0	7.5
5	360	6.0	4.3
6	350	5.5	6.5
7	370	6.0	4.3
8	380	6.0	4.0
M±SE	374±5	6.8±0.7	5.2±0.4
WKY			
1	340	≥16	9.0
2	340	11.3	7.0
3	420	10.0	9.5
4	360	≥16	10.0
5	360	7.0	4.5
6	360	≥16	10.5
7	330	≥16	13.0
8	380	9.0	7.5
9	360	7.0	9.0
10	310	≥16	11.0
M±SE	356±10	12.4±1.3	9.1±0.8
	ns	p<0.01	p<0.01

Values in arterioles and venules express the number of laser irradiation necessary to induce occlusive thrombus formation. ns: not significant.

Table 2 Platelet aggregation in SHR and WKY

	ADP	collagen	thrombin
SHR	6.0±0.5	9.0±0.7	11.8±2.4
WKY	4.0±0.7	5.5±0.8	16.0±1.8
	p<0.05	p<0.02	ns

Change of the impedance at three minutes after the addition of each agonists (ohm/3min) is expressed in mean±SE (n=8 in SHR, n=10 in WKY). The final concentrations of ADP, collagen and thrombin were 200 μM, 20 μg/ml and 2 u/ml, respectively. ns: not significant.

Table 3 Blood parameters in SHR and WKY

	PLT	RBC	WBC	HCT
SHR	107±3	1219±16	50±6	49.8±1.0
WKY	67±6	1112±25	39±4	48.6±1.3
	p<0.01	p<0.01	ns	ns

Values are expressed in mean±SE (n=8 in SHR, n=10 in WKY). ns: not significant. PLT: platelet count ($\times 10^4/\mu l$), RBC: erythrocyte count ($\times 10^4/\mu l$), WBC: leukocyte count ($\times 10^2/\mu l$) and HCT: hematocrit (%).

cells leading to platelet thrombus formation. But denudation of endothelial cells depends on the power of He-Ne laser and microvessels used^{5,22}. It is speculated that both of injury and denudation of endothelial cells of microvessels might be occurred by exposure to laser in the present study because of the He-Ne laser power used⁵. Argon laser irradiation has also been reported to cause damage

to endothelial cells followed by platelet thrombus formation⁸). Biolaser, however, induces the formation of plasma protein precipitates and the destruction of erythrocytes as well as endothelial cells injury leading to platelet thrombus formation²³). Fibrin can rarely be seen in Argon- and biolaser-induced thrombus cases^{8,23}).

The significantly higher thrombotic ten-

dency of SHR was estimated in the present study. This confirmed the previously reported results employing the laser-induced thrombus formation method¹⁷⁾¹⁸⁾.

On the other hand platelet aggregability was significantly higher in SHR. This differs from results reported previously in which platelet aggregation was measured in platelet rich plasma^{19)~21)}. The difference could be explained by the influence of erythrocytes and leukocytes co-existing in whole blood as reported previously²⁴⁾. However, the most dominant factor may be platelet count, because platelet count in SHR was 1.6 times higher than that in WKY. It is speculated that results of platelet aggregations in whole blood may reflect those in the circulatory system.

The higher thrombotic tendency in SHR shown by the He-Ne laser-induced thrombus formation method may be partially due to the higher platelet aggregability. The laser-induced thrombus formation method is a promising method for measurement of the thrombotic tendency in experimental animals.

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