EXPERIMENTAL ISCHEMIC HEART DISEASE INDUCED BY THROMBOXANE A₂ IN RABBITS

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How an acute ischemic attack is induced in a patient with coronary atherosclerosis is unknown and we carried out studies using thromboxane A₂ (TXA₂) to determine if acute myocardial ischemia and necrosis could be induced in rabbits.

TXA₂ was perfused through the coronary artery for 5 seconds by means of a Swan-Ganz catheter through the right common carotid artery. Significant serial changes of ST-T on ECG and hypotension were observed from 1 minute to more than 1 day after the perfusion in all 22 rabbits. The TXA₂ that was composed of both aggregated platelets and prostaglandin H₂ induced the same response, and such was dose dependent. The inactivated TXA₂ was without effect.

Seventeen of the experimental rabbits were autopsied. Histological studies of the hearts showed focal myocardial ischemia and necrosis in all rabbits except one autopsied 10 minutes after the perfusion.

TXA₂ is apparently capable of inducing acute myocardial ischemia and necrosis.

SEVERE coronary atherosclerosis was reported in 85% of patients with ischemic heart disease. It is not clear however, how myocardial ischemia occurs in the chronic progress of coronary atherosclerosis!¹⁻⁴

Recently, Samuelsson et al^{5,6} found that thromboxane A₂ (TXA₂) was a potent vasoconstrictor, and induced aggregation of platelets, and that it was released from aggregated platelets.

Therefore, the possibility that TXA₂ may be released from aggregated platelets on an atherosclerotic plaque⁷ of a coronary artery and might

have a direct and immediate influence on the peripheral circulation in the perfused myocardium had to be considered⁸.

We perfused TXA₂ into the coronary artery of rabbits to determine the effects of this prostaglandin endoperoxide on ischemia and myocardial necrosis.

MATERIALS AND METHODS

Twenty-five rabbits (2.5–3.0 kg) were anesthetized with Nembutal sodium 20 mg/kg i.v. A 4F Swan-Ganz catheter was inserted to 1 cm above the aortic valve in the ascending aorta through the right common carotid artery. The position of the catheter tip was determined by measurement of the inserted catheter length.

Two ml of TXA₂ solution was injected into the proximal portion of the ascending aorta soon after inflation of the balloon which obstructed

Key Words:

Vasoconstriction
Platelet aggregation
Myocardial ischemia
Coronary atherosclerosis
Prostaglandin H₂
Thromboxane A₂

(Received on July 19, 1977; Accepted on September 13, 1977)

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Fig.1. Method of perfusion in coronary arteries: The balloon was inflated and soon after thromboxane A₂ was perfused into coronary arteries for 5 sec.

Fig.2. Serial changes in ECG and blood pressure after the perfusion of 2 ml solution A. The ST on aVr elevated at 1 minute and even on 1 hour. The blood pressure also decreased at 1 minute and was still lower than control at 30 minutes.

Fig.3. Changes in ECG and blood pressure by the perfusion of solution A.
abbreviations; BP: blood pressure, T: T wave on ECG, ST-MV: magnitude of ST vector.

the ascending aorta. The inflation remained for 5 seconds, the solution was perfused into coronary arteries, and then the balloon was deflated (Fig. 1). Blood pressure was recorded using a stathum transducer by puncture of the femoral artery. ECG of leads I, aVf and V2, electroencephalogram and respiration were also recorded on a polygraph.

In a few rabbits, the catheter went down to the descending aorta. In these cases, the position of the catheter tip was confirmed at autopsy after the experiment, as no response appeared after the injection. These rabbits were excluded from the study.

TXA₂ solution was prepared by the following two methods: 1) Rabbit platelets were washed and suspended in 6×10^8 /ml in Krebs solution. This was aggregated by 3 units of thrombin (Mochida Co., Ltd.) by incubation at 37°C for 10 seconds. The filtrate through a Sartorius membrane filter 11301 of the reacted solution was used as TXA₂ solution (solution A). This

Fig.4. Focal myocardial necrosis and fibrosis was observed one week after the perfusion of solution A (Mallory-Azan stain).

solution had the equivalent potency to 670 ng/ml noradrenaline in contraction of an aortic strip, but lost it in 6 minutes at 37°C. The period from the start of incubation to the end of injection was 25 seconds. Two other different TXA₂ solutions such as 3×10^8 platelets/ml and 1.5 units thrombin (solution B), and 6×10^7 platelets/ml and 0.3 units thrombin (solution C) were also prepared. 2) TXA₂ was synthesized from 1 µg prostaglandin H₂ and 1.5 mg microsome enzyme of platelets by 5 minutes incubation at 0°C (solution D). This solution had the same potency as solution A.

In this study, quantitative analysis of ST change on ECG was made by an approximate calculation (ST-MV) of the magnitude of ST vector on Frank lead Vector-cardiogram, as such can be used for an index of myocardial ischemia.⁹ Usually the magnitude on the Frank lead was calculated as; Magnitude of ST vector = $\sqrt{ST^2x + ST^2y + ST^2z}$, ST = change of ST segment, x, y, z = 3 scalar recordings of Frank lead. In this

TABLE I ECG AND HISTOLOGICAL CHANGES OF 19 RABBITS

	Period D=day H=hour	Infarct (Histology)		ECG (within 1 hour)	
		M·A H-E	HBFP	ST	T
TXA ₂ -Mixture	15 D	++	—	↑	↓
	14 D	+(SE)	—	↑	0
	9 D	++	—	↓	↓
	7 D	++	—	↓	~
	7 D	+	—	↑	↓
	6 D*	+	—	↓	0
	5 D	++	—	↑	↓
	2 D	+(SE)	—	↑	↓
	2 D	++	+	↑	↓
	3 H	+(SE)	+	↓	0
	1 H	—	+	↓	0
	1 H*	—	+	↑	↑
	1 H	—	+	↑	↓
TXA ₂ (Synthetized)	2 H	—	+	↓	0
	1 H	—	+	↑	↓
	1 H	—	+	↑	0
	10 minutes	—	—	↓	↓
Inactivated TXA ₂ -Mixture	1 D	—	—	0	0
	1 H	—	—	0	0
Control		—	—	0	0

The period between the perfusion and the autopsy was 10 minutes to 15 days. Thirteen in the table were perfused with TXA₂ mixture (solutions A and B*) composed of aggregated platelets. Four were perfused with the TXA₂ (solution D) synthetized from prostaglandin H₂. HBFP stains revealed myocardial ischemia, and HE and M.A. stain myocardial necrosis and fibrosis.

study, $ST-MV = \sqrt{ST^2 I + ST^2 aVf + ST^2 V2}$, I, aVf, V2 = the recordings of 12 lead. Student's t-test was used for statistical evaluations.

The rabbits were autopsied at 10 minutes to 15 days after the TXA₂ perfusion. The excised heart was fixed by 10% formalin solution immediately and embedded in paraffin for histological examination. The specimens were cut and stained with Hematoxylin-Eosin, Mallory-Azan and Hematoxylin Basic Fuchsin Picric acid (HBFP).

RESULTS

1. Perfusion of solution A

Twelve out of 12 rabbits showed significant changes in both ECG and blood pressure (Fig. 2).

Of the serial observations of 6 rabbits followed for over 2 days, 4 showed ST elevation in any lead and there was ST depression in the other 2. T wave also changed in all 6 rabbits. The ST

change occurred significantly in ST-MV at 1 minute after the perfusion, increased significantly at 5 and 10 minutes, and maintained the same grade for 30 minutes. A significant recovery began 1 hour but the changes continued for over one day. The T wave changed in parallel with the ST changes. Inverted T wave appeared by 2 out of the 6 rabbits at 1 minute, 5 out of 5 at 10 minutes, 3 at 30 minutes and 1 at 1 hour. The control blood pressure of the rabbits before the perfusion was 180 ± 23 mmHg in systole and 100 ± 15 mmHg in diastole (mean $\pm$ SD). After the perfusion, the systolic pressure decreased significantly to $61 \pm 19\%$ of the control at 1 minute, and then recovered significantly to $81 \pm 18\%$ at 5 minutes, but still remained at $84 \pm 16\%$ at 30 minutes (Fig. 3).

Transient arrhythmias such as bigemina, conduction defect and short run of ventricular tachycardia were observed in some rabbits during

Fig.5-1. Perfusion of solution B caused minimal ST-T changes in ECG and transient hypotension.

Fig.5-2. No change was observed after the perfusion of solution C. The recording speed was twice faster than that in Fig.5-1.

the periods.

Eleven of the 12 rabbits were autopsied. All had a broad myocardial ischemic lesion in the left ventricle (Table I, Fig. 4). Focal myocardial ischemia was observed with HBFP stain in 2

rabbits autopsied one hour after the perfusion. Local hemorrhage, infiltration of polynuclear leucocytes, and myocardial cell damage were observed at 3 hours, and infiltration of fibroblasts with fiber formation was seen on 2 days in

Fig.6-1. Focal myocardial ischemia was evident 1 hour after the perfusion of solution B (HBFP stain).

Fig.6-2. Small fibrous foci in the ventricular wall were observed 6 days after the perfusion of solution B (Mallory-Azan stain).

tissues stained with HE and Mallory-Azan.

2. Perfusion of solutions B and C

Five rabbits were perfused with solution B. Mild or minimal changes of ST-T on any lead of the ECG and mild hypotension were observed in all 5 rabbits (Fig. 5-1). These changes were transient and disappeared completely within 15 minutes after the perfusion. Two rabbits were perfused with solution C. Neither showed any change of in ECG and blood pressure (Fig. 5-2).

Three of the 5 rabbits perfused with solution B were autopsied. Two showed focal myocardial ischemia (Fig. 6-1) at 1 hour after the perfusion and small fibrous foci in the ventricular wall on 6 days was evident in another (Fig. 6-2).

3. Perfusion of solution D

Four rabbits were perfused with the solution D. Significant changes of ST-T on ECG and hypotension appeared in all 4 (Table I). The serial changes were the same as those seen with solution A.

All 4 rabbits were autopsied. In one animal autopsied 10 minutes after the perfusion, there was no evident change. In two autopsies at 1 hour and one at 2 hours myocardial ischemia was clearly evident in tissues stained with HBFP.

COMMENTS

In this study, 5 seconds perfusion of thromboxane A₂ in the coronary arteries induced serial changes of ST-T on the ECG. Hypotension was a concomitant occurrence with these ECG changes.

These functional changes were dose dependent. Solution A induced a potent response, solution B only a transient change and with solution

C there were no changes. The TXA₂ solution which was composed of aggregated platelets also contained histamine, serotonin, prostaglandins, etc. However, TXA₂ that was synthesized from prostaglandin H₂ induced the same response. The inactivated TXA₂ solution produced no response, nor did the injection of TXA₂ into the descending aorta.

Morphological examination of the same animals showed focal myocardial ischemia 1 hour after the perfusion and serial histological changes and focal myocardial necrosis and fibrosis were seen in the following period.

TXA₂ is an unstable substance and the active components decrease to half in 32 seconds at 37°C^{5,6}. However, more than 30 minutes is required for an irreversible myocardial lesion to occur with ligation of a coronary artery branch^{11,12}. Therefore vasoconstriction by TXA₂¹⁰ only cannot induce the ischemic response. However, Jørgensen¹³ reported that the microthrombi of aggregated platelets were produced in vivo by ADP infusion and myocardial infarction ensued. The myocardial ischemia and necrosis in our study may also be the result microthrombosis of aggregated platelets by TXA₂ in addition to the vasoconstriction effect of the compound.

Most patients with ischemic heart disease have severe coronary artery disease. In these cases, atherosclerotic plaques are disseminated diffusely along the extramural coronary artery, and more than 75% stenosis and dilatation are evident in one or more coronary main branches^{7,14-16}. Changes in the coronary artery diameter of course induce changes in the blood flow.

It is probable that in patients with atherosclerotic plaques and a change in blood flow an aggregation of platelets occurs thus releasing thromboxane A₂ in the coronary artery.

Acknowledgement

Thanks are due to M. Ohara for assistance with the manuscript and to K. Moriya, T. Takahashi, Y. Takashima, and M. Sakamoto for technical assistance.

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