An Observation of Thromboxane A₂ in Arterial Blood after Cholesterol Feeding in Rabbits

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SUMMARY

Agents responsible for the entry of cholesterol-bearing lipoproteins into the arterial wall represent local risk factors in atherogenesis. In an attempt to identify these agents, we inserted a catheter into the ascending aorta of rabbits and 5 ml of arterial blood sample was withdrawn. The contraction of the aortic strip of rabbit against Piper and Vane's antagonists with application of this sample was observed. Samples obtained after feeding of 1 Gm/Kg of cholesterol and after an intravenous administration of thromboxan A₂ (TXA₂) contracted the aortic strip. Samples from non-treated rabbits or those obtained after intravenous administration of 10 ng of epinephrine or norepinephrine did not contract the strip. Previous administration of 20 mg/Kg of indomethacin decreased the contraction developed after feeding of 1 Gm/Kg of cholesterol. It was suggested that TXA₂ might be released into the arterial blood by the ingestion of cholesterol and might be one of the agents responsible for the entry of lipoproteins.

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It is now generally accepted since Anitschkow (1913)¹ that the repetitious administration of cholesterol produces experimental atherosclerosis in susceptible animal species and atherosclerosis progresses under states of hypercholesteremia both in human and laboratory animals.¹

The agents responsible for the entry of cholesterol-bearing lipoproteins into the arterial wall and for the deposition of cholesterol in the arterial wall are local risk factors in atherogenesis, but are not yet identified.

In the light of the fact that thromboxane A₂ (TXA₂) has highly potent permeability increasing activity, contracts arteries, aggregates platelets, and

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has lipid-release inhibiting activity in adipocytes,\textsuperscript{2},\textsuperscript{3} the role of TXA\textsubscript{2} in atherogenesis may be significant.

We report herein our observations that administration of cholesterol releases TXA\textsubscript{2} into the arterial blood of rabbits.

**Materials and Methods**

New Zealand male white rabbits (2.5–3.0 Kg) were anesthetized with Nembutal sodium (20 mg/Kg i.v.). A polyethylene siliconized catheter (1.2 mm in diameter) was inserted into the ascending aorta through the right common carotid artery and 5 ml of arterial blood was withdrawn through the catheter into a plastic syringe containing heparin (50 U, 0.05 ml) and indomethacin (100 μg/ml, 0.5 ml) (Fig. 1).

The 5 ml of blood sample was put into the bath of 5 ml Krebs solution (37°C, pH 7.4), which was saturated with 95% O\textsubscript{2} and 5% CO\textsubscript{2} and contained twice the concentration of Piper and Vane’s\textsuperscript{4} combined antagonists to 5-hydroxytryptamine, histamine, acetylcholine, and catecholamine in order to increase the sensitivity and selectivity of the assay tissue. Piper and Vane’s antagonists employed herein consisted of methysergide bimaleate (4×10\textsuperscript{-7} Gm/ml, May and Baker), hyoscine hydrobromide (2×10\textsuperscript{-7} Gm/ml, Tokyo Kasei), phenoxybenzamine hydrochloride (2×10\textsuperscript{-7} Gm/ml, Tokyo Kasei), propranolol (4×10\textsuperscript{-6} Gm/ml, ICI), and indomethacin (2×10\textsuperscript{-6} Gm/ml, Merck S.D.) in order to prevent endogeneous synthesis of prostaglandins by the tissues.

A rabbit thoracic aorta strip which has been soaked in Krebs solution containing phenoxybenzamine hydrochloride (1 μg/ml) for 30 min in order to block the α-effects of catecholamines, was then soaked in the bath of the above mentioned Krebs solution with the antagonists, and used for measurement of the isometric contraction which was registered through isometric transducer 88-1T on the recorder RJR-32024, Nihonkoden Co, Ltd (Fig. 1).

![Diagram](image)

**Fig. 1.** Five ml of arterial blood was withdrawn through a catheter and was mixed with 5 ml Krebs solution with Piper and Vane’s antagonists. Isometric contraction of an aortic strip in the bath with application of the blood sample was recorded.
Heparin (100 U, 0.1 ml) was added to the bath 2 min previously, and then the arterial blood sample was mixed with the solution in the bath immediately after sampling through the catheter. The presence in the sample of rabbit aorta contracting substance (RCS) by Piper and Vane⁴ was confirmed by the isometric contraction of the aortic strip, since the final concentrations of antagonists in the bath corresponded exactly with those of Piper and Vane’s combined antagonists which also contained indomethacin (1 × 10⁻⁶ Gm/ml).

RESULTS

1) TXA₂ solution synthesized from 20 ng/ml prostaglandin H₂ and TXA₂ synthetase in 32 μg/ml of rabbits platelet microsome,⁴⁻⁷ contracted the aortic strip, and an antagonist to TXA₂, phthalazinol (EG626),⁸ suppressed the contraction (Fig. 2).

2) The arterial blood samples obtained from untreated 3 rabbits which did not contract the aortic strip, showed no detectable amount of RCS (Fig. 3). The arterial blood samples obtained from the same 3 rabbits 10 and 30 min after the intravenous injection of 10 μg/Kg of epinephrine or 10 μg/Kg of norepinephrine also did not contract the aortic strips (Fig. 4).

3) The arterial blood samples obtained from 3 rabbits 1 min after intravenous injection of TXA₂ which was released from 9.0 × 10⁹/ml of rabbit platelets suspension stimulated by 50 units of thrombin⁷ induced a rapid contraction of the aortic strip (Fig. 5).

4) The arterial blood samples obtained from 3 rabbits fed of 1 Gm/Kg of cholesterol 1 or 2 hours before sampling, induced a rapid contraction of the aortic strip (Fig. 5).

The maximum contraction was observed at 30 sec, after which the tissue

* Thromboxane A₂ was produced by the mixture of rabbit platelets microsomes (32 μg/ml) and PGH₂ (20 ng/ml)

Fig. 2. The aortic strip contraction was induced by TXA₂ which was put directly into the bath, and was suppressed by an antagonist (EG626) of TXA₂.
Fig. 3. The blood sample from untreated rabbits did not contract the aortic strip. Blood samples after administration of indomethacin or cholesterol with indomethacin induced minimal contraction.

Fig. 4. The blood samples after intravenous injection of 10 µg/kg of epinephrine or norepinephrine did not contract the aortic strip.

Fig. 5. The blood samples 1 and 2 hours after feeding of 1 Gm/Kg of cholesterol or after intravenous injection of TXA2 contracted the aortic strip.
gradually relaxed. The half life of RCS in the sample was about 7 min at 37°C. Both samples obtained 1 and 2 hours after administration of cholesterol contracted the aortic strip to the same degree.

5) The arterial blood samples of 3 rabbits given 20 mg/Kg of indomethacin 1 hour before sampling induced a minimal contraction of the aortic strip (Fig. 3).

These rabbits were given 1 Gm/Kg of cholesterol soon after the sampling and a second arterial blood sample was withdrawn 1 hour after the feeding. This sample also induced only a minimal contraction (Fig. 3).

**DISCUSSION**

Piper and Vane\(^4\) in 1969 discovered a new rabbit aorta contracting substance (RCS) which maintained a potency against several antagonists to known vasoactive substances. Hamberg et al\(^2\) reported that the active agent of the RCS was thromboxane A\(_2\) and that this agent induced a potent edematous arterial reaction.

In our study herein, the RCS in arterial blood was detected by the contraction of an aortic strip against Piper and Vane's antagonists.

The arterial blood sample obtained after cholesterol feeding contracted the aortic strip. Epinephrine and norepinephrine were given intravenously to rabbits, but the arterial blood samples failed to contract the aortic strip. When TXA\(_2\) was given intravenously, the arterial blood did contract the aortic strip. Indomethacine was administered to rabbits 1 hour before the cholesterol feeding to prevent TXA\(_2\) synthesis, and this treatment suppressed the contraction.

Therefore, the arterial blood after cholesterol feeding apparently contained the RCS reported by Piper and Vane, and according to Samuelsson's studies,\(^3\) cholesterol feeding may release TXA\(_2\) into the arterial blood. Although the mechanism of release of TXA\(_2\) in arterial blood by cholesterol feeding remains to be elucidated, it is known that TXA\(_2\) synthetase\(^9\) exists in large quantities in platelets and in the spleen.

During the early stage of analysis on atherogenicity by the classic technique of the repetitious administration of cholesterol, the edematous arterial reaction was first demonstrated in the aorta of rabbits.\(^10,11\) A single-dose of cholesterol (1 Gm/Kg, p.o.) also given to rabbits induced an edematous reaction.\(^10,11\) These studies suggested that active agents in the blood stimulated arterial endothelial cells which, by their contracting and phagocytic activity, transported the plasma substances including beta-lipoprotein from the blood into the arterial wall to the susceptible segments.\(^10,13\)
TXA$_2$ contracts arteries and induces edematous arterial reaction. In addition, our present data suggested that TXA$_2$ might appear in arterial blood after cholesterol feeding. Therefore, cholesterol feeding seems to induce TXA$_2$ release in arterial blood, which causes the edematous arterial reaction of the early stage of atherosclerosis. According to these observations, TXA$_2$ might be one of local risk factors in atherogenesis.

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**References**