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A Study of Mechanism Involved in Cadmium-induced Platelet Aggregation

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카드뮴중독으로 혈소판응집이 항진되어 고혈압 또는 동맥경화증이 발생한다는 연구보고를 감안하여 가토와 흰쥐의 생체내 실험으로서 미세혈전의 형성, Malondialdehyde(MDA) 및 thromboxane B_2 (TXB_2)치에 미치는 카드뮴의 효과를 검토하고, in vitro 실험으로 카드뮴을 처치한 가토 대동맥절편이 혈소판응집에 미치는 영향을 관찰하였으며, prostacyclin의 합성능력을 측정하여 그 결과를 다음과 같이 요약하였다.

1) Cadmium chloride 2 mg/kg을 매주 동물의 복강내에 주입하였을 때 미세혈전형성이 증가되었다.

2) 카드뮴 중독동물의 platelet-rich plasma (PRP)는 MDA와 TXB_2형성이 정상동물에서 보다 현저히 증가되었다.

3) 카드뮴을 중독시킨 가토의 platelet-poor plasma (PPP)에서의 lipid peroxide치는 대조군과 차이가 없었다.

4) In vitro 실험으로, 가토대동맥 절편에서의 6-keto-$PGF_{1\alpha}$의 생성은 카드뮴 농도에 비례하여 억제되었고 이때 혈소판 응집률의 증가와 평행하였다.

5) 이상의 결과로서 카드뮴은 동맥내피세포에서 prostacyclin 합성을 억제할 뿐만 아니라 혈소판에서 TXA_2합성을 촉진시켜 그 결과로 혈소판 응집률을 증가시켰음을 알 수 있었다.

INTRODUCTION

It has been previously reported that Cd-induced arterial hypertension in animals may be due to the accumulation of high amounts of Cd in kidney (Lener et al., 1971 a) or indirectly due to the increase in tubular sodium reabsorption (Lener et al., 1971 b). However, the mechanism of the elevated arterial blood pressure by Cd remains uncertain despite of numerous efforts to elucidate it.

Since the patients with certain cardiovascular diseases including vascular insufficiency, congestive heart failure, and diabetic microangiopathy have shown an increase in platelet aggregate formation activity (Wu et al., 1974; Preston et al., 1977; Mehta et al., 1979), a causal relationship may exist between the increased platelet aggregation and arterial hypertension. Furthermore, this relationship

coupled with the recent findings that platelet aggregation is modulated by prostacyclin (PGI_2) as an antiaggregatory substance (Needleman et al., 1978; Moncada et al., 1979) and thromboxane A_2 as an aggregator (Hamberg et al., 1975) suggest the possibility that the damage of venous endothelium (Schlaepfer, 1971) and arterial hypertension (Schroeder et al., 1966; Caruthers et al., 1979) may be related to the increased platelet aggregation by the inhibition of the PGI_2 production and an increased synthesis of TXA_2.

In this study, the *in vivo* effect of Cd on the level of MDA and TXB_2 in platelet-rich plasma(PRP) in rabbits and rats was determined. The *in vitro* effect of Cd-treated rabbit aorta ring on the platelet aggregation was also observed and confirmed by measuring the rate of PGI_2 synthesis.

METHODS

Rabbits (2 kg) and rats (200～250 g) were poisoned by a series of weekly intraperitoneal injections of a 2 mg/kg dose of cadmium chloride for 4 weeks. The control animals were sham injected with normal saline. In order to observe the effect of aorta ring on platelet aggregation, the whole blood was collected from the carotid artery under ether anesthesia and transferred immediately into the polypropylene tube containing 3.8% sodium citrate (9 : 1 v/v). PRP was obtained by centrifuging at 150 g for 8～10 minutes and the platelet count in PRP was adjusted at approximately 250,000/ml by adding a tris buffer (pH 7.4). The experiment was completed at a room temperature within 3 hours of blood collection. The rabbit aorta freed from its surrounding tissues was cut into rings of an average 20～25 mg in wet tissue weight and immersed in the oxygenated physiological salt solution (PSS) at 37°C for 60 minutes. Platelet-poor plasma (PPP) was used for the determination of lipid peroxide (Yagi, 1976). Both MDA and TXB_2 levels in plasma were assessed in the presence of 2 mM sodium arachidonate (Doni et al., 1981). MDA was determined by using spectrofluorometer (Doni et al., 1981), and TXB_2 by radioimmunoassay using the New England Nuclear kit (Granström et al., 1978).

The aorta ring was preincubated at 37°C for 30 minutes with tris-PSS (6 mM tris was added instead of sodium bicarbonate and the pH was adjusted to 7.4 with 1 N HCl) in which Cd was dissolved. Afterwards, the ring was rinsed in the tris buffer, and immediately removed into the polypropylene tube with 1.0 ml of PRP. After 10 minutes, 0.5 ml of PRP in the tube was transferred into the vial containing 10 μg indomethacin for determination of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and stored at -20°C until the assay was performed. The remaining PRP (0.5 ml) was also removed into the siliconized cuvette and 3 μM ADP was added to induce the platelet aggregation. A continuous changes of light transmission due to aggregation was monitored with an automatic aggregometer (Chrono-Log, Model 440). The production of 6-keto-$PGF_{1\alpha}$ was measured using the radioimmunoassay kits of New England Nuclear (Granström et al., 1978).

Drugs used: Sodium arachidonate (Sigma Chemical Co., St. Louis, MO), indomethacin (Sigma), ADP (Sigma), and cadmium chloride (Hayashi Chemical Co.). All the values are expressed as the mean $\pm$SE of the mean. The statistical analysis of the data was performed by Student's t-test.

RESULTS

As shown in Table 1, the MDA level in PPP was significantly increased by Cd-poisoning. The MDA formation activity in PRP from the control group was 5.83±0.26 nmol/ml, and the activity of the Cd-poisoned group was significantly increased to 6.74±0.26 and 7.76±0.31 nmol/ml in 2- and 4-weeks, respectively. The plasma lipid peroxide level showed no difference between the control and Cd-poisoned groups.

An enhanced TXB_2 formation in PRP by Cd was shown in Table 2. The TXB_2 level in PRP from 2-weeks Cd-poisoned rabbits and rats was significantly higher than that of the control group. However, the level of TXB_2 in PPP was not much different between the control and 2-weeks Cd-poisoned rabbits.

The tracing of platelet aggregation and its mean change is shown in Fig. 1 and Table 3. When the intact aorta ring was incubated with PRP and ADP, the platelet aggregability was 20.3±3.2, 16.2±4.3, and 9.9±3.3% at 30 seconds, 2 and 3 minutes, respectively. However, after the ring was pretreated with Cd, it was significantly increased in a dose-dependent manner.

A dose-dependent inhibition of vascular 6-keto-$PGF_{1\alpha}$ production by Cd was apparent when the experiment was performed *in vitro*.

Table 1. Malondialdehyde(MDA) and lipid peroxide concentrations in plasma of the control and Cd-poisoned rabbits

	Control	Cd-poisoned	
		2-weeks	4-weeks
Lipid peroxide in PPP (nmol MDA/ml)	3.51±0.20[a] (21)[b]	3.95±0.29 (10)	3.25±0.24 (9)
MDA in PPP (nmol/ml)	2.32±0.10 (9)	2.71±0.09[c] (9)	2.89±0.08[c] (8)
MDA in PRP (nmol/ml)	5.83±0.26 (8)	6.74±0.26[c] (13)	7.76±0.31[c,d] (8)

[a] The mean value±SE of the mean.
[b] Numbers in parentheses indicate the number of experiments.
[c] Significantly different from the corresponding value of the control, $p<0.05$.
[d] Significantly different between 2- and 4-weeks of Cd-poisoned groups, $p<0.05$.

Table 2. Thromboxane B_2 production in the control and Cd-poisoned animals

	N	Control (pg/ml)	2-weeks Cd-poisoned (pg/ml)	% Increase
Rabbits				
PPP	9	81.4±30.1[a]	93.1±23.4	14.4
PRP	10	551.4±64.5	743.8±48.8[b]	34.9
Rats				
PRP	10	605.5±45.9	847.0±37.3[b]	39.9

[a] The mean value±SE of the mean.
[b] Significantly different from the control, $p<0.05$.
N means the number of experiments.

Table 3. Enhancement of platelet aggregation by pretreatment of rabbit aorta ring with Cd

Pretreatment	N	% of Aggregation		
		30 Sec	2 Min	3 Min
PSS	15	20.3 ± 3.2^{a}	16.2 ± 4.3	9.6 ± 3.3
No aorta ring	12	46.8 ± 4.5^{b}	77.9 ± 5.8^{b}	70.8 ± 6.5^{b}
5×10^{-3}g/ml Cd	15	33.7 ± 4.0^{b}	45.9 ± 6.8^{b}	36.9 ± 6.7^{b}
10^{-3}g/ml Cd	15	26.1 ± 3.9^{c}	33.2 ± 6.5^{c}	24.7 ± 5.5^{c}
10^{-4}g/ml Cd	15	24.1 ± 3.4	29.1 ± 6.1	18.2 ± 3.7

[a] The mean value±SE of the mean.
[b] Significantly different from the corresponding value in PSS, $p<0.001$.
[c] Significantly different from the corresponding value in PSS, $p<0.05$.
N means the number of experiments.

Table 4. 6-keto-$PGF_{1\alpha}$ production by the rabbit aorta ring

Incubation medium	6-keto-$PGF_{1\alpha}$ (pg/mg/min)
PSS	2.0 ± 0.2^{a}
5×10^{-3}g/ml Cd	0.7 ± 0.2^{b}
10^{-3}g/ml Cd	1.3 ± 0.3^{c}
10^{-4}g/ml Cd	1.5 ± 0.4

[a] The mean value±SE of the mean for 8 experiments.
[b] Significantly different from the level in PSS, $p<0.001$.
[c] Significantly different from the level in PSS, $p<0.05$.

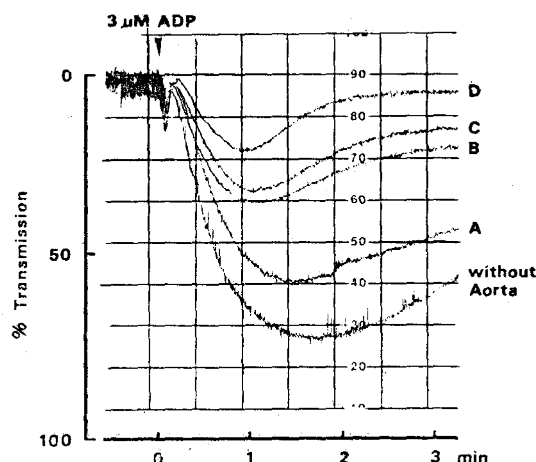

Fig. 1. The superimposed tracings of the platelet aggregation. The aorta ring was previously treated with cadmium chloride for 30 minutes at different concentrations(A, 5×10^{-3} g/ml; B, 10^{-3} g/ml; C, 10^{-4} g/ml; D, tris buffer only), The ring was rinsed with a tris buffer before incubation.

As shown in Table 4, when the intact aorta ring was incubated with PRP, the rate of production of 6-keto-$PGF_{1\alpha}$ was 2.0 ± 0.2 pg/mg/min. However, after the aorta ring was pretreated in tris-PSS containing 5×10^{-3}, 10^{-3} and 10^{-4} g/ml Cd, the concentration of assayable 6-keto-$PGF_{1\alpha}$ was dose-dependently decreased. Tris-PSS, when used instead of PSS did not make any difference.

DISCUSSION

It was hypothetized with the evidence of enhanced MDA and TXB_2 formation by Cd-poisoning that Cd must increase the prostaglandin endoperoxide production in platelets and subsequently enhance the platelet aggregation. The balance between TXA_2 and PGI_2 formation is considered to be responsible for the control of platelet aggregation under physiological conditions (Srivastava, 1978). The experiment was done on the presumption

that an increased aggregate formation by Cd may contribute to the arterial hypertension.

A number of mechanisms that enhance the platelet aggregation by Cd are considered. One possible mechanism is supported by the findings that both MDA and TXB_2 levels of PRP from Cd-poisoned animals were significantly higher than those of the control group. When MDA, an index of prostaglandin endoperoxide (Smith et al., 1976; Doni et al., 1981), was measured to deduce the platelet function, the MDA level was much elevated. It has been reported that the circulating platelet is aggregated by TXA_2 (Hamberg et al., 1975; Needleman et al., 1976). However, it is not known whether Cd stimulates the cyclooxygenase activity. The other mechanism is the inhibition of PGI_2 synthesis in the vessel wall by Cd poisoning since PGI_2 is known to be the most potent antiaggregatory prostaglandin in all platelet preparations as so far investigated (Bunting et al., 1976a; 1976b; Moncada et al., 1977; Needleman et al., 1978; Bayer et al., 1979).

Recently, lipid peroxide, a product of arachidonic acid by lipoxygenase has been reported to inhibit the PGI_2 synthetase (Ham et al., 1979; Gryglewski, 1980). Furthermore, the hypothesis has been made that lipid peroxide may be responsible for triggering the diseases such as atherosclerosis and hypertension. However, in this study plasma lipid peroxide level was not influenced by Cd-poisoning. Caprino et al. (1979) reported that Cd in vitro has reduced the prostacyclin-like activity released from rat arterial tissue, and recently they demonstrated the biphasic effect of Cd on PGI_2 release (1982). However, in this study Cd-poisoning not only inhibited PGI_2 production but also stimulated TXA_2 production and subsequently resulted in the increased platelet aggregation since the prostaglandin endoperoxides are the common substrate for TXA_2 and PGI_2 (Bayer et al., 1979).

The present data demonstrated that Cd has an effect to induce an alteration of prostaglandin biosynthesis, which enhances the platelet aggregation, and furthermore, it may be related to the development of arterial hypertension in Cd-poisoned animals.

SUMMARY

Cadmium (Cd) was administered by a series of weekly intraperitoneal injections at dose of 2mg/kg in rabbits and rats. The levels of malondialdehyde (MDA) and thromboxane B^2 (TXB_2) in platelet-rich plasma from Cd-poisoned animals were significantly higher than those of the control group. Furthermore, the inhibition of 6-keto-prostaglandin $F_{1\alpha}$ pɹoduction in Cd-treated aorta riug was inversely related to the enhancement of platelet aggregation. These results suggest that Cd not only inhibits prostacyclin synthesis in the arterial endothelium, but also stimulates the platelet aggregation by enhancing thromboxane A_2 production. These findings are assumed to support the evidence of an effect of Cd toxicity on the vascular wall and platelet function in raising arteriall pressure.

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