

Effects of Sulfinpyrazone, Aspirin and Propranolol on the Isoproterenol-induced Myocardial Necrosis

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SUMMARY

Prophylactic effects of sulfinpyrazone (100 mg/Kg), aspirin (5 mg/Kg, 50 mg/Kg), and propranolol (2 mg/Kg, 10 mg/Kg) on myocardial necrosis and hypertrophy induced by isoproterenol were examined. Drugs were administered to rats daily by gavage for a period of 2 weeks and after that isoproterenol (40 mg/Kg) was injected subcutaneously. The control group received gavage of water and isoproterenol injection. The "no infarct" group received gavage of water and saline injection. Premedication of sulfinpyrazone and propranolol significantly preserved myocardial CK activity and decreased cardiac hypertrophy compared with control group (24 hours after isoproterenol injection) while aspirin did not have such effects. Myocardial cyclic AMP concentration significantly increased 30 min after isoproterenol injection in control and all the premedicated rats compared with the "no infarct" rats. The increment of cyclic AMP was not suppressed by sulfinpyrazone and propranolol during this period. Plasma levels of prostaglandins were significantly suppressed by the administration of sulfinpyrazone and 50 mg/Kg of aspirin, and were not suppressed by 5 mg/Kg of aspirin.

It was concluded that premedication of sulfinpyrazone and propranolol reduced cardiac necrosis and hypertrophy induced by isoproterenol, but aspirin did not have such cardioprotective effects.

Additional Indexing Words:

Myocardial infarction Creatine kinase Cyclic AMP Prostaglandin E₁ Prostaglandin F_{2α}

THE role of catecholamines in the development of myocardial infarction has been stressed.^{1),2)} Experimentally, it is well known that the administration of large doses of β -adrenergic stimulant isoproterenol causes myocardial necrosis in rats without coronary ligation.³⁾ Such an isoproterenol-induced "infarct-like" lesion is morphologically similar to a certain lesion described in acute myocardial infarction and sudden death in man.¹⁾ On the

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other hand, sulfinpyrazone, aspirin, and propranolol are supposed to have some cardioprotective effects on the ischemic heart. Aspirin and sulfinpyrazone affect the prostaglandin (PG) synthetase system,^{4),5)} and they manifest their cardiovascular effects mainly by regulating platelet function.⁶⁾ Propranolol is an established β -adrenergic blocking agent and it works in opposition to the effects of isoproterenol.^{7),8)}

The purpose of this study is to investigate whether or not the premedication of sulfinpyrazone, aspirin, and propranolol could be cardioprotective against the isoproterenol-induced myocardial necrosis. The degree of myocardial necrosis is estimated by changes in myocardial creatine kinase (CK) activity.^{9),10)} The degree of cardiac β -adrenoreceptor stimulation is estimated by determining myocardial 3',5'-cyclic adenosine monophosphate (cyclic AMP) content.^{7),11)} The magnitude of intrinsic PG suppression by sulfinpyrazone and aspirin can be estimated by measuring plasma PG concentration.

MATERIALS AND METHODS

Male Wister rats, weighing about 300 Gm each and fed a standard pellet diet and water ad libitum, were used in this study.

Estimation of myocardial necrosis by determining myocardial CK activity

Sixty rats were divided into 6 groups. Each group consisted of 10 rats. Drugs were given once a day via a stomach tube for 14 days to each group in the following doses: (1) 100 mg/Kg sulfinpyrazone, (2) 5 mg/Kg aspirin, (3) 50 mg/Kg aspirin, (4) 2 mg/Kg propranolol, (5) 10 mg/Kg propranolol. Water was given to the sixth group in the same way and this group consisted the control group. Sulfinpyrazone was dissolved in equimolar sodium hydroxide solution and then prepared to pH 7.5 by adding 0.1 M phosphate buffer. Aspirin and propranolol were directly dissolved in water. All these solutions were prepared just before gavage and were administered in a volume of 10 ml/Kg. After 14 days' administration of drugs, 40 mg/Kg of isoproterenol hydrochloride was injected subcutaneously into each rat. This injection was performed 1.5 hour after the last gavage. Isoproterenol was dissolved into a 0.9% sodium chloride solution just before the injection. Rats were sacrificed 24 hours after isoproterenol injection under ether anesthesia and their hearts were excised immediately. The atrium, great vessels and clots were removed and the myocardium was weighed. Creatine kinase (CK) activity of the whole myocardium was then measured according to the method of Sobel.⁹⁾ Ventricular myocardium was minced with scissors, homogenized by high speed Ultra-Turrax (Ika-Werk Co) for 2 min in 5 ml of homogenizing medium consisting of 0.25 M sucrose, 0.001 M EDTA and 0.1 mM mercaptoethanol. The homogenate was then centrifuged at 1100 g for 30 min and the supernatant fraction was removed. All these procedures were performed at 0 to 4°C. CK activity in the supernatant was then assayed by the Rosalki's method.¹²⁾ Total protein in the supernatant was assayed by the biuret procedure.¹³⁾ Results were expressed in international units (IU)/mg protein. Another group of rats, which

received water via stomach tube for 14 days and was injected with saline instead of isoproterenol, was also prepared and named the "no infarct" group ($n=10$). Myocardial CK activity in this group was also measured by the same method. The ratio of heart to body weight (heart/body weight ratio) was calculated by using the body weight just before the isoproterenol injection to eliminate the extra-cardiac effects of isoproterenol.

As a preliminary study to investigate the time course of myocardial CK activity, rats, which had been administered water for 14 days by gavage, were injected with 40 mg/Kg of isoproterenol and sacrificed 12 hours ($n=5$), 24 hours ($n=10$), and 48 hours ($n=5$) after the injection to determine myocardial CK activity and the heart/body weight ratio.

Determination of myocardial cyclic AMP content

Twenty-eight rats were divided into 4 groups. Each group consisted of 7 or 8 rats. One hundred mg/Kg of sulfinpyrazone, 50 mg/Kg of aspirin, and 10 mg/Kg of propranolol were given to each group once a day via a stomach tube for 14 days. Water was given to the control group in the same way. After 14 days' administration of these drugs, 40 mg/Kg of isoproterenol was injected subcutaneously. This injection was performed 1.5 hour after the last gavage. Rats were sacrificed under ether anesthesia 30 min after isoproterenol injection. Beating hearts were rapidly excised and about 800 mg of myocardium was cut from the apical portion of the ventricles and quickly immersed in liquid nitrogen. Samples were weighed and stored at -80°C . Myocardial cyclic AMP was determined by the following procedure according to the method of Honma,¹⁴⁾ using a cyclic AMP assay kit of the Yamasa Shoyu Co. Frozen tissue was homogenized by high speed Ultra-Turrax for 1 min in 4 ml of 6% trichloroacetic acid. The homogenate was then centrifuged at 1000 g for 15 min at 0 to 4°C and the supernatant fraction was washed 3 times with water-saturated diethyl ether. The sample was diluted to 10 times by adding distilled water and then succinylated, and cyclic AMP was determined by radioimmunoassay using auto well type scintillation counter (ARC-251; Aloka Co). Results were expressed in pmoles/Gm wet weight tissue. Another group of rats, which received water via stomach tube for 14 days and was then injected with saline instead of isoproterenol, was also prepared and named the "no infarct" group ($n=5$). Myocardial cyclic AMP content was also measured in this group in the same manner.

Determination of plasma PGE_1 and $\text{PGF}_{2\alpha}$ concentrations

Thirty-four rats were divided into 4 groups. Each group consisted of between 8 to 10 rats. One hundred mg/Kg of sulfinpyrazone, 5 mg/Kg of aspirin, and 50 mg/Kg of aspirin were given to each group once a day via a stomach tube for 14 days. Water was given to the control group in the same way. On the 14th day, about 5 ml of blood was drawn with small amount of heparin by percutaneous cardiac puncture 1.5 hour after the last gavage. Plasma (1 ml) was acidified with hydrochloric acid to pH 3.0 and then PGs were extracted with diethyl ether according to the method of Hennam.¹⁵⁾ PGE_1 and $\text{PGF}_{2\alpha}$ were determined by radioimmunoassay described by Caldwell,¹⁶⁾ using PGE_1 and $\text{PGF}_{2\alpha}$ RIA kits of the Clinical Assays Inc and Beckman's liquid scintillation counter (LS-7500). In this procedure, PGE_1 was converted to PGB_1 and then assayed. Results were expressed in pg/ml.

Reagents used in this study were generally those of a special grade and were

purchased from the Katayama Chemical Co. Sulfipyrazone was a gift from Ciba-Geigy Co. Aspirin was obtained from the Katayama Chemical Co. Propranolol was a gift from ICI Pharma Co, and isoproterenol HCl was obtained from the Sigma Chemical Co.

Statistical analysis was performed according to Student's t-test. A p-value of 0.05 or less was considered to be statistically significant in this study.

RESULTS

Myocardial CK activity

In the preliminary study, myocardial CK activity significantly decreased 24 and 48 hours after isoproterenol injection, and was most depleted 24 hours after the injection (Fig. 1). Mean myocardial CK activities of sulfipyrazone, propranolol, and "no infarct" groups 24 hours after the isoproterenol injection were significantly higher than that of the control group. In propranolol pretreated groups, CK was higher in the 10 mg/Kg group than in the 2 mg/Kg group, but the difference was not significant statistically. The "no infarct" group showed higher CK and this was thought to be the CK activity of the intact rat heart. Myocardial CK activity of the sulfipyrazone pretreated group was significantly ($p < 0.005$) higher than that of the propranolol group and was as high as that of the "no infarct" group. CK of the sulfipyrazone group was not different statistically from that of the "no infarct" group. On the other hand, mean myocardial CK activities of aspirin pretreated groups were not different statistically from that of the control group.

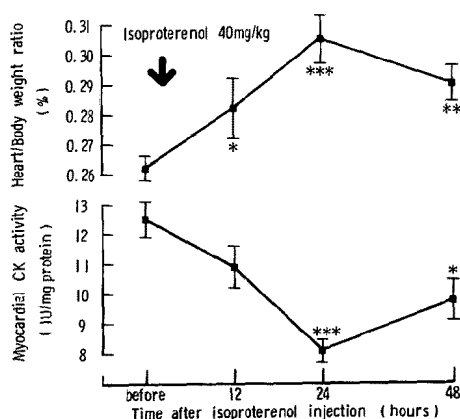


Fig. 1. Preliminary study. Effects of isoproterenol (40 mg/Kg) injection on the time course of myocardial CK activity and heart/body weight ratio. Vertical bar indicates mean \pm SEM. * $p < 0.05$ compared to control (before), ** $p < 0.005$ compared to control (before), *** $p < 0.001$ compared to control (before).

CK activity of the 50 mg/Kg aspirin group was higher than that of the 5 mg/Kg aspirin group, but the difference was not significant statistically (Table I).

The ratio of heart to body weight (heart/body weight ratio)

In the preliminary study, the heart/body weight ratio of the animals increased significantly 24 and 48 hours after isoproterenol injection, and manifested its peak value at 24 hours after the injection (Fig. 1). The mean heart/body weight ratios of the sulfinpyrazone, propranolol and "no infarct" groups 24 hours after isoproterenol injection were significantly lower than that of the control group. In propranolol pretreated groups, the heart/body weight ratio was lower in the 10 mg/Kg group than in the 2 mg/Kg group, but the difference was not significant statistically. The "no infarct" group showed the lowest heart/body weight ratio and this was thought to be the heart/body weight ratio of an intact animal. As in the case of CK activity, the mean heart/body weight ratios of the aspirin pretreated groups were not different statistically from that of the control group. The heart/body weight ratio of the 50 mg/Kg aspirin group was lower significantly ($p < 0.05$) than that of the 5 mg/kg aspirin group. The heart/body weight ratios of the propranolol and sulfinpyrazone groups were not different statistically from that of the "no infarct" group, while the heart/body weight ratios of the

Table I. Effects of Drugs on Myocardial CK Activity, Heart/Body Weight Ratio and Myocardial Cyclic AMP Content after Isoproterenol Injection

	24 hours after isoproterenol injection		30 min after isoproterenol injection
	Myocardial CK activity (IU/mg protein)	Heart/Body weight ratio (%)	Myocardial cyclic AMP content (pmol/Gm wet wt. tissue)
Control	8.10 ± 0.36	0.305 ± 0.008	1203 ± 99
Aspirin 5 mg/Kg	7.33 ± 0.62	0.318 ± 0.008	
Aspirin 50 mg/Kg	8.74 ± 0.32	0.288 ± 0.009	1257 ± 60
Propranolol 2 mg/Kg	$10.23 \pm 0.44^{**}$	$0.276 \pm 0.008^{*}$	
Propranolol 10 mg/Kg	$11.09 \pm 0.38^{***}$	$0.264 \pm 0.004^{***}$	1492 ± 114
Sulfinpyrazone 100 mg/Kg	$12.83 \pm 0.29^{***}$	$0.270 \pm 0.004^{***}$	1373 ± 74
"No infarct"	$12.52 \pm 0.61^{***}$	$0.262 \pm 0.004^{***}$	$713 \pm 51^{**}$

Myocardial CK activity and heart/body weight ratio were determined 24 hours after isoproterenol (40 mg/Kg) injection. Myocardial cyclic AMP content was determined 30 min after isoproterenol (40 mg/Kg) injection. Control group received isoproterenol injection without premedication. "No infarct" group received neither premedication nor isoproterenol injection. Values are mean \pm SEM.

* $p < 0.05$ compared to control, ** $p < 0.005$ compared to control, *** $p < 0.001$ compared to control.

aspirin groups were higher significantly ($p < 0.02$) than that of the "no infarct" group (Table I).

Myocardial cyclic AMP content

Mean myocardial cyclic AMP contents of the sulfinpyrazone, aspirin and propranolol pretreated groups 30 min after isoproterenol injection were not different statistically from that of the control group. In addition, cyclic AMP revealed no statistical differences among the sulfinpyrazone, aspirin and propranolol groups. The "no infarct" group showed the lowest myocardial cyclic AMP content, and this was considered to be the cyclic AMP of an intact rat heart. The cyclic AMP content of the "no infarct" group was significantly lower than that of the sulfinpyrazone, aspirin, propranolol, and control groups (Table I).

Plasma PGE₁ and PGF_{2α} concentrations

Mean plasma PGE₁ and PGF_{2α} concentrations of the sulfinpyrazone and 50 mg/Kg aspirin groups were both significantly lower than those of the control group. However, plasma PGE₁ and PGF_{2α} levels of the 5 mg/Kg aspirin group showed no statistical difference compared with the control group. Plasma PGE₁ and PGF_{2α} levels of the sulfinpyrazone group were significantly lower than those of the 50 mg/Kg aspirin group (Table II).

Table II. Effects of Drugs on Plasma Prostaglandin (PG) Concentration

	PG E ₁ (pg/ml)	PG F _{2α} (pg/ml)
Control	100.9±6.7	76.8± 6.3
Aspirin 5 mg/Kg	93.9±5.4	77.1±10.0
Aspirin 50 mg/Kg	66.4±8.1**	50.0± 1.9**
Sulfinpyrazone 100 mg/Kg	19.9±5.3***	14.8± 3.3***

Drugs were administered for 14 days successively. Control group received only water. Values are mean ± SEM.

** $p < 0.005$ compared to control, *** $p < 0.001$ compared to control.

DISCUSSION

The enzymatic method to estimate myocardial necrosis consists of successive measurement of creatine kinase (CK) activity in blood or myocardium.^{9),10)} Mueller and his co-workers have reported that myocardial CK activity begins to decrease 6 hours after isoproterenol (5 and 80 mg/Kg) injection, and is most depleted from 24 to 48 hours after the injection in the rat. It then begins to increase again.¹⁷⁾ Using 40 mg/Kg of isoproterenol injection, our preliminary study also supported Mueller's results. So we determined myocardial CK activity 24 hours after isoproterenol injection and

thought that this might reflect the minimum myocardial CK and the maximum necrotic zone. Previously, we had also tried histopathological determination of myocardial necrosis in rats injected with isoproterenol by using nitro-blue tetrazolium and Masson's trichrome stainings, but these methods were somewhat subjective and complicated. Therefore, we preferred the enzymatic method to the histological technique.

Myocardial 3', 5'-cyclic adenosine monophosphate (cyclic AMP) content is considered to be a good index to represent the level of cardiac β -adrenergic drive.^{7),11)} When rat myocardium is incubated with isoproterenol *in vitro*, myocardial cyclic AMP rapidly increases within 2 min and becomes highest 5 min after starting the incubation.¹⁸⁾ When a rat is injected with isoproterenol (5.25 mg/Kg) in *in vivo* study, myocardial cyclic AMP showed its peak value 1 hour after the injection.⁷⁾ Taking such reports into regard, we measured the myocardial cyclic AMP content 30 min after isoproterenol injection, expecting that cyclic AMP during this period might reflect its peak value.

Both sulfinpyrazone and aspirin were reported to inhibit the cyclooxygenase in the prostaglandin (PG) synthetase system,^{4),5)} and intrinsic PGs have been purported to modulate the effects of catecholamines on various organs.¹⁹⁾ Therefore, we measured plasma PGE₁ and PGF_{2 α} levels after the administration of sulfinpyrazone and aspirin to estimate the magnitude of the intrinsic PG suppression.

Rona stated that isoproterenol injection of 85 mg/Kg or less brought no necrotic change in any organs except the heart.³⁾ He also found that the coronary artery was intact and patent. Accordingly, the isoproterenol-induced myocardial necrosis is a "noncoronarogenic" infarction and the pathogenesis of the necrosis is different from that of the ligation-induced infarction. Rona thought that relative hypoxemia induced by the vasodepressive and inotropic effects of isoproterenol was the macroscopic cause of cardiac necrosis.³⁾ Fleckenstein considered this model on cellular and molecular levels and stressed the role of calcium ions (Ca⁺⁺). He thought that the effect of isoproterenol was directly cardiotoxic and was due to excess Ca⁺⁺ inflow to the myocardial fiber, resulting in energy consumption, adenosine triphosphate and creatine phosphate depletion, and cellular death.²⁰⁾ Haft thought that catecholamine-induced myocardial injury was secondary to platelet aggregation in the coronary microvasculature,²¹⁾ and showed that antiplatelet agents such as aspirin and dipyridamole had a prophylactic effect in dogs.²²⁾ But recently, Moschos showed the opposite result by describing that the induced myocardial necrosis was not related to microvascular occlusion by platelets.²³⁾ In the canine infarction model, he also reported that aspirin did not have a myocardial preserving effect, but had an antiarrhythmic effect which

was independent of the platelet aggregation inhibiting action of aspirin.²⁴⁾ Sulfinpyrazone has not been tried in this model.

According to our results, the premedication of sulfinpyrazone and propranolol reduced isoproterenol-induced myocardial necrosis, while that of aspirin did not reduce the necrosis compared with the control group. Increase in heart weight was also prevented by the premedication of sulfinpyrazone and propranolol, and was not prevented by that of aspirin. Since heart weight increases in proportion to the dosage of injected isoproterenol,³⁾ this result suggests the reduced cardiotoxic effect of isoproterenol due to sulfinpyrazone and propranolol pretreatment and supports the CK preserving effect of the 2 drugs. Myocardial cyclic AMP content increased to about twice of the initial ("no infarct" group) value 30 min after isoproterenol injection. The degree of increment was not suppressed in sulfinpyrazone and propranolol pretreated groups during this period. Plasma PGs were suppressed by sulfinpyrazone and 50 mg/Kg of aspirin, and were not suppressed by 5 mg/Kg of aspirin.

If platelet aggregation is not a major cause of the isoproterenol-induced myocardial necrosis,²³⁾ we have to consider other mechanisms to account for the cardioprotective effect of sulfinpyrazone. As myocardial cyclic AMP was not suppressed in sulfinpyrazone group compared with the control group, so sulfinpyrazone might have been cardioprotective against β -adrenergic overstimulation by acting posterior to the second messenger (i.e. cyclic AMP) or by affecting some other system independent of myocardial cyclic AMP. However, since cyclic AMP elevation was not depressed by the β -blocker during this period, further examination is needed to conclude these hypotheses. Another explanation is that sulfinpyrazone was cardioprotective due to intrinsic PG suppression. However, 50 mg/Kg of aspirin was not cardioprotective in spite of plasma PG suppression. Therefore, the suppression of intrinsic PGs does not seem to result in the reduction of β -stimulation and the cardiac protection. Other effects of sulfinpyrazone such as an anti-inflammatory effect or protective effect on vascular endothelium²⁵⁾ should also be considered. Propranolol preserved myocardial CK activity in a dose related manner. However, myocardial cyclic AMP was not suppressed in the propranolol group 30 min after isoproterenol injection. One explanation of such a discrepancy is that the β -blocking effect of propranolol was overcome by a larger dose of isoproterenol since the β -blocking action of propranolol is competitive, and the non-specific direct effect of propranolol on the cell membrane^{8),26)} might have been responsible for the myocardial CK preservation. Another possibility is that cyclic AMP 30 min after isoproterenol injection did not represent the peak level of myocardial cyclic AMP after the injection.

Moncada showed that a high dose of aspirin (50–150 mg/Kg) blocked both vessel wall prostacyclin (prostaglandin I_2) and platelet thromboxane A_2 formation, but a low dose of aspirin (5–10 mg/Kg) blocked only thromboxane A_2 and decreased platelet aggregability.²⁷⁾ Therefore, we tried both a large (50 mg/Kg) and a small (5 mg/Kg) doses of aspirin, but the myocardial CK preservation was not different between the 2 groups.

Our results showed that aspirin had no prophylactic effect on the cardiac necrosis while sulfinpyrazone and propranolol were cardioprotective. Clinically, sulfinpyrazone reduced cardiovascular mortality and sudden death in patients who had survived a myocardial infarction,²⁸⁾ and the β -adrenergic blockade also reduced reinfarction and sudden death.²⁹⁾ On the other hand, aspirin reduced the frequency of transient cerebral ischemic attack and cerebral infarction.³⁰⁾ The prophylactic effect of aspirin against myocardial infarction or its recurrence has not been established yet,³¹⁾ but the pessimistic results have been reported.^{32), 33)} Our results may provide an experimental basis to such differences in clinical medicine between sulfinpyrazone and propranolol on the one hand and aspirin on the other.

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