Protection of Rats from Isoproterenol Induced Myocardial Necrosis by Trolnitrate Phosphate (Metamine®)

Experimental myocardial necrosis in rats following isoproterenol administration was first observed by RONA et al.[1] and CHAPPEL et al.[2] and adapted to evaluation of drugs with a possible protective action in myocardial infarction. The technique was used for evaluation of antianginal drugs by ZBINDEN and BAGDON[3], who suggested that isoproterenol-induced lesions may be due to hypoxia, since isoproterenol is known to cause myocardial ischemia[4] and to increase oxygen consumption and heart work. This hypothesis was strengthened by the observation that isoproterenol-induced myocardial necrosis is more severe in animals placed in an oxygen-deficient atmosphere[5].

Hypoxia may not be the only mechanism of isoproterenol-induced myocardial necrosis. RONA et al.[6] observed that a potassium deficient diet increases the severity of lesions. According to BAJUSZ[7] decrease in intracellular potassium and in glycogen deposits in myocardial cells follows administration of sympathomimetic amines and precedes any structural changes. Another possible contributing factor may be the effect of isoproterenol on lipid metabolism. Lipid mobilization follows the administration of isoproterenol, and in the early stages of isoproterenol-induced myo-cardial necrosis lipid droplets accumulate in the myocardium[8].

Various drugs have been evaluated for their activity in preventing isoproterenol-induced myocardial necrosis. ZBINDEN[5,9,10] described the activity of hydrazine type monoamine oxidase inhibitors in reducing the severity of isoproterenol-induced myocardial necrosis. He suggested that monoamine oxidase inhibitors may prevent myocardial necrosis by decreasing myocardial oxygen requirements. The protective activity of nialamide (Niamid®) against plasmocid- or isoproterenol-induced myocardial necrosis in rats was reported by BAJUSZ and JASMIN[11] and was observed in our laboratories by FINKELSTEIN and STEBBINS[12].

There is no evidence in the literature that nitroglycerin or nitrites prevent isoproterenol-induced necrosis. ZBINDEN[10] administered sodium nitrite to rats in the diet at dose levels from 7 to 178 mg/kg/day and found no protection from myocardial necrosis induced by isoproterenol at a high dose level (80 mg/kg s.c.). Despite this negative result, we decided to evaluate trolnitrate phosphate by a

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Effect of trolnitrate phosphate on isoproterenol-induced myocardial necrosis in rats

Exp. No.	Treatment	Dose of isoproterenol	Dose of trolnitrate phosphate	No. of rats	No. of deaths during treatment	Myocardial necrosis, average scores ± S.E.		
						Right ventricle	Intra-ventricular septum	Left ventricle
1	Isoproterenol + 1 h later trolnitrate phosphate	2 mg/kg/day i.p. for 5 days	20 mg/kg/day p.o for 5 days	10	1	0.8 ± 0.03	2.8 ± 0.48	1.7 ± 0.48[a]
	Isoproterenol + 1 h later H_2O	same	none	10	0	0.3 ± 0.11	3.8 ± 0.07	3.3 ± 0.11
2	Trolnitrate phosphate + 1 h later isoproterenol	same	20 mg/kg/day p.o. for 5 days	30	2	0.5 ± 0.10	2.7 ± 0.16[a]	2.1 ± 0.13[a]
	H_2O + 1 h later isoproterenol	same	none	30	0	0.4 ± 0.09	3.8 ± 0.08	3.4 ± 0.13
3	Trolnitrate phosphate + 1 h later isoproterenol	same	5 mg/kg/day p.o. for 5 days	10	0	1.0 ± 0.80	3.9 ± 0.10	3.3 ± 0.10
	Lactose + 1 h later isoproterenol	same	none	10	0	0.8 ± 1.40	4.0 ± 0	3.9 ± 0.10
4	Trolnitrate phosphate + 1 h later isoproterenol	10 mg/kg i.p. on the 4th day only	20 mg/kg/day p.o. for 5 days	20	0	1.5 ± 0.30	2.7 ± 0.20	2.8 ± 0.20
	H_2O + 1 h later isoproterenol	same	none	20	5	2.0 ± 0.30	3.2 ± 0.20	3.1 ± 0.30

[a] $p < 0.01$.

similar technique, using lower, but repeated doses of isoproterenol.

It has been previously observed in our laboratory that trolnitrate phosphate reduced left ventricular work and myocardial oxygen consumption in dogs[13]. It was reasonable to assume that under the condition of reduced myocardial work and reduced oxygen requirement, isoproterenol might be less likely to produce myocardial necrosis if hypoxia were a contributing factor to this condition. This report summarizes our experiments on the protective effect of trolnitrate phosphate on isoproterenol-induced myocardial necrosis in rats.

$$N \begin{cases} CH_2CH_2\text{–}ONO_2 \\ CH_2CH_2\text{–}ONO_2 \\ CH_2CH_2\text{–}ONO_2 \end{cases} \cdot 2\,H_3PO_4$$

Trolnitrate phosphate – Metamine®

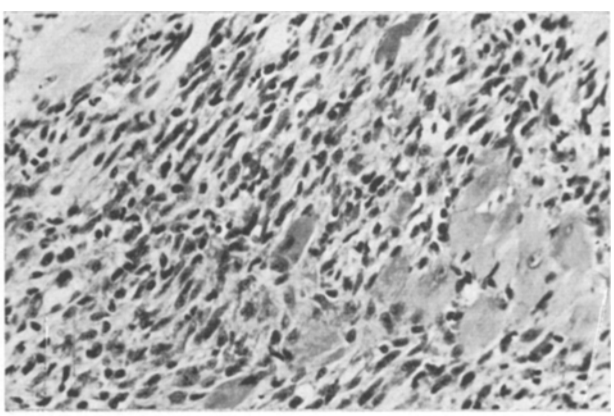
Male Wistar rats of 200 to 300 g body weight were used in all experiments. Unless otherwise specified the animals were kept on Rockland pellets and water, both ad libitum. Isoproterenol was given in most of our experiments at 2 mg/kg i.p., a lower dose than that used by other investigators. The isoproterenol injections were repeated daily for 5 days. In one experiment (No. 4) isoproterenol was given at 10 mg/kg i.p., but only on the 4th day of treatment. Trolnitrate phosphate was given either at 5.0 or at 20.0 mg/kg by gavage, daily for 5 days.

Microscopic sections were prepared from three different myocardial regions: right ventricle, intraventricular septum, and left ventricle; they were examined microscopically for the presence of lesions. The scores 0–4 were given to each section: 0, no lesions; 1, just detectable lesions; 2, moderate, but isolated lesions; 3, severe, still isolated lesions; 4, massive diffuse areas of necrosis. The statistical significance of the difference in scores for myocardial necrosis in animals treated with trolnitrate phosphate and for the control groups was evaluated with the Student '*t*' test.

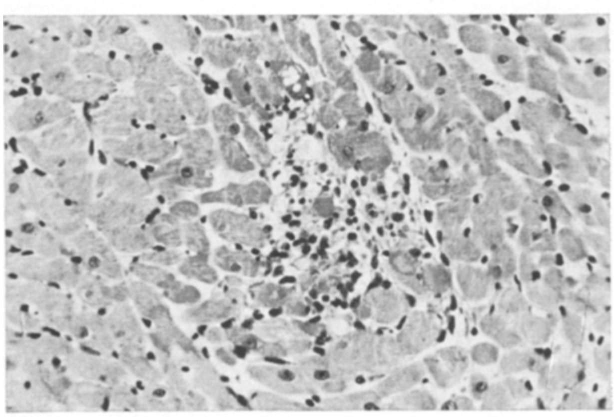
Our results indicated that trolnitrate phosphate protects rats from isoproterenol-induced myocardial necrosis. The protective activity was demonstrable at 20 mg/kg per day of trolnitrate phosphate but not at 5 mg/kg per day. If isoproterenol was given at 10 mg/kg i.p. on the 4th day of therapy instead of daily administration at 2 mg/kg i.p., the protective activity of trolnitrate phosphate was less pronounced (Table). The typical lesions in animals treated with isoproterenol only and with isoproterenol and trolnitrate phosphate are shown in the Figure (a and b).

Trolnitrate phosphate, like other antianginal agents[14], reduces left ventricular work[13]. Under conditions of reduced work, isoproterenol may produce less hypoxic changes in myocardium, and ischemia was shown to precede the development of isoproterenol-induced necrosis in hamsters (HANDFORTH[4]). The observed protective effect of trolnitrate phosphate may be explained by reduction in cardiac work induced by trolnitrate phosphate leading to an unspecific antagonism of cardiac stimulant actions of isoproterenol.

Other explanations for the protective effect of trolnitrate phosphate are possible. Compensatory mechanisms activated in response to stress which could have been produced by the drug at this relatively high dose level, may account for the protection. Also, absorption of injected isoproterenol may have been delayed by circulatory changes induced by trolnitrate phosphate. Nevertheless, the demonstration of protective effect of trolnitrate phosphate suggests the possibility, that isoproterenol-induced myocardial necrosis may be a useful experimental procedure in the evaluation of potential antianginal agents.



a



b

a, Typical massive necrosis in the left ventricle of a rat heart. The animal received isoproterenol, 2.0 mg/kg/day i.p., for 5 days. b, Typical isolated area of myocardial necrosis in the left ventricle of a rat heart. The animal received trolnitrate phosphate, 20 mg/kg/day orally for 5 days followed by isoproterenol at 2.0 mg/kg/day i.p. for 5 days. The animals were sacrificed 2 h after the last treatment. Bouin fixation. Paraffin section. Hematoxylin and eosin. ×400.

Zusammenfassung. Isoproterenol (Isoprenalin), 2 mg/kg pro Tag für 5 Tage i.p., ruft Myocardnekrosen in Ratten hervor, die durch wiederholte Nach- oder Vorbehandlung mit Metamin® (Trolnitrat, Triäthanol-amintrinitrat), 20 mg/kg pro Tag für 5 Tage p.o., abgeschwächt werden können.

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Department of Pharmacology, Medical Research Laboratories, Chas. Pfizer & Co., Groton (Conn. USA), October 1, 1965.

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