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Effect of Policosanol on Isoprenaline-induced Myocardial Necrosis in Rats

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Abstract—Policosanol is a mixture of higher aliphatic primary alcohols isolated from sugar cane (*Saccharum officinarum* L.) and octacosanol represents its main component. This study was conducted to examine the effects of policosanol on myocardial necrosis induced by subcutaneous injection of isoprenaline in rats. A significant reduction ($P < 0.01$) of infarct size, polymorphonuclear cells and mast cells was observed in animals treated with policosanol at 5 or 25 mg kg^{-1}, while animals receiving only acetysalicylic acid pretreatment showed a significant decrease in the infarct area ($P < 0.05$). No significant differences in polymorphonuclear and mast cells were obtained when compared with positive control data. It is concluded that policosanol delays the evolution of infarction, showing a protective effect on the myocardial necrosis induced by isoprenaline in this experimental model.

Policosanol is a mixture of high molecular weight aliphatic primary alcohols, isolated from sugar cane (*Saccharum officinarum* L.). Octacosanol is the major component, followed by triacontanol and hexacosanol, while minor components are tetracosanol, heptacosanol, nonacosanol, dotriacontanol and tetratriacontanol.

Thromboxane A_2 (TxA_2) has been implicated in the pathophysiology of experimental myocardial ischaemia (Brezinski et al 1985, 1987; Hock et al 1986) and in human ischaemic heart disease (Walinsky et al 1984; Vesterqvist et al 1985). Since orally administered policosanol produced a significant reduction of thromboxane B_2 (TxB_2) (a stable metabolite of TxA_2), an increase of prostacyclin levels in rodents (Arruzazabala et al 1991, 1992) and protection in experimental models of brain ischaemia (Carbajal et al 1993), this work was undertaken to investigate whether single oral doses of policosanol also had a protective effect on isoprenaline-induced myocardial necrosis in rats.

Materials and Methods

Effects of policosanol on isoprenaline-induced myocardial necrosis

Forty-eight Wistar male rats, 200±20 g, from Cuba-Veterinaria were adapted to laboratory conditions for one week with free access to food and water. Rats were randomly distributed into six groups (8 animals/group).

Policosanol or acetylsalicylic acid was orally administered as a suspension in 2% Tween 20/water by gastric gavage (1 mL kg^{-1}). After 2 h, myocardial damage was induced by subcutaneous injection of isoprenaline (25 mg kg^{-1}) and after a further 24 h, the animals were killed by ether anaesthesia.

Hearts were rapidly removed, sliced, photographed and fixed in a 10% buffer formaldehyde. Subsequently, they were embedded in paraffin, sectioned and stained with haematoxylin and eosin for light microscopy. Toluidine blue and Stevenel blue (Noa et al 1985) stains were used to study mast cells and glycosaminoglycans. Fifteen myocardial sections were studied for each animal.

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Infarct areas were measured according to their weight as described previously (Kormoczy et al 1987). In brief, the stained serial sections prepared were projected onto a paper. The infarct areas were marked with a pencil, cut out from the paper and weighed. From these data the mean values for each heart and group were calculated. Infarct areas were recognized only if necrosis of cardiac cells, exudative infiltration and oedema were present. The infarct size observation and quantification were performed by an uninformed observer.

Quantification of polymorphonuclear neutrophils (PMN) and mast cells was carried out by cell counts in 10 infarct-slide areas chosen at random after a 40× magnification for all studied groups (Wargovich et al 1987; Seibold et al 1990). This quantification was also carried out by an uninformed observer.

The occurrence of glycosaminoglycans was semiquantitatively identified in terms of unchanged, increased or decreased, relative to the appearance of the negative control groups.

The comparison between groups of PMN and mast cells as well as the infarct area data was performed using the Mann-Whitney U-test.

Effects of policosanol on isoprenaline-induced chronotropism

Twenty Sprague-Dawley rats, 250±20 g, from Centro Nacional de Producción de Animales de Laboratorio (Cuba) were adapted to laboratory conditions for one week and randomly distributed in two groups (10 animals/group). Food and water were freely available.

Policosanol was suspended in 2% Tween 20/water and orally administered at 25 mg kg^{-1} by gastric gavage, 2 h before subcutaneous injection of isoprenaline.

Animals were anaesthetized with endovenous injection of 30 mg kg^{-1} sodium pentobarbitone and electrocardiograms recorded on a Nihon Kohden polygraph before (t = 0) and 5, 10 and 20 min after isoprenaline injection.

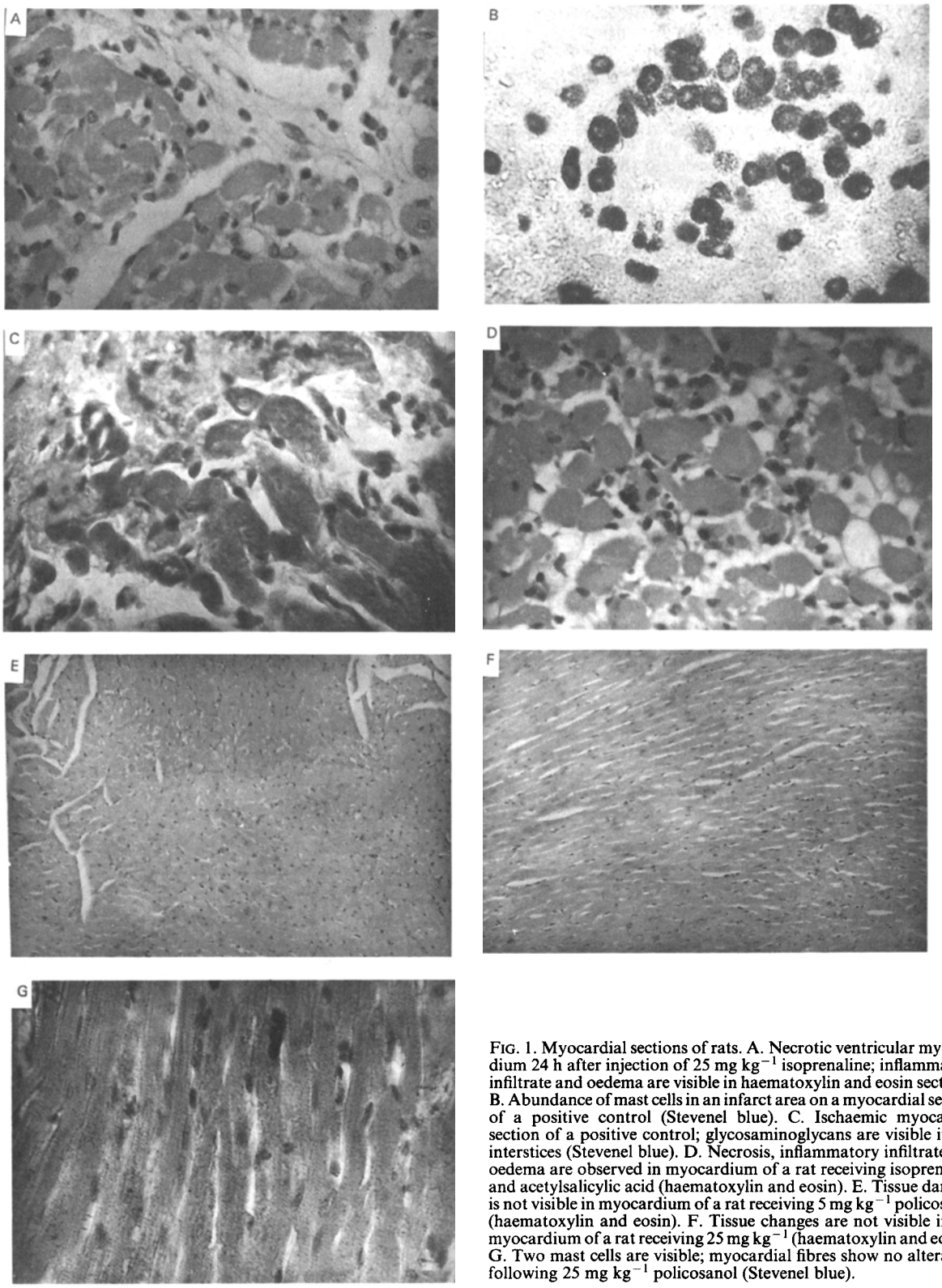

FIG. 1. Myocardial sections of rats. A. Necrotic ventricular myocardium 24 h after injection of 25 mg kg^{-1} isoprenaline; inflammatory infiltrate and oedema are visible in haematoxylin and eosin sections. B. Abundance of mast cells in an infarct area on a myocardial section of a positive control (Stevenel blue). C. Ischaemic myocardial section of a positive control; glycosaminoglycans are visible in the interstices (Stevenel blue). D. Necrosis, inflammatory infiltrate and oedema are observed in myocardium of a rat receiving isoprenaline and acetylsalicylic acid (haematoxylin and eosin). E. Tissue damage is not visible in myocardium of a rat receiving 5 mg kg^{-1} policosanol (haematoxylin and eosin). F. Tissue changes are not visible in the myocardium of a rat receiving 25 mg kg^{-1} (haematoxylin and eosin). G. Two mast cells are visible; myocardial fibres show no alteration following 25 mg kg^{-1} policosanol (Stevenel blue).

Table 1. Effects of policosanol on myocardial necrosis induced by isoprenaline in rats. Values are % change from control values.

Dose (mg kg^{-1})	Infarct area (g)	PMN (±s.d)	Mast cells	Glycosaminoglycans
No treatment	0·0	0·0	0·0	—
Isoprenaline only	1·91±0·13	27·8±3·56	3·0±0·71	Increased
Policosanol				
1	1·85±0·15 97·3%	26·3±3·18	2·7±0·94	Increased
5	0·41±0·14** 21·4%	7·2±2·59**	1·4±0·55*	Decreased
25	0·63±0·29** 32·9%	4·6±1·81**	1·2±0·45*	Decreased
Acetylsalicylic acid				
30	1·41±0·17* 73·2%	28·0±4·85	2·8±0·84	Increased

*$P<0·05$; **$P<0·01$ (Mann-Whitney U-test) compared with the positive control.

Table 2. Effect of policosanol on isoprenaline induced chronotropic action in rats.

	Time (min)		
	5	10	20
Controls	93·00±27·29	70·5±23·07	36±19·59
Policosanol (25 mg kg^{-1})	95·14±35·56	76·8±32·26	52±33·65

The Mann-Whitney U-test indicated no significant differences between controls and policosanol-treated animals. Values are mean±s.d.

Results

Effects of policosanol on isoprenaline-induced myocardial necrosis

Three rats receiving isoprenaline and acetylsalicylic acid and one from the positive control group (isoprenaline) died before animal necropsy was performed. No deaths occurred among policosanol-treated groups.

Autopsies of the rats showed that animals in the negative control group showed no heart microscopic alterations. All surviving animals in the positive control group developed a whitish-yellowish colour, especially on heart tips, as usually found in isoprenaline-induced damage.

All rats receiving 1 mg kg^{-1} policosanol showed a similar pattern to the positive controls.

Three rats receiving 5 mg kg^{-1} policosanol showed some heart tip areas slightly paler than those in negative controls. Rats receiving 25 mg kg^{-1} policosanol showed no gross colour changes in the heart, except one which showed a somewhat yellowish area. All surviving animals receiving 30 mg kg^{-1} acetylsalicylic acid showed injuries similar to those in positive controls.

Histology of the hearts of isoprenaline-treated animals, including those also receiving acetylsalicylic acid showed characteristic cardiac cell necrosis, inflammatory infiltrate and the presence of glycosaminoglycans, oedema and mast cells (Fig. 1A–D). These characteristics were also observed in animals receiving 1 mg kg^{-1} policosanol and to a lesser extent in those receiving the higher doses (Fig. 1E–G).

Table 1 shows infarct areas, PMN and mast cell values for the different groups. Glycosaminoglycans are also presented.

Table 2 summarizes data obtained when effects of policosanol on isoprenaline-induced chronotropic action were investigated. Policosanol orally administered 2 h before isoprenaline injection did not affect the positive chronotropic effect induced by isoprenaline.

Discussion

Our results confirm that subcutaneous injection of 25 mg kg^{-1} isoprenaline induced cardiac necrosis in rats (Wexler & Kittinger 1963; White & White 1986; Pérez-Cao et al 1989). This experimental model of myocardial infarction is not frequently used, as it shows some differences from the normal human myocardial infarction pattern, including the development of contraction band necrosis in this model and no coronary occlusion.

The present study has demonstrated that oral pretreatment with single doses of policosanol produces a beneficial effect on the myocardial injury developed in this model.

The significant decrease in the number of PMN cells in damaged areas of policosanol-treated rats was an interesting aspect of this study. It is known that PMN cells play an important role in cardiac damage during infarction (Romson et al 1982; Mullane et al 1984; Engler 1987), because these leucocytes release three groups of compounds participating in cell responses: proteases and lipases, free radicals as the superoxide anion, and lipid products resulting from phospholipase activation (Weissman et al 1980; Fantone & Ward 1982). Consequently, it has been reported that drugs reducing infarct size in experimental animals through different mechanisms also reduce the number of PMN cells in the

infarct zone. Thus, non-steroidal anti-inflammatory agents such as ibuprofen diminished infarct sizes due to a reduction of PMN cells present in the infarct area (Romson et al 1983), while thromboxane synthetase inhibitors such as U-63 and 557A also show a myocardial infarct area reduction, associated with a decrease of the PMN cells in the area (Wargovich et al 1987).

Our results indicate that policosanol was more effective than acetylsalicylic acid in preventing the development of infarction in this model. Moreover, while animals treated with acetylsalicylic acid did not show a decrease in PMN and mast cells, policosanol reduced not only the size of isoprenaline-induced myocardial infarction in rats, but also the presence of PMN and mast cells in the area, indicating a global action related to the decrease of myocardial tissue injury.

Previous studies demonstrated that policosanol not only significantly decreased TxB_2 levels in rats 2 h after oral administration (Arruzazabala et al 1991), but also increased prostacyclin levels (Arruzazabala et al 1992). This is consistent with differences between effects of acetylsalicylic acid and policosanol in this model, with acetylsalicylic acid acting by cyclo-oxygenase inhibition, reducing not only TxB_2 but also prostacyclin levels (Goodman 1987) and policosanol effecting TxA_2 and prostacyclin. It has been shown that TxA_2 may be important in the initiation of ischaemia and in the subsequent propagation of ischaemic damage because of its vasocontractor and platelet aggregatory activities (Ellis et al 1976).

Glycosaminoglycans decreased in policosanol-treated animals compared with positive controls. Wexler & Kittinger (1963) reported that the high concentration of this ground substance promptly disappeared if a good repair was produced in this experimental model. On the other hand, White & White (1986) also demonstrated that glycosaminoglycans disappeared from myocardial tissue by three to four days after isoprenaline injection. Since glycosaminoglycans have a high water binding capacity (Murata & Yokoyama 1989) they may play a role in the occurrence of oedema described in this model.

Further evaluation of policosanol oral treatment on other experimental models of myocardial infarction is needed to predict the clinical relevance of these effects.

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