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Effect of Policosanol on Foam-cell Formation in Carrageenan-induced Granulomas in Rats

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Abstract

Policosanol is a new cholesterol-lowering drug isolated and purified from sugar-cane wax, which prevents the development of lipofundin-induced lesions and foam-cell formation in New Zealand rabbits and Wistar rats. This study was conducted to examine the effects of policosanol on foam-cell formation in carrageenan-induced granulomas in rats.

Eighteen Wistar rats were randomly distributed in three experimental groups which received orally for 20 days Tween 20 H_2O as vehicle (control group) or policosanol at 2·5 or 25 mg kg^{-1}. At the 11th day, lipofundin was injected intraperitoneally for 8 days to induce formation of foam cells in the granuloma. At day 13, carrageenan was injected subcutaneously for granuloma induction and seven days later animals were killed. A significant reduction of the foam-cell formation in granulomas of policosanol-treated rats was observed.

It is concluded that policosanol prevents the development of foam cells in carrageenan-induced granulomas (extravascular medium) in rats.

The infiltration of monocyte-macrophage in the intima plays an important role in atherogenesis and provides further evidence of the inflammatory nature of the disease process as described previously (Kelley et al 1985). The presence of lipids in macrophages of the arterial wall with foam-cell formation is a prominent feature of the atherosclerotic lesion in man (Haust 1983; Munro & Cotran 1988) and in different non-human species.

Schwartz et al (1985) described the development of an inflammatory granuloma model to study the differentiation of monocyte-macrophage in foam cells. Policosanol is a mixture of higher aliphatic primary alcohols isolated from sugar cane (*Saccharum officinarum* L.) wax, the main component of which is octacosanol, followed by triacontanol and hexacosanol, while the other alcohols are minor components. Cholesterol-lowering effects of policosanol have been demonstrated in experimental models (Arruzazabala et al 1991a, b, 1992), healthy volunteers (Hernández et al 1992) and patients with type II hypercholesterolaemia (Illnait et al 1991).

We have also demonstrated that policosanol reduces lipofundin-induced lesions in rabbits and rats, including foam-cell formation (Noa et al 1992, 1995).

The aim of this work was to study the effect of policosanol on foam-cell formation in carrageenan-induced granulomas in rats (extra-vascular medium).

Materials and Methods

Animals

Eighteen Wistar male rats weighing, 200 ± 20 g, from Cenpalab were used and adapted to laboratory conditions for one week with free access to food and water. Rats were randomly distributed into 3 groups.

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Administration and dosage

Policosanol was orally administered as a suspension in a 2% Tween 20/water vehicle by gastric gavage (1 mL kg^{-1}) for 20 days.

Granuloma induction. Granulomas were induced as described by Schwartz et al (1985). In brief, rats were anaesthetized, the anterior abdominal wall was shaved, the skin sterilized and 10 mL sterile 1% (w/v) solution of carrageenan in physiological saline (0·85%, w/v) at 37 C was slowly injected subcutaneously in the midline of the anterior abdominal wall. Lipofundin S (Braun, Melsungen), a soy oil suspension (which contains medium chain triglycerides, phospholipids and glycerol) stabilized at a particle size of 1 μm was used for inducing foam-cell formation in granulomas.

Experimental groups

Group 1. Rats were treated with Tween 20/H_2O (1 mL kg^{-1}) for 20 days. From day 11, lipofundin was injected intraperitoneally (2 mL kg^{-1}) for 8 days. Two days after starting the lipofundin treatment, carrageenan was injected and after seven days animals were killed.

Groups 2 and 3. Animals were treated orally with policosanol at 2·5 or 25 mg kg^{-1} respectively. Lipofundin and carrageenan were administered as previously described.

Morphology

Rats were anaesthetized and the granulomas were harvested. Granuloma tissue samples (2–4 mm) were studied by light microscopy with the use of paraffin sections and

stained with hematoxylin and eosin and also by frozen sections that were stained with oil red O for neutral lipids (Martínez 1979).

For transmission electron microscopy, tissue samples were cut into 1–2 mm-thick slices (maximum) and kept for 1 h in 3·2% glutaraldehyde, 0·1 M phosphate buffer and then for another hour in 1% osmium tetroxide. After dehydration the tissue was included in Epon 812. Ultrathin sectioning was performed using an LKB ultramicrotome, Ultratome III model and stained with uranyl acetate and lead citrate. The study was conducted in a JEOL 100S electron microscope in a blind fashion.

Statistical analysis

For the extent of foam-cell formation, cells of sections stained with oil red O were counted and classified into 4 categories according to the extent of lipid storage as described by Yamamoto et al (1986). Class (−): cells without fat droplets; class (+): less than 20 tiny droplets were seen only in the peripheral zone of the cytoplasm, class (++): many small lipid droplets scattered all over the cytoplasm (>20) class (+ + +): cells are markedly enlarged with large vacuoles in the cytoplasm.

For statistical analysis, judgement of the foam cell was made when more than 10 clear vacuoles were found in cytoplasm by electron microscopy or when many lipid droplets were seen to be scattered all over the cytoplasm by light microscopy (+ of 20).

Statistical analysis was performed using the contingence table of square chi test of the package of programs of Microstat for the percent of cell type in rat granuloma and the Mann Whitney U-test for the values of foam cells on light and electron microscopy.

Results

Gross appearance of granulomas were indistinguishable between groups. In all animals they were of irregular form, each exhibiting a myxoid consistency with zones firmer, and a grey appearance with haemorrhagic mottling.

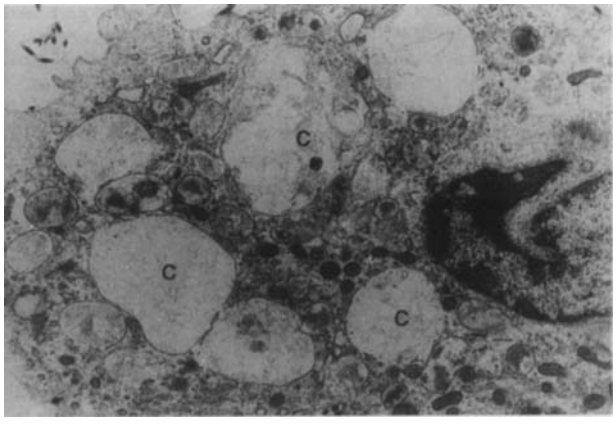

FIG. 2. A policosanol (2·5 mg kg^{-1})-treated macrophage showing large and small membrane bound inclusions containing a floccular material with a reticular pattern resembling ingested carrageenan (C). No lipid inclusions are seen. (×13 800).

Light microscopy

In haematoxylin and eosin-stained paraffin sections, granulomas from the control group showed groups of large macrophages distended by clear vacuolar lipid inclusions and could be recognized as foam cells. Some epithelioid cells, granulocytes and lymphocytes were also observed. The macrophages from these granulomas revealed considerable oil red O-stainable lipid in large and small cytoplasmic droplets.

In policosanol-treated groups (2 and 3), granulomas exhibited abundant macrophages with reticular inclusions, which were smaller than those of the control group: occasionally epithelioid cells, granulocytes and lymphocytes were observed.

Lipid staining with oil red O of frozen sections revealed that macrophages from these granulomas were essentially free of stainable lipid; only some cells showed the presence of fine cytoplasmic droplets.

Ultrastructure

There was agreement between the observations made at the light and electron microscopic levels. In the control group,

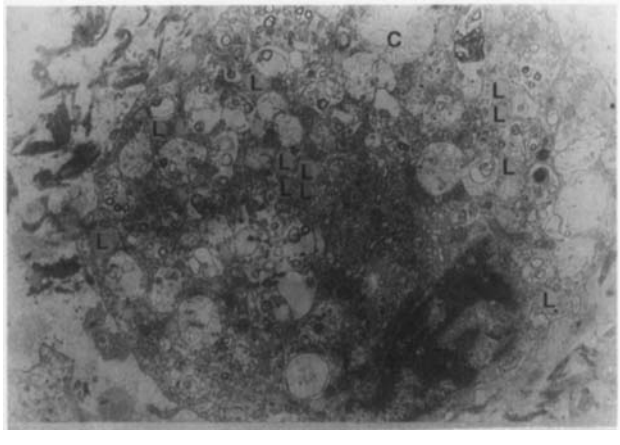

FIG. 1. A control group developing foam cell with large lysosomal carrageenan inclusions, more than ten lipid inclusions (L) and some myelin forms. Peripheral condensation of nuclear heterochromatin can be seen (×10 000).

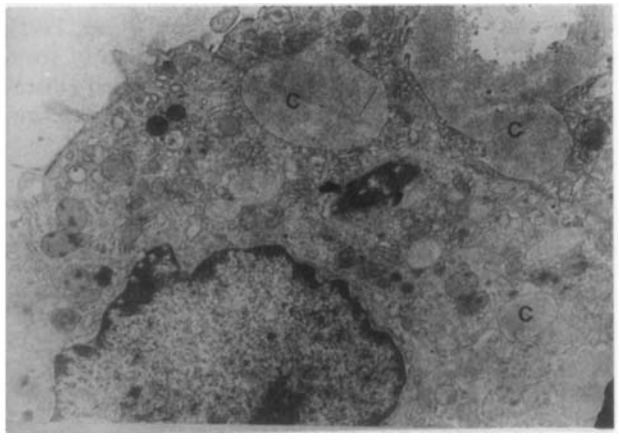

FIG. 3. A policosanol (25 mg kg^{-1})-treated macrophage showing some lysosomal carrageenan inclusions (C). No lipid inclusions are seen (×16 000).

Table 1. Effects of policosanol on distribution of foam cells and macrophages in rat granulomas.

Treatment	Dose mg kg^{-1}	Percent of each cell type referred to the total number of cells: Foam cells	Macrophages
Control	0	52*	44
Policosanol	2·5	3	90
Policosanol	25	2	92

* $P < 0{\cdot}05$ contingence table square chi test.

44% of granuloma cells were macrophages and 52% were identified as foam cells. Macrophages exhibited peripheral cytoplasmic projections, lysosomal carrageenan inclusions, and numerous electron-translucent inclusions. Foam cells showed numerous cytoplasmic inclusions some of them membrane-bound and others non-membrane-bound, some showing fusion between them. Furthermore, large and small carrageenan-containing inclusions were observed with a reticular pattern. Both the macrophages and foam cells contained numerous myelin bodies in the cytoplasm. Nuclear heterochromatin showed peripheral condensation (Fig. 1).

The granulomas of policosanol-treated groups (2 and 3) showed 90 and 92% of macrophages and 3 and 2% of foam cells, respectively. Macrophages from these two groups showed carrageenan-containing inclusions with reticular pattern. Lipid inclusions and myelin bodies were practically absent (Figs 2 and 3). Foam cells were scarce in these two groups (Tables 1, 2 and 3).

Discussion

In our work, lipofundin instead of a cholesterol-rich diet was used to induce the formation of foam cells in the carrageenan-induced granuloma. Lipofundin is a soy oil extract clinically used for parenteral nutrition. Jellinek et al (1982) described a model for inducing atherosclerosis in rats with this product. This model has the advantage of causing atherosclerotic lesions in only 8 days unlike hypercholesterolaemic diets where morphologic lesions appear only after several months. Noa et al (1992, 1995), described the effect of policosanol on reduction of foam cells of rabbits and rats with lipofundin-induced atherosclerotic lesions. A significant reduction of the atherosclerotic lesions in the treated animals was also observed.

Table 2. Foam cell formation.

Treatment	Dose (mg kg^{-1})	Number of foam cells (mean ± s.d.): Light microscopy	Electron microscopy
Controls	0	52 ± 2·3	58 ± 2·3
Policosanol	2·5	3 ± 0·3**	3 ± 0·2**
	25	2 ± 0·3**	2 ± 0·4**

Values are the average of 10 countings from different visual fields of each animal per group. ** $P < 0{\cdot}003$ (Mann Whitney U-Test).

Table 3. Effect of policosanol on foam-cell formation. Classification of lipid-laden cells into 3 categories according to the extent of lipid storage.

	Controls	Policosanol (mg kg^{-1})	
		2·5	25
Non-foam cells class (−)	44	90	91
Foam cells			
class (+)	2	2	1
class (++)	12	1	1
class (+ + +)	40	2	1

Values show the percentage of the cells belonging to each class with respect to the total number of cells.

In the present work characteristics of the carrageenan-induced granulomas in rats which had received lipofundin injection were similar to those reported by Schwartz et al (1985) in rabbits which became hypercholesterolaemic with a cholesterol-rich diet. A significant reduction of foam-cell formation in granulomas of policosanol-treated rats (2·5 and 25 mg kg^{-1}) was demonstrated.

Skrinska et al (1988) showed that rabbits fed with a hypercholesterolaemic diet and treated with the thromboxane-synthetase inhibitor TXA_2 (UK-38485) developed significantly less lesions by foam cells than those fed the atherogenic diet only. In addition, they suggested that TXA_2 inhibition might prevent monocyte activation when PGE_2 synthesis increases and may affect occurrence of early atherosclerotic lesions when preventing monocyte function.

Thus, the results of this work on the prevention of foam-cell development in carrageenan-induced granulomas in rats could be related to the reduction of TXA_2 levels (Arruzazabala et al 1991a), and the increase of prostacyclin levels (Arruzazabala et al 1993), induced by policosanol. Taking into account these results, as well as previous data showing inhibitory effects of policosanol on foam-cell formation in lipofundin-induced atherosclerotic lesions in rabbits and rats (Noa et al 1992, 1995), we can conclude that policosanol prevents the development of foam cells in the atherosclerotic lesions as well as in the granulomas (extravascular medium).

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