J. Pharm. Pharmacol. 1995, 47: 289–291
Received July 22, 1993
Accepted September 7, 1994

Effect of Policosanol on Lipofundin-induced Atherosclerotic Lesions in Rats

M. NOA, R. MÁS, M. C. DE LA ROSA AND J. MAGRANER

National Center for Scientific Research, Havana, Cuba

Abstract

Policosanol is a mixture of higher aliphatic alcohols isolated from sugar cane wax, showing cholesterol-lowering effects and preventing the development of lipofundin-induced lesions in New Zealand rabbits. This study was conducted to determine whether policosanol orally administered to rats also protects against the development of lipofundin-induced atherosclerotic lesions.

Fifty four male Wistar rats were randomly distributed amongst a negative control group, a positive control group intravenously injected with lipofundin for eight days, and four experimental groups also injected with lipofundin, but orally receiving policosanol at 0·5, 2·5, 5 and 25 mg kg^{-1}, respectively. Policosanol treatment was orally administered once-a-day for eight days, while control groups similarly received equivalent amounts of vehicle. A significant reduction of the atherosclerotic lesions in the treated animals was observed.

It is concluded that policosanol has a protective effect on lipofundin-induced aortic lesions in Wistar rats.

In different experimental models many authors have studied the ultrastructural characteristics of atherosclerotic lesions, both spontaneous (Haust 1978a,b, 1983) and induced (Vesselinovitch & Wissler 1980; Kritchevsky et al 1982; Joris et al 1983; Noa & Illnait 1987).

Jellinek et al (1982) described a model for inducing atherosclerosis with the use of lipofundin, an emulsion containing soy oil extract, middle chain triglycerides, phospholipids and glycerol, which is clinically used for parenteral nutrition. This model has the advantage of causing atherosclerotic lesions in only 8 days, unlike hypercholesterolaemic diets where morphologic lesions appear only after several months.

Policosanol is a natural mixture of eight high-molecular weight alcohols isolated and purified from sugar cane (*Saccharum officinarum* L.) wax, whose main component is octacosanol, followed by triacontanol and hexacosanol, while the other alcohols are minor components. It has been demonstrated that oral treatment with policosanol induces cholesterol-lowering effects in animal models (Arruzazabala et al 1991a, 1992b; Cruz-Bustillo et al 1991; Rodríguez Echenique et al 1992), in healthy volunteers (Hernández et al 1992) and in patients with type II hypercholesterolaemia (Illnait et al 1991; Castaño et al 1991; Pons et al 1992, 1993; Aneiros et al 1993). In all cases, treatment significantly reduced total cholesterol and low-density lipoprotein cholesterol (LDL-C). Since hypercholesterolaemia and mainly elevated LDL-C levels are considered as major risk factors for atherosclerosis (Keys 1970; Kannel et al 1971), drugs which decrease levels of both total cholesterol and LDL-C theoretically would have an impact on atherosclerotic lesion development.

As policosanol reduces both cholesterol and LDL-C levels, the aim of this study was to investigate whether policosanol treatment to rats protects the development of lipofundin-induced aortic lesions.

Correspondence: M. Noa, Laboratory of Histology, National Center for Scientific Research, PO Box 6880, Havana, Cuba.

Materials and Methods

Animals

A group of 54 young adult male Wistar rats (4 weeks old) (200 ± 20 g) was used. They were randomly distributed in six groups and were housed in an air-conditioned room (23 ± 2°C, 60 ± 10% r.h.) under an artificial 12 h light/dark cycle for one week, with water and food freely available. Animals were fed a standard chow for rats made by the National Center for Laboratory Animals Production (CENPALAB).

Administration and dosage

Lipofundin S (Braun, Melsungen), a soy oil suspension stabilized at a particle size of 1 μm is clinically used for parenteral nutrition. Policosanol was orally administered as a suspension in a Tween 20/water (2%) vehicle.

Experimental groups comprised a negative control (nine animals without treatment); a positive control (rats daily injected intraperitoneally with lipofundin (2 mL kg^{-1}) for eight days); animals injected with lipofundin and a daily oral dose of policosanol (0·5 mg kg^{-1}); animals receiving lipofundin + policosanol (2·5 mg kg^{-1}); animals receiving lipofundin + policosanol (5 mg kg^{-1}); and animals receiving lipofundin + policosanol (25 mg kg^{-1}). Policosanol was orally administered through a gastric probe (1 mL kg^{-1}), while the rats in the control groups received equivalent volumes of vehicle Tween 20/H_2O by the same route. The last dose was administered 16 h before the animals were killed.

Morphological study

At the end of the experiment, animals were anaesthetized with ether, and aortas were removed and analysed macroscopically. Samples were taken from the aortas and processed for electron microscopy. The samples were cut into 1 to 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

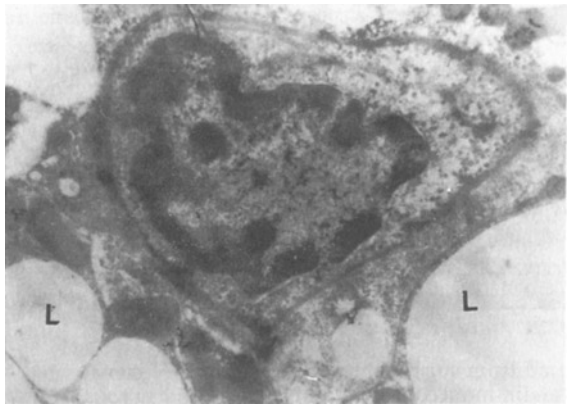

FIG. 1. Foam cell of aortic intima from a positive control rat showing many droplets of lipids (L) in the cytoplasm. Magnification ×8700.

was included in Epon 812, and ultrathin sections were prepared using an LKB ultramicrotome (Ultratome III) and stained with uranyl acetate and lead citrate. The study was conducted in a JEOL 100 S electron microscope in a double-blinded fashion. Aortic lipofundin-induced lesions were identified when lipid droplets in cells, nuclear envelope dilatation and abundant collagenous and elastic fibres were present in the intima.

Comparison among groups of rats presenting lipofundin-induced lesions was performed using the Fisher exact probability test. The occurrence of a foam cell was judged when more than 10 lipid droplets were present in the cytoplasm of intimal cells. Comparison among groups of the mean number of foam cells per animal was by the Mann-Whitney U-test.

Results

In the macroscopic study, no atherosclerotic lesions were found in any group, including the positive control group. The ultrastructural study showed that the aortic intimal cells in all rats (9/9) treated only with lipofundin had lipid droplets in their cytoplasm (foam cells), nuclear envelope dilatation, and fibrosis with abundant collagenous and elastic fibres, characteristics of lipofundin-induced lesions (Figs 1–3). The group of rats daily dosed with lipofundin + policosanol at the low dose of 0·5 mg kg^{-1} did not show

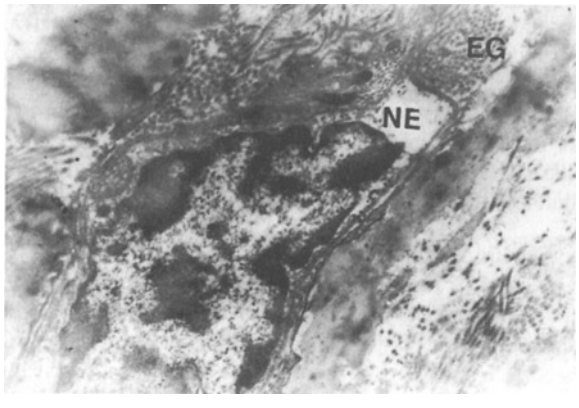

FIG. 2. Another cell of aortic intima from a positive control rat with nuclear envelope dilatation (NE) and abundant elastic granules (EG) with microfibrils. Magnification ×8700.

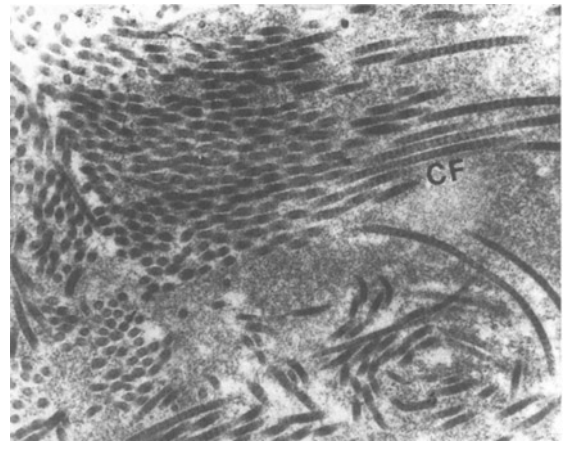

FIG. 3. Numerous collagen fibres (CF) with axial periodicity in the aortic intima from a positive control rat. Magnification ×8700.

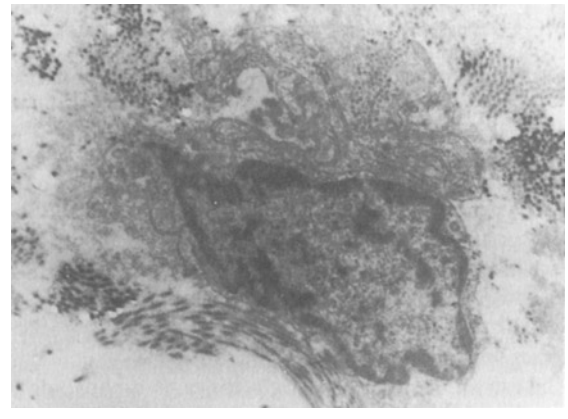

FIG. 4. Cells of aortic intima from policosanol-treated rat (5 mg kg^{-1}). No lipid droplets or nuclear envelope dilatation can be seen. Magnification ×5200.

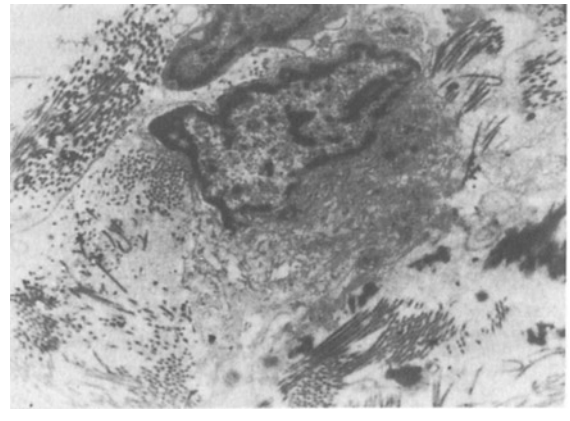

FIG. 5. Cells of aortic intima from policosanol-treated rat (25 mg kg^{-1}). No lipid droplets or nuclear envelope dilatation can be seen. Magnification ×5200.

significant differences when compared with the positive control group, showing in most of the animals (7/9) the simultaneous presence of all the ultrastructural characteristics of the lipofundin-induced lesions.

None of the rats treated with lipofundin + policosanol at 2·5, 5 and 25 mg kg^{-1} showed intracytoplasmic lipids or nuclear envelope dilatation. Moreover, a reduction of

Table 1. Effects of policosanol on foam cells present in lipofundin-induced lesions in rats.

	Dose (mg kg^{-1})	Foam cells (± s.d.)
Positive control	—	23 ± 2·3
Negative control	—	0***
Policosanol	0·5	18 ± 10·6
	2·5	0***
	5	0***
	25	0***

***$P < 0{\cdot}0003$ (Mann-Whitney U-test).

fibrosis was found in aortas of most of the rats corresponding to these groups (Figs 4, 5, Table 1).

Discussion

These results corroborate those obtained when the lesions were induced in rabbits (Noa & Más 1992) and confirm the protective effect of this treatment in the development of this type of atherosclerotic lesion. The physiological basis of this action can be related not only to the cholesterol-lowering effects of policosanol (Arruzazabala et al 1991a; Cruz-Bustillo et al 1991; Illnait et al 1991), but also to the reduction of thromboxane A_2 and an increase of prostacyclin induced by policosanol in rats (Arruzazabala et al 1991b, 1992a,c).

Skrinska et al (1988) showed that rabbits fed with a high cholesterol diet and treated with a thromboxane synthetase inhibitor (UK-38485,3-(1H-imidazol-1-yl-methyl)-2-methyl-1-H-indole-1-propanoic acid) developed significantly less lesions of the type described than those fed the atherogenic diet only.

These results suggest that thromboxane A_2 inhibition might prevent monocyte activation when prostaglandin E_2 synthesis increases and may affect occurrence of early atherosclerotic lesions when preventing monocyte function. It has been also reported that BM 13505, a specific thromboxane A_2-receptor antagonist, showed an antiatherogenic effect by reducing the deposition of cholesterol in the arterial wall and by retarding plaque formation in coronary arteries of cholesterol-fed rabbits (Osborne & Lefer 1988).

Acknowledgements

A. Capote provided excellent technical assistance. The authors wish to thank Esteban Pérez and Josefina Mederos for the preparation of the manuscript.

References

Aneiros, E., Calderón, B., Más, R., Illnait, J., Castaño, G., Fernández, L., Fernández, J. C. (1993) Effect of successive dose increases of policosanol on the lipid profile and tolerability of treatment. Curr. Ther. Res. 54: 304–312

Arruzazabala, M. L., Carbajal, D., Más, R., Castaño, G., Sotolongo, R., Mesa, R. (1991a) Efecto del Ateromixol (PPG) sobre los niveles de colesterol en perros Beagle. Revista CENIC Ciencias Biológicas 22: 50–51

Arruzazabala, M. L., Carbajal, D., Más, R., Garcia, M. (1991b) Efectos del Ateromixol (PPG) sobre la agregación plaquetaria. Revista CENIC Ciencias Biológicas 22: 52–53

Arruzazabala, M. L., Carbajal, D., Más, R., Garcia, M., Fraga, V., Molina, V., Valdés, S. (1992a) Estudio experimental de los efectos del Policosanol sobre la agregación plaquetaria. Revista de Farmacología Clínica y Experimental, España

Arruzazabala, M. L., Carbajal, D., Más, R., Illnait, J., Laguna, A., Castaño, G. (1992b) Efecto del Ateromixol sobre el perfil lipídico de conejos hipercolesteriolémicos. Archivos Venezolanos de Farmacología y Terapéutica 11: 80–86

Arruzazabala, M. L., Carbajal, D., Molina, V., Valdés, S., Más, R., García, M., Fraga, V. (1992c) Effects of Ateromixol on platelet aggregation in rats. Thromb. Res. 69: 321–327

Castaño, G., Zardoya, R., Illnait, J., Más, R., Fernández, L., Surribas, E., Nodarse, M., Fernández, J. C. (1991) Efectors del tratamiento con PPG (5 mg) en pacientes con hiperlipoproteineimai tipo II. Progresos en Ciencias Médicas (Venezuela) 5: 21

Cruz-Bustillo, D., Mederos, C. M., Más, R., Arruzazabala, M. L., Barreto, B., Martinez, O. (1991) Efecto hipocolesterolémico del Ateromixol (PPG) en el cerdo en ceba. Revista CENIC Ciencias Biológicas 22: 54–55

Haust, M. D. (1978a) Atherosclerosis in childhood. In: Rosemberg, H. S., Bolande, R.P. (eds) Perspectives in Pediatric Pathology, vol. 4. Year Book Medical Publishers, Chicago

Haust, M. D. (1978b) Light and electron microscopy of human atherosclerotic lesions. In: Chandler, R. F. (ed.) The Thrombotic Process in Atherogenesis. Eurenius. Plenum Publishing Corporation, New York

Haust, M. D. (1983) Derivation and progression of atherosclerotic plaques. In: Schettler, F. G. (ed.) Proceedings of the 6th International Symposium of Atherosclerosis. Springer-Verlag, New York

Hernández, F., Illnait, J., Más, R., Castaño, G., Fernández, L., González, M., Cordoví, N., Fernández, J. C. (1992) Effect of Ateromixol (policosanol) on serum lipids and lipoproteins in healthy volunteers. Curr. Ther. Res. 51: 1–8

Illnait, J., Castaño, G., Nodarse, M., Pontigas, V., Hernández, L., Más, R. (1991) Efectos del Ateromixol (PPG) sobre la hiperlipoproteinemia del tipo II. Revista CENIC Ciencias Biológicas 22: 80–83

Jellinek, H., Harsing, J., Fuzcesi, Sz. (1982) A new model for arteriosclerosis. An electron microscopy study of the lesions induced by i.v. administered fat. Atherosclerosis 43: 7–18

Joris, I., Zand, T., Majno, G. (1983) Studies on the pathogenesis of atherosclerosis. Am. J. Pathol. 113: 341–358

Kannel, W. B., Catelli, W. P., Gordon, T., McNamara, P. M. (1971) Serum cholesterol, lipoproteins and risk of coronary heart disease. Ann. Intern. Med. 74: 1–12

Keys, A. (1970) Coronary heart disease in seven countries. Circulation 41 (Suppl. 1): I1–I211

Kritchevsky, D., Davidson, L. M., Kriek, N. P., Du Plessis, J. P. (1982) Influence of native and randomized peanut oil on lipid metabolism and aortic sudanophilia in the vervet monkey. Atherosclerosis 4: 53–58

Noa, M., Illnait, J. (1987) Induction of aortic plaques in guinea pigs by exposure of kerosene. Arch. Environ. Health 42: 320–324

Noa, M., Más, R. (1992) Effect of Ateromixol on Lipofundin-induced atherosclerotic lesion in rabbits. Progresos en Ciencias Médicas (Venezuela) 6: 14–19

Osborne, J., Lefer, A. (1988) Cardioprotective actions of thromboxane receptor antagonism in ischemic atherosclerotic rabbits. Am. J. Physiol. 255 (Heart Circ. Physiol. 24): H318

Pons, P., Más, R., Illnait, J., Fernández, L., Rodríguez, M., Robaina, C., Fernández, J. C. (1992) Efficacy and safety of policosanol in patients with primary hypercholesterolemia. Curr. Ther. Res. 52: 507–512

Pons, P., Jiménez, A., Rodríguez, M., Illnait, J., Más, R., Fernández, L., Fernández, J. C. (1993) Effects of policosanol in elderly hypercholesterolemic patients. Curr. Ther. Res. 53: 265–269

Rodríguez Echenique, C., Mesa, R., Más, R., Amor, A. M., Castaño, G. (1992) Estudio del efecto sobre lípidos y lipoproteinas séricos y la tolerancia al tratamiento oral con dosis crecientes de policosanol en monos Macaca arctoides. Arch. Venezolanos Farmacol. Terapéutica 11: 74–79

Skrinska, V., Konieczkowski, M., Gerrity, R., Galang, C., Revec, M. (1988) Suppression of foam cell lesions in hypercholesterolemic rabbits by inhibition of thromboxane A_2 synthesis. Arteriosclerosis 8: 220–225

Vesselinovitch, D., Wissler, R. W. (1980) Reversal of atherosclerosis. Comparison of non human primate models. In: Gotto, A. M., Smith L. C. (eds) Atherosclerosis. Springer-Verlag, New York, pp 369–274