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Effect of Policosanol on Circulating Endothelial Cells in Experimental Models in Sprague-Dawley Rats and in Rabbits

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Abstract

The effect of policosanol on circulating endothelial cells has been studied in different experimental models with endothelium damage.

Oral administration of 25 mg kg^{-1} policosanol to Sprague-Dawley rats resulted in significant protection of the endothelial lining against the desquamating effect of citrate. Oral administration of 5 mg kg^{-1} policosanol to spontaneously hypertensive rats (SHR) resulted in a significant reduction of circulating endothelial cells compared with controls. Moreover, comparison between groups revealed a lower frequency of aortic lesions in policosanol-treated animals than in controls. On the other hand, administration of 5 mg kg^{-1} policosanol to rabbits with intimal hyperplasia induced by cuff placement in the carotid artery resulted in levels of circulating endothelial cells significantly lower than in controls.

These results demonstrate the protective effect of policosanol in different experimental models and suggest its potential for endothelial protection.

Quantification of circulating endothelial cells has been taken as an indicator of vascular endothelial damage (Hladovec 1978; Hladovec et al 1978; Davis et al 1985, 1987a, b, 1994; Friman et al 1988; Casacó et al 1990; Hladovec & Kornalik 1990).

Policosanol is a natural mixture of higher aliphatic primary alcohols isolated from sugar cane (*Saccharum officinarum*, L) wax; its cholesterol-lowering effects have been demonstrated in different experimental models (Rodríguez et al 1992; Arruzazabala et al 1994), healthy volunteers (Hernández et al 1992) and in patients with type II hypercholesterolaemia (Castaño et al 1991; Pons et al 1992, 1994a, b; Aneiros et al 1993, 1995; Soltero et al 1993a, b; Torres et al 1995).

Thromboxane A_2 (TxA_2) has been implicated in vascular endothelial injury in some experimental models (De Clerck et al 1985). Because oral administration of policosanol induces a significant reduction of thromboxane B_2 (TxB_2) (a stable metabolite of TxA_2), (Arruzazabala et al 1993a) and increases prostacyclin levels in rodents (Arruzazabala et al 1993b), this work was undertaken to investigate whether policosanol prevents endothelial cell injury in different experimental models of endothelial damage.

Materials and Methods

Vascular endothelial injury induced by sodium citrate in Sprague-Dawley rats

Eighteen young Sprague-Dawley adult male rats, 200 ± 20 g, from the National Centre for Laboratory Animals (Cenpalab, Cuba) were left to adapt to laboratory conditions for one week with free access to food and water. Rats were randomly distributed into three experimental groups of six.

Policosanol was suspended in 2% Tween 20 in H_2O (as vehicle) and administered orally by gastric gavage (1 mL kg^{-1}) for 20 days. Group 1, negative controls, were untreated animals; saline solution (0·9%, 2 mL (200 g)$^{-1}$) was injected into the penile vein 5 min before blood was drawn. Group 2 were positive controls; endothelial damage was induced by injection of sodium citrate (1·52%, 2 mL) into the penile vein 5 min before blood was drawn. Group 3 rats were pre-treated with policosanol (25 mg kg^{-1}) for 20 days then endothelial damage was induced by intravenous injection of sodium citrate (1·52%, 2 mL (200 g)$^{-1}$).

Endothelial cell counting. Circulating anuclear carcasses of endothelial cells were counted by the technique of Hladovec & Rossman (1973). After treatment, blood samples were drawn by cardiac puncture after 12-h fasting; sodium citrate 3·7% was added in a 1 : 9 (v/v) ratio. The mixture was centrifuged at 395 *g* for 20 min at 4°C to give platelet-rich plasma (PRP). ADP (1 mg mL^{-1}, 0·2 mL) was added to the PRP (1 mL). The mixture was shaken mechanically for 10 min. Further centrifugation (395 *g* for 20 min) served to remove platelet aggregates. The supernatant was centrifuged at 2100 *g* for 20 min and the scanty sediment was carefully suspended in NaCl (0·9%, 0·1 mL). Counting was performed in a Fuchs 'Rosenthal' chamber and results were expressed as the average of four counts.

Effects of policosanol on circulating endothelial cells and aortas in spontaneously hypertensive rats (SHR)

Twenty SHR, 200 ± 20 g, from Cenpalab were adapted to laboratory conditions and were distributed randomly into two experimental groups of ten rats.

Policosanol was suspended in 2% Tween 20 in H_2O (as vehicle) and administered orally by gastric gavage (1 mL kg^{-1}) for 7 days. Group 1 rats were controls treated with the 2% Tween 20 in water vehicle (1 mL kg^{-1}). Group 2 animals were treated with policosanol (5 mg kg^{-1}). After treatment the animals were anaesthetized and blood samples

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were drawn by cardiac puncture for endothelial cell counting as previously described.

Light microscopy studies. Animals were killed and samples were taken of all the aortae at the first intercostal branch and between the renal and iliac branches. Samples were fixed in 10% buffer formaldehyde and processed for study by light microscopy. Sections were prepared in paraffin and stained with haematoxylin and eosin. Arterial lesions were defined as occurring when animals showed intimal lesions with the presence of leukocytes between the endothelium and the internal elastic lamina.

Effect of policosanol on circulating endothelial cells in rabbits with intimal hyperplasia induced by a cuff

Eighteen male New Zealand rabbits (2–2·5 kg) from Cenpalab were left to adapt to laboratory conditions. They were randomly distributed into three groups.

Policosanol was suspended in 2% Tween 20 in H_2O (as vehicle) and administered orally by gastric gavage ($1\,mL\,kg^{-1}$) for 15 days. Group 1 rabbits were treated with Tween 20–water vehicle ($1\,mL\,kg^{-1}$). Group 2 animals were treated with policosanol ($5\,mg\,kg^{-1}$). Group 3 rabbits were not treated (negative control).

Experimental induction of neointimal formation. The day before the beginning of the treatment the rabbits were anaesthetized with sodium pentobarbital ($30\,mg\,kg^{-1}$, i.v.). The carotid arteries were exposed and dissected from the surrounding tissues. A non-occlusive, biologically inert, soft and flexible silicone cuff (20 mm × 4·7 mm i.d.) was placed around the left carotid artery as described by Kockx et al (1992).

Fifteen days after therapy, blood samples were drawn by cardiac puncture for endothelial cell counting as aforementioned.

Statistical analysis

Between groups, plasma endothelial cell counts were compared by use of the Mann-Whitney *U*-test and the occurrence of intimal lesions in SHR was analysed by use of Fisher's exact probability test.

Results

Vascular endothelial injury induced by sodium citrate in Sprague-Dawley rats

Table 1 summarizes the effect of policosanol on plasma endothelial cells in Sprague-Dawley rats with endothelial lesions induced by sodium citrate. The number of circulating endothelial cells was significantly lower for the control and policosanol-treated groups than for the positive control. In addition, the values for the policosanol-treated group ($25\,mg\,kg^{-1}$) were also significantly lower than those for the negative control.

Effects of policosanol on circulating endothelial cells and on aortas in spontaneously hypertensive rats (SHR)

Table 2 summarizes the effect of policosanol on plasma-circulating endothelial cells in SHR. It was observed that the number of circulating endothelial cells in the plasma of SHR treated with policosanol was significantly lower in that of the controls.

Microscopic study revealed morphological changes characteristic of the arteries of this strain of rat, for example, medial thickening by myocyte hypertrophy and proliferation, and increase of the extracellular matrix of the connective tissue. Moreover, the intimal lesion described in Materials and Methods was observed in four of the ten rats (40%) of the control group, whereas no such lesions were observed in the policosanol-treated SHR rats. Consequently, between-group comparisons showed that the frequency of these lesions in the control groups was significantly higher than in the policosanol-treated group ($P < 0·01$; Fisher's Exact Test).

Effect of policosanol on circulating endothelial cells in rabbits with intimal hyperplasia induced by a cuff

Table 3 summarizes the effect of policosanol on plasma endothelial cells in rabbits with intimal hyperplasia induced by a cuff. The numbers of circulating endothelial cells in the negative control and in the policosanol cuffed group were statistically similar and significantly lower than for the positive control.

Discussion

Experiments conducted in three different experimental models revealed that administration of repeated doses of policosanol significantly reduced the number of endothelial cells circulating in the plasma of Sprague-Dawley rats, SHR and cuffed rabbits compared with the number in animals with endothelial lesions.

In-vivo, the injection of citrate in rats results in damage of the endothelial cells and detachment or desquamation. The endothelial damage can be quantified by counting the detached endothelial cells in blood (Hladovec & De Clerck 1981). Oral pre-treatment of Sprague-Dawley rats with $25\,mg\,kg^{-1}$ policosanol resulted in significant protection of the endothelial lining against the desquamating effect of citrate.

Table 1. Effect of policosanol on the number of endothelial cells circulating in the plasma of Sprague-Dawley rats.

Group	Treatment	Dose ($mg\,kg^{-1}$)	Number of cells mL^{-1} platelet-rich plasma
Negative control	Vehicle	0	182·3 ± 42·69*
Positive control	Vehicle + sodium citrate	0	218·7 ± 65·54
Sodium citrate + policosanol	Policosanol + sodium citrate	25	114·6 ± 53·85*,**

Values are means ± s.d. *$P < 0·05$, significant compared with positive control group; *,**$P < 0·05$, significant compared with negative control group (Mann-Whitney *U*-test).

Table 2. Effect of policosanol on the number of endothelial cells circulating in the plasma of spontaneously hypertensive rats.

Group	Dose ($mg\,kg^{-1}$)	Number of cells mL^{-1} platelet-rich plasma
Control	0	267·1 ± 156·24
Policosanol	5	164·0 ± 84·6*

Values are means ± s.d. *$P < 0.05$, significant compared with control group (Mann-Whitney U-test).

Table 3. Effect of policosanol on the number of endothelial cells circulating in the plasma of cuffed rabbits.

Group	Dose ($mg\,kg^{-1}$)	Number of cells mL^{-1} platelet-rich plasma
Positive control (cuff)	0	302·12 ± 34·23
Negative control	0	88·54 ± 21·34*
Cuff + policosanol	5	60·26 ± 14·05*

Values are means ± s.d. *$P < 0.001$ compared with positive control (Mann-Whitney U-test)

On the other hand, SHR are considered the best animal model for the study of essential hypertension, wherein thromboxane A_2 (TxA_2) plays a role in the development of hypertension (Stier & Itskovitz 1988). Spontaneous endothelial damage has also been described in this strain (Mrhova et al 1976; Shimamoto et al 1976; Hadjiisky 1980, 1984). These factors support the opinion that this strain of rats is particularly useful for study of drugs acting on the vascular endothelium. In this work policosanol administered orally at $5\,mg\,kg^{-1}$ resulted in significant reduction of circulating endothelial cells compared with those circulating in controls. Moreover, comparison of groups revealed a lower frequency of aortic lesions in policosanol-treated animals than in controls, suggesting a protective effect on the vascular endothelium.

Cuff placement in the rabbit carotid artery has been used to study the proliferation of smooth muscle cell (intimal hyperplasia) in arteries (Booth et al 1989; Kockx et al 1992). Kockx et al (1993) demonstrated alterations to the endothelial cells in cuff-induced neointima formation in the rabbit carotid artery. So this experimental model is also useful for study of drugs acting on the vascular endothelium.

In this case similar results were also observed. Thus, a significant reduction of circulating endothelial cells was observed in cuffed rabbits treated with policosanol ($5\,mg\,kg^{-1}$) compared with controls.

Because only one dose was investigated, we selected in each instance doses previously established as preventing the development of arterial lesions (Noa & Más 1992; Noa et al 1995, 1996).

Much evidence supports the concept that endothelial cell injury might initiate a number of vascular disorders including the development of atherosclerosis (Haust 1983) and thrombus formation (Cadroy & Harker 1990). For these reasons, several authors have studied the effect of different drugs on endothelial cell levels (Hladovec & De Clerck 1981; De Clerck 1982; Hladovec 1989; Hladovec & Kornalik 1990; Davis et al 1994).

It has been reported (Friman et al 1988; Hladovec 1989; Casacó et al 1990) that a reduction of plasma endothelial cell levels is observed in experimental animals treated with inhibitors of TxA_2 synthesis, for example dazoxiben (Hladovec 1989) and levamisol (Arruzazabala et al 1990), and with TxA_2 antagonists such as lobenzarit (Carbajal & Gonzáles 1990). In this sense, policosanol significantly reduces TxB_2 levels and increases prostacyclin levels in rats, mice and Mongolian gerbils, significantly reducing the TxA_2/prostacyclin ratio (Arruzazabala et al 1993a, b). These effects are in accord with a beneficial action on vascular endothelium and could be the physiological basis of the aforementioned effects.

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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

Aneiros, E., Más, R., Calderón, B., Illnait, J., Fernández, L., Castaño, G., Fernández, J. C. (1995) Policosanol for cholesterol-lowering levels in patients with type II hypercholesterolemia. Curr. Ther. Res. 56: 176–182

Arruzazabala, M. L., Carbajal, D., García, M. (1990) Levamisol: un inhibidor de la enzima tromboxano sintetasa. Rev. Latinoamericana de Trombosis y Hemostasis III: 19–21

Arruzazabala, M. L., Carbajal, D., Más, R., García, M., Fraga, V. (1993a) Effects of ateromixol on platelet aggregation in rats. Thromb. Res. 69: 321–327

Arruzazabala, M. L., Molina, V., Carbajal, D., Valdés, S., Más, R. (1993b) Effect of policosanol on cerebral ischemia in Mongolian gerbils: role of prostacyclin and thromboxane A_2. Prostaglandins Leukot. Essent. Fatty Acids 49: 695–699

Arruzazabala, M. L., Carbajal, D., Más, R., Molina, V., Valdés, S., Laguna, A. (1994) Cholesterol-lowering effects of policosanol in rabbits. Biol. Res. 27: 205–208

Booth, R., Martinn, J., Honey, A., Hassall, D., Beesley, J., Moncada, S. (1989) Rapid development of atherosclerotic lesions in the rabbit carotid artery induced by perivascular manipulation. Atherosclerosis 76: 257–268

Cadroy, Y., Harker, L. (1990) Platelets, thrombosis and antithrombotic therapies. In: Antonaccio, M. (ed.) Cardiovascular Pharmacology. 3rd edn, Raven Press, New York

Carbajal, D., González, R. (1990) Efecto del CNIC-4 (Lobenzarit) como antagonista del TxA_2. Revista CENIC Ciencias Biológicas. 21: 2–3

Casacó, A., Carbajal, D., Friman, M., Noa, M. (1990) Interference of levamisole with Forssman Shock. Thromb. Res. 59: 629–638

Castaño, G., Zardoya, R., Illnait, J., Más, R., Fernández, L., Surribas, E., Nodarse, M., Fernández, J. C. (1991) Efectos del tratamiento con PPG (5 mg) en pacientes con hiperlipoproteinemia tipo II. Progresos en Ciencias Médicas (Venezuela) 5: 21–28

Davis, J. W., Shelton, L., Eigenberg, D. A., Hignite, C. E., Watanabe, I. S. (1985) Effects of tobacco and non-tobacco cigarette smoking on endothelium and platelets. Clin. Pharmacol. Ther. 37: 529–533

Davis, J. W., Shelton, L., Eigenberg, D. A., Hignite, C. E. (1987a) Lack of effect of aspirin on cigarette smoke-induced increase in circulating endothelial cells. Haemostasis 17: 66–69

Davis, J. W., Shelton, L., Watanabe, I. S., Arnold, J. (1987b) Passive smoking affects endothelium and platelets. Arch. Intern. Med. 149: 386–389

Davis, J., Lewis, H. D., Francis-Oliver, A. (1994) Prevention of exercise-induced endothelemia by isradipine in men with angina pectoris. Cardiology 84: 375–379

De Clerck, F. (1982) Effects of pharmacological agents on erythrocyte deformability and endothelial cell injury. J. Cereb. Blood Flow Metab. 2 (suppl. 1): S50–S52

De Clerck, F., Loots, W., Somers, Y., Van Gorp, L., Verheyen, A., Wouters, L. (1985) Thromboxane A_2-induced vascular endothelial cell damage and respiratory smooth muscle cell contraction: inhibition by flunarizine, a Ca-overload blocker. Arch. Int. Pharmacodyn. Ther. 274: 4–23

Friman, M., Noa, M., Pauste, H., Quintela, A., Illnait, J. (1988) Effect of CNIC-4 (lobenzarit) on circulating endothelial cells of rats. In: Proc. 6th Int. Dresden Lipid Symp., Adv. Lipoprotein and Atherosclerosis Res. Diagn. Treat.

Hadjiisky, P. (1980) Morphogenese de l'artheriopathie centrale peripherique du rat spontanement hypertendu. 1. Characteristiques histologiques. Paroi Arterielle VI: 169–182

Hadjiisky, P. (1984) Metabolic and structural behaviour of hypertensive arteries: possible relationships with atherogenesis and tensiogenesis. In: Balas, P. (ed.) Angiology, Plenum Publishing Corporation

Haust, M. D. (1983) Derivation and progression of atherosclerotic plaque. In: Schetler, F. B. (ed.) Proc. 6th Int. Symp. Atherosclerosis, Springer, 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: 568–575

Hladovec, J. (1978) Endothelial injury by nicotine and its prevention. Experientia 34: 1585–1586

Hladovec, J. (1989) Antithrombotic Drugs in Thrombosis Models, CRC Press, Boca Ratón, FL

Hladovec, J., De Clerck, F. (1981) Protection by flunarizine against endothelial cell injury in vivo. Angiology 32: 448–462

Hladovec, J., Kornalik, F. (1990) Antithrombotic activity of an unsaturated fatty acid preparation. Thromb Res. 58: 505–510

Hladovec, J., Rossman, P. (1973) Circulating endothelial cells isolated together with platelets and the experimental modification of their counts in rats. Thromb. Res. 3: 665–674

Hladovec, J., Prerovsky, I., Stanek, V., Fabian, J. (1978) Circulating endothelial cells in acute myocardial infarction and angina pectoris. Klin. Wochenschr. 56: 1033–1036

Kockx, M., De Meyer, G., Jacob, W., Bult, H., Herman, A. (1992) Triphasic sequence of neointimal formation in the cuffed artery of the rabbit. Arteriosclerosis Thromb. 12: 1447–1457

Kockx, M., De Meyer, G., Andries, L., Bult, H., Jacob, W., Herman, A. (1993) The endothelium during cuff-induced neointima formation in the rabbit carotida artery. Arteriosclerosis Thromb. 13: 1874–1884

Mrhova, O., Albretch, I., Urbanova, D. (1976) Vessel wall metabolism in SHR rats in relation to atherosclerosis. Ann. New York Acad. Sci. 275: 302–310

Noa, M., Más, R. (1992) Ateromixol y lesión aterosclerótica en conejos inducida por lipofundin. Progresos en Ciencias Médicas (Venezuela) 6: 14–19

Noa, M., Más, R., De la Rosa, M. C., Magraner, J. (1995) Effect of policosanol on lipofundin-induced atherosclerotic lesions in rats. J. Pharm. Pharmacol. 47: 289–291

Noa, M., Más, R., De la Rosa, M. C. (1996) Effect of policosanol on foam-cell formation in carrageenan-induced granulomas in rats. J. Pharm. Pharmacol 48: 306–309

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 hypercholesterolaemia. Curr. Ther. Res. 52: 507–512

Pons, O., Rodríguez, M., Robaina, C., Illnait, J., Más, R., Fernández, L., Fernández, J. C. (1994a) Effects of policosanol successive dose increases on lipid profile of patients with type II hypercholesterolaemia and tolerability to treatment. Int. J. Clin. Pharmacol. Res. 14: 27–33

Pons, P., Rodríguez, M., Más, R., Illnait, J., Fernández, L., Robaina, C., Fernández, J. C. (1994b) One year efficacy and safety of policosanol in patients with type II hypercholesterolaemia. Curr. Ther. Res. 55: 1084–1092

Rodríguez, C., Mesa, R., Más, R., Illnait, J., Laguna, A., Castaño, G. (1992) Estudio del efecto sobre lípidos y lipoproteinas séricos y de la tolerancia al tratamiento oral con dosis creciente de policosanol en monos (*Macaca arctoides*). Arch. Venezol. Farmacol. Terap. 11: 74–79

Shimamoto, T., Hidaka, H., Moriya, K., Kobayashi, M., Takahashi, T., Numan, F. (1976) Hyperreactive arterial endothelial cells: a clue for treatment of atherosclerosis. Ann. New York Acad. Sci. 275: 266–285

Soltero, I., Fuenmayor, I., Colmenares, J., Arias, F. (1993a) Ensayo doble ciego para la evaluación del policosanol en el tratamiento de la hiperlipoproteinemia tipo II. Arch. Venezol. Farmacol. Terap. 12: 65–70

Soltero, I., Fuenmayor, I., Colmenares, J. (1993b) Estudio comparativo doble ciego de la eficacia y tolerancia del policosanol vs bezafibrato en pacientes con hiperlipidemias tipo II. Arch. Venezol. Farmacol. Terap. 12: 71–76

Stier, Ch., Itskovitz, H. (1988) Thromboxane A_2 and the development of hypertension in spontaneously hypertensive rats. Eur. J. Pharmacol. 146: 129–135

Torres, O., Agramonte, S. J., Illnait, J., Más, R., Fernández, L., Fernández, J. C. (1995) Treatment of hypercholesterolaemia in non-insulin-dependent diabetes mellitus with policosanol. Diabetes Care 18: 393–397