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Picotamide, an antithromboxane agent, inhibits the migration and proliferation of arterial myocytes [1]

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Abstract

Picotamide is an antiplatelet drug with a peculiar dual mechanism of action: it inhibits thromboxane A_2 synthase and antagonizes the pharmacological responses mediated by thromboxane A_2 receptor. We investigated the in vitro effect of picotamide on smooth muscle cell migration and proliferation. Picotamide (1–500 μM) decreased human and rat smooth muscle cell proliferation, evaluated as cell number, in a concentration-dependent and reversible manner. Picotamide inhibited DNA synthesis induced by fetal calf serum (10%), platelet-derived growth factor (PDGF-BB (20 ng/ml)), epidermal growth factor (EGF (1 nM)) and (15*S*)-hydroxy-11,9-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid (U46619 (10 μM, thromboxane A_2 receptor agonist)). Co-incubation of U46619 together with EGF or PDGF-BB resulted in a marked amplification of [^{3}H]thymidine incorporation that was completely reversed by picotamide. The drug also inhibited smooth muscle cell migration induced by fibrinogen (600 μg/ml) or PDGF-BB (20 ng/ml) in a concentration-dependent manner. The ability of picotamide to interfere with myocyte migration and proliferation confers, at least in vitro, a pharmacological interest on the compound in atherogenesis.

Keywords: Smooth muscle cell; Atherosclerosis; PDGF (platelet-derived growth factor); EGF (epidermal growth factor)

1. Introduction

Migration and proliferation of smooth muscle cells in the arterial wall are early prominent features of atherogenesis and represent major mechanisms involved in vascular occlusion in both atherosclerosis and restenosis after angioplasty (Ip et al., 1990; Ross, 1993; Isner et al., 1994). An arterial injury leads to extensive platelet activation, adhesion, followed by platelet aggregation and secretion (Ip et al., 1990; Ross, 1993; Le Breton et al., 1996). Platelet secretion results in the local release of intracellular granuli constituents, including platelet-derived growth factor (PDGF), as well as epidermal growth factor (EGF) (Oka and Orth, 1983; Assoian et al., 1984; Ross, 1993); these factors have been identified as potent endogenous smooth muscle cell mitogens (Ross, 1993). PDGF-BB is also one of the most potent chemoattractive agents (Ross, 1993). Platelets themselves also synthesize and release the pro-aggregatory agent thromboxane A_2 at the sites of vascular injury (Moncada and Vane, 1979). Although well known as a potent vasoconstrictor (Moncada and Vane, 1979), thromboxane A_2 may also induce smooth muscle cell proliferation (Hanasaki et al., 1990; Nagata et al., 1992; Morinelli et al., 1994) and contribute to lesion formation. Recently, it has been shown that PDGF-stimulated cell proliferation was potentiated two-fold by thromboxane A_2 agonist (15*S*)-hydroxy-11,9-(epoxymethano)prosta-5*Z*,13*E*-dienoic acid (U46619) (Zucker et al., 1995; Grosser et al., 1997) supporting the role of vasoconstrictory eicosanoids released from activated platelets in sustaining the proliferation of smooth muscle cells during atherogenesis. Drugs affecting the biological effects of thromboxane A_2 may be, therefore, potential antiatherosclerotic agents (Grosser et al., 1997; Pakala et al., 1997).

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Picotamide (*N*,*N*′-bis-(3-picolyl)-4-methoxy-isophthalamide) is an antiplatelet drug with an original dual mechanism of action: it inhibits thromboxane A_2 synthase and antagonizes the pharmacological responses mediated by thromboxane A_2 receptor at equivalent concentrations, while enhancing endothelial formation of prostacyclin (Gresele et al., 1989; Modesti et al., 1989). Both activities result in a synergic inhibition of thromboxane A_2-mediated actions (Giustina et al., 1993). A recent double-blind, placebo-controlled study showed that long term treatment (24 months) with picotamide can slow the evolution of early carotid atherosclerotic lesions in diabetic patients (Cocozza et al., 1995). These premises have provided a rationale for a study addressing the effect of picotamide on arterial myocyte migration and proliferation. Toward that end, we examined the action of picotamide on DNA synthesis and migration of smooth muscle cells elicited by growth factors secreted by activated platelets (Ip et al., 1990; Ross, 1993).

2. Materials and methods

2.1. Materials

Eagle's Minimum Essential Medium (MEM), trypsin–EDTA, penicillin (10 000 U/ml), streptomycin (10 mg/ml), tricine buffer (1 M, pH 7.4) and non-essential amino acid solution (100×) were purchased from Gibco (Grand Island, NY, USA); fetal calf serum was from Mascia Brunelli (Milan, Italy). Disposable culture flasks and petri dishes were from Corning, Glassworks (Corning, NY, USA), and filters from Gelman Sciences (Ann Arbor, MI, USA). [6-^{3}H]Thymidine (s.a., 27 Ci/mmol) was from Amersham (Amersham Place, Buckinghamshire, UK). Isoton II was purchased from Coulter (Coulter Instruments, Milan, Italy). Picotamide, kindly provided by Novartis Farma (Milan, Italy), was dissolved in 1 M HCl/distilled water (1:4); U46619, provided by Cayman Chemical (USA), was dissolved in ethanol. PDGF-BB and EGF provided by Calbiochem (La Jolla, CA, USA), were dissolved in 10 mM acetic acid/2.5% w:v bovine serum albumin and phosphate buffered saline/0.1% bovine serum albumin, respectively. The solutions were sterilized by filtration.

2.2. Cell culture

Smooth muscle cells were cultured, according to Ross (1971), from intimal–medial layers of aorta of male Sprague–Dawley rats (200–250 g). Cells were grown in monolayers at 37°C in a humidified atmosphere of 5% CO_2 in MEM supplemented with 10% (v:v) fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 20 mM buffer tricine and 1% (v:v) non-essential amino acid solution (Corsini et al., 1993). The medium was changed every third day. Cells were used between the fourth and tenth passage. Smooth muscle cells were identified for growth behaviour, morphology and using monoclonal antibody specific for α-actin, the actin isoform typical of smooth muscle cells (Skalli et al., 1986). The cells grew out of explants after 12–16 days, piled up after confluency and contained numerous myofilaments and dense bodies, as observed by transmission electron microscopy (Skalli et al., 1986; Ross, 1993). Human femoral artery myocytes (A 617) were grown in the same culture conditions (Corsini et al., 1993).

2.3. Proliferation of smooth muscle cells

Myocytes were seeded at a density of 5×10^4 per 35 mm dish (human) or 2×10^5 per 35 mm dish (rat), and incubated with MEM supplemented with 10% fetal calf serum (Corsini et al., 1993, 1995). 24 h later, the medium was changed to one containing 0.4% fetal calf serum to stop cell growth and the cultures incubated for 72 h. At this time (time 0), the medium was replaced with one containing 10% fetal calf serum in the presence or absence of known concentrations of the tested compound and the incubations were continued for further 72 h at 37°C. At time 0, just before the addition of the substances to be tested, three petri dishes were used for cell counting. The reversibility of the inhibitory effect of picotamide on cell growth was also investigated. Human arterial myocytes were treated with the tested compound for 72 h, after which the incubation medium was removed and replaced with fresh culture medium for further 48 h (Corsini et al., 1995, 1996). Cell proliferation was evaluated by cell count after trypsinization of the monolayers using a Coulter Counter model ZM (Corsini et al., 1993). Smooth muscle cell doubling time was computed according to Elmore and Swift (Elmore and Swift, 1976).

In another set of experiments, synchronization of smooth muscle cells to the G_0/G_1 interphase of the cell cycle was accomplished by incubating logarithmically growing cultures (3×10^5 myocytes/petri dish) for 5 days in a medium containing 0.4% fetal calf serum. Quiescent cells were then incubated for 20 h in a fresh medium containing 10% fetal calf serum in the presence or absence of picotamide. When PDGF-BB and U46619 were investigated, quiescence medium was replaced at the fourth day with one containing 1% plasma-derived bovine serum. After 24 h, mitogenic stimuli were added directly to the medium in the presence or absence of picotamide. A medium containing 0.1% bovine serum albumin was utilized when EGF was employed as mitogenic stimulus. After 20 h, DNA synthesis was estimated by nuclear incorporation of [^{3}H]thymidine, incubated with the cells (2 μCi/ml medium) for 2 h according to Corsini et al. (1995, 1996) and radioactivity was measured with Aquasol scintillation cocktail.

Cell viability was assessed by trypan blue exclusion, and found to be higher than 95% at the drug concentrations used.

2.4. Migration of smooth muscle cells

Migration of rat smooth muscle cells was examined by using a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, MD, USA). Freshly trypsinized smooth muscle cells were suspended in a medium supplemented with 5% fetal calf serum (assay medium). The lower wells, containing 27 μl of the assay medium including fibrinogen (600 μg/ml) or PDGF-BB (20 ng/ml) as chemotactic agents (Nakao et al., 1982; Naito et al., 1990), were covered with a polyvinylpyrrolidone-free polycarbonate filter (8-μm pore size). 50 μl of the cell suspension (1×10^6 cells/ml) were placed in the upper compartment with the tested compound and the incubation was carried out for 5 h at 37°C in an atmosphere of 95% air and 5% CO_2. On removal from the chamber, non-migrated cells were scraped from the upper surface of the filter, which was washed three times with phosphate buffered saline, pH 7.4. Filters were stained with Diff-Quik (Baxter Diagnostics AG, Düdingen, Switzerland). The number of smooth muscle cells per 100 × high power field that had migrated to the lower surface of the filters was determined microscopically, and the mean was obtained (Corsini et al., 1993, 1996).

2.5. Statistics

Data are expressed as mean ± S.D. The effect of the drug vs. control on cell proliferation and migration was analyzed by two-tailed Student's *t*-test for unpaired data and by Duncan's test.

3. Results

The potential antiproliferative action of picotamide was studied in rat aortic myocytes at concentrations ranging from 1 to 500 μM. In a first set of experiments, the effect of the tested compound was evaluated by cell counting after 3 days of growth and exposure to the drug. Picotamide decreased rat and human myocytes proliferation in a concentration-dependent manner and treated cells had longer doubling times than controls (Table 1). This action was not the result of cytotoxicity. In fact, when human myocytes were treated with the tested compound for 72 h, and the incubation medium was removed and replaced with fresh medium, cells recovered from the inhibitory effect (Fig. 1).

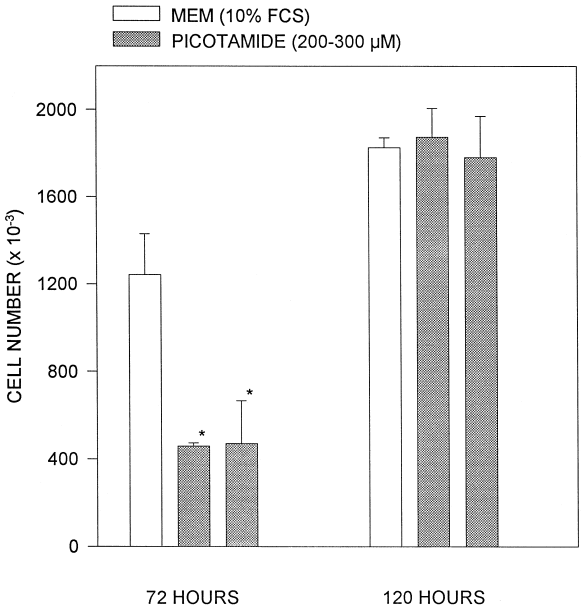

Fig. 1. Reversibility of the antiproliferative effect of picotamide on human myocytes growth. Picotamide-containing media were removed after 72 h and new media (without picotamide) supplemented with 10% FCS were added for additional 48 h. Each bar represents the mean ± S.D. of triplicate dishes. Drug vs. control: * $P < 0.01$ (Student's *t*-test).

Picotamide also inhibited DNA synthesis induced by several mitogenic stimuli in a concentration-dependent manner (Table 2, Fig. 2). Since fetal calf serum contains several potential growth factors, it could be expected that higher concentrations of picotamide are necessary to elicit

Table 1
Effect of picotamide on proliferation and on doubling time of rat and human myocytes

Addition (μM)	Cell number (% of control)		Doubling time (h)	
	Mean ± S.D.		Mean ± S.D.	
	Rat	Human	Rat	Human
None	100.0 ± 23.6	100.0 ± 12.2	41.0 ± 0.8	21.6 ± 1.2
1	91.8 ± 14.3	124.9 ± 4.0[a]	39.6 ± 1.6	19.9 ± 0.3[a]
5	86.3 ± 6.7	107.1 ± 17.0	45.8 ± 0.4[b]	21.3 ± 1.7
10	86.1 ± 8.6	127.4 ± 12.0	43.4 ± 1.4	19.7 ± 1.0
50	75.2 ± 10.8	122.5 ± 10.0	50.5 ± 3.7[b]	19.9 ± 0.7
100	71.3 ± 9.8[b]	78.6 ± 6.5[a]	48.8 ± 2.2[b]	25.3 ± 1.2[a]
500	44.6 ± 9.2[c]	1.0 ± 0.1[c]	64.2 ± 3.4[c]	

Each point represents the mean ± S.D. of four and two experiments for rat and human myocytes, respectively. The doubling time was measured after 72 h of incubation. The average cell number in controls was 1 399 009 ± 330 166 (rat) and 633 067 ± 77 234 (humans).
Drug vs. control: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ (Student's *t*-test).

Table 2
Effect of picotamide on [^{3}H]thymidine incorporation in rat aortic myocytes

Addition	[^{3}H]thymidine incorporation (dpm × 10^{-3}/mg prot.) (mean ± S.D.)
MEM (0.4% FCS)	77 ± 17
MEM (10% FCS)	770 ± 74
+ picotamide 50 μM	708 ± 154
+ picotamide 100 μM	538 ± 186
+ picotamide 500 μM	445 ± 52

Each point represents the mean ± S.D. of seven experiments run in triplicate.
* $P < 0.01$ (Student's *t*-test).

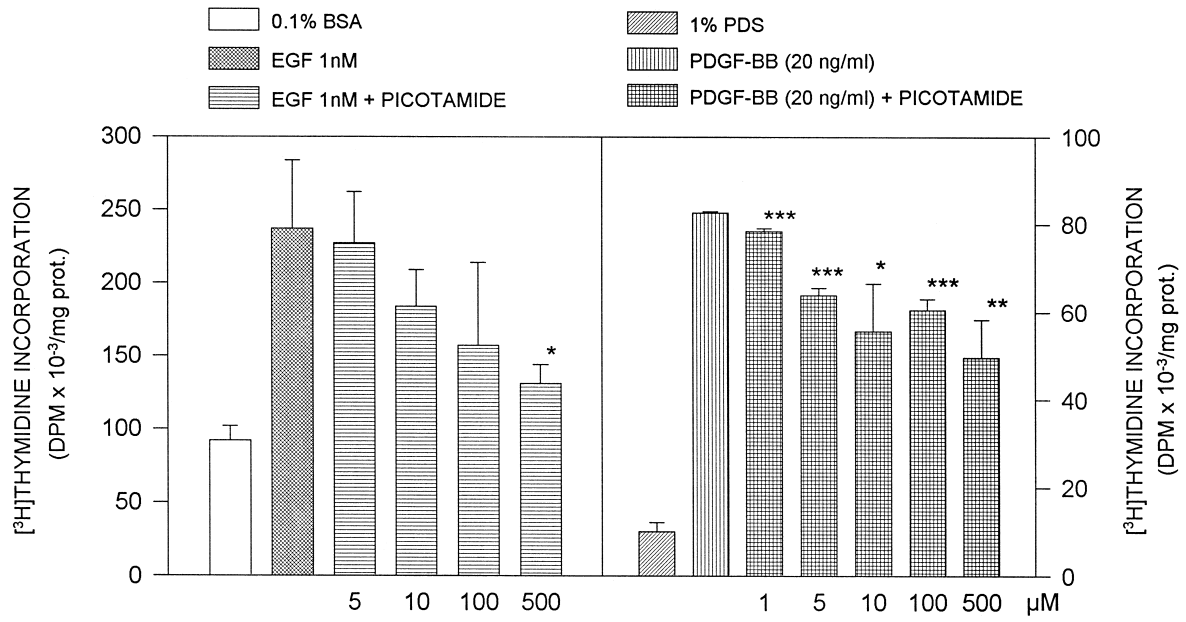

Fig. 2. Effect of picotamide on EGF- and PDGF-BB-induced [^{3}H]thymidine incorporation by rat aortic myocytes. Each bar represents the mean ± S.D. of triplicate dishes. Drug vs. control: $*\ P < 0.05$; $**\ P < 0.01$; $***\ P < 0.001$ (Student's *t*-test).

an inhibitory effect on both parameters (Freshney, 1986). We then determined whether picotamide interfered with DNA synthesis induced by the simultaneous presence in the culture medium of the peptide growth factor EGF and the non-peptide growth factor thromboxane A_2-mimetic U46619. U46619 alone did not significantly stimulate DNA synthesis in rat arterial myocytes, while a pronounced stimulation by EGF (two-fold) was detected; the

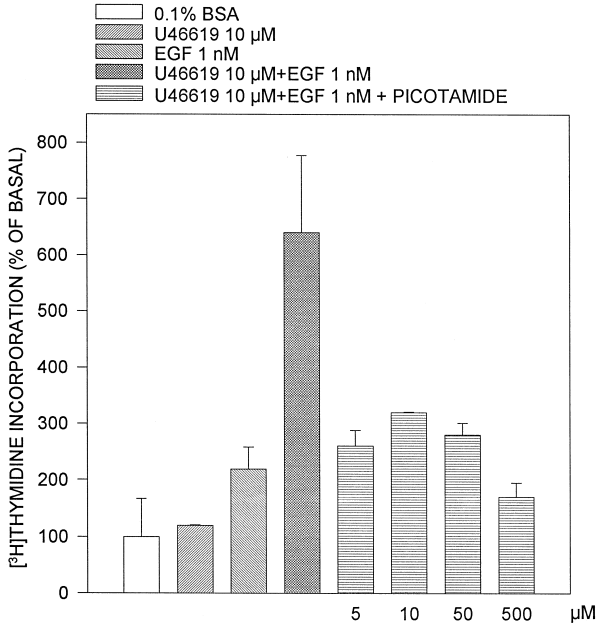

Fig. 3. Effect of picotamide on EGF- and U46619-induced [^{3}H]thymidine incorporation by rat aortic myocytes. The mean value of basal (0.1% bovine serum albumin) was 10,632 ± 7218 DPM/mg prot. and is designed as 100%. Duncan's test ($P < 0.05$): 6 vs. 8; 5 vs. 2; 3 vs. 1; ($P < 0.01$): 4 vs. 1, 2, 3, 5, 6, 7, 8; 6 vs. 1, 2; 7 vs. 1, 2; 5 vs. 1. 0.1% bovine serum albumin = 1; U46619 = 2; EGF = 3; U46619 + EGF = 4; picotamide 5 μM = 5; picotamide 10 μM = 6; picotamide 50 μM = 7; picotamide 500 μM = 8.

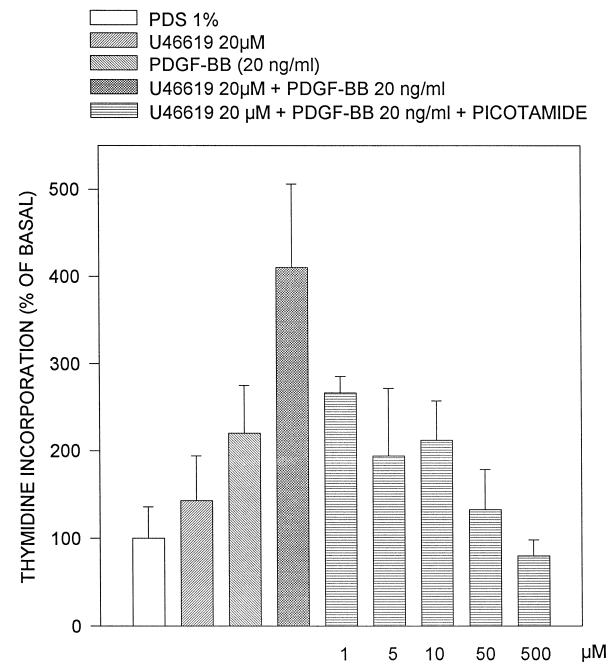

Fig. 4. Effect of picotamide on PDGF-BB- and U46619-induced [^{3}H]thymidine incorporation by rat aortic myocytes. Each bar represents the mean ± S.D. of triplicate dishes. The mean value of basal (1% plasma-derived bovine serum) was 38,830 ± 14,026 DPM/mg prot. and is designed as 100%. Duncan's test ($P < 0.05$): 6 vs. 1, 8; 3 vs. 1, 8; 5 vs. 8; ($P < 0.01$): 4 vs. 1, 2, 3, 5, 6, 7, 8, 9. 1% PDS = 1; U46619 = 2; PDGF = 3; U46619 + PDGF = 4; picotamide 5 μM = 5; picotamide 10 μM = 6; picotamide 50 μM = 7; picotamide 500 μM = 8.

Table 3
Effect of picotamide on the fibrinogen- and PDGF-BB-induced migration of rat aortic myocytes

Addition (μM)	SMC migration (% of control) (mean ± S.D.)	
	Fibrinogen	PDGF-BB
None	100.0 ± 31.0	100.0 ± 5.6
1	74.4 ± 21.4	
5	65.4 ± 4.0	13.8 ± 8.7[b]
10	44.3 ± 7.7[a]	
50	31.6 ± 18.0[a]	12.8 ± 5.8[b]

Data are the mean ± S.D. of triplicate dishes. The average cell number in controls was 4.2 ± 1.3 (fibrinogen) and 10.7 ± 0.6 (PDGF-BB).
Drug vs. control: [a] $P < 0.05$; [b] $P < 0.001$ (Student's *t*-test).

co-incubation with both mitogens resulted in a synergic response reversible by picotamide (Fig. 3), which displayed an inhibitory effect when DNA synthesis was induced by co-incubation of PDGF-BB together with U46619 (Fig. 4).

The effect of picotamide on cell migration was explored in rat aortic myocytes incubated for 5 h in the presence of fibrinogen or PDGF-BB as chemotactic factors. Picotamide inhibited myocyte migration in a concentration-dependent manner, with a more pronounced effect on PDGF-BB induced chemotaxis (Table 3).

4. Discussion

Migration and proliferation of smooth muscle cells are predominant features of atherogenesis (Ip et al., 1990; Ross, 1993). Several studies have provided evidence that platelet-derived products are mitogenic for vascular myocytes (Friedman et al., 1977; Ross, 1986; Willerson et al., 1991; Le Breton et al., 1996). Platelet-induced smooth muscle cell proliferation may not simply depend on peptide growth factors (i.e., PDGF and EGF) (Oka and Orth, 1983; Assoian et al., 1984; Ross, 1993) but may involve multiple growth signals mediated in part by other platelet-derived products including thromboxane A_2 (Hanasaki et al., 1990; Nagata et al., 1992; Morinelli et al., 1994).

In the present study, we show the effect of picotamide, an antiplatelet agent with dual antithromboxane activity (Gresele et al., 1989; Modesti et al., 1989) to inhibit arterial myocyte migration and proliferation. The antiproliferative effect of the drug, assayed as cell number and nuclear incorporation of [^{3}H]thymidine, was concentration-dependent. Similar results (Pakala et al., 1997) have been recently reported with ridogrel, a combined thromboxane A_2 synthase inhibitor and receptor antagonist (DeClerck et al., 1989). Recently, through the use of stable mimetics, thromboxane A_2 has been shown to be mitogenic or even to act as progression factor for vascular smooth muscle cells (Uehara et al., 1988; Hanasaki et al., 1990; Nagata et al., 1992; Grosser et al., 1997; Pakala et al., 1997). Nagata et al. (1992), in fact, have shown that 9,11-epithio-11,22-methano-thromboxane (STA_2), a stable analogue of thromboxane A_2, significantly enhanced DNA synthesis and shortened the doubling time in randomly cycling smooth muscle cells. However, data describing another thromboxane A_2 mimetic, U46619, as a mitogenic or hypertrophic agonist for smooth muscle cells, are contradictory and inconsistent (Akopov et al., 1988; Morisaki et al., 1988; Uehara et al., 1988; Hanasaki et al., 1990; Ali et al., 1993; Morinelli et al., 1994; Grosser et al., 1997; Pakala et al., 1997). Nevertheless, thromboxane A_2 could behave as an amplification factor for cellular growth response to locally released mitogenic peptides (Zucker et al., 1995; Grosser et al., 1997; Pakala et al., 1997). As expected, the addition of U46619 to EGF or PDGF elicited a clear-cut increase of [^{3}H]thymidine incorporation into DNA of aortic myocytes, which is completely prevented by picotamide. This result is consonant with the observation of Grosser et al. (1997), who recently showed that a specific thromboxane A_2 receptor antagonist inhibited the potentiating effect of U46619 on mitogenesis induced by PDGF-BB. The observation that picotamide inhibits human smooth muscle cell proliferation with a IC_{50} (174 μM) similar to those required to inhibit thromboxane A_2 synthesis in human platelets, as well as their aggregation induced by thromboxane A_2 ($IC_{50} = 100$ μM) (Gresele et al., 1989), suggests a potential relationship between its antithromboxane activity and inhibition of cell growth. On the other hand, since picotamide shows both competitive and non-competitive activities against thromboxane A_2 binding sites (Modesti et al., 1994), it is difficult to relate the binding kinetics with cell proliferation.

The mechanism whereby picotamide affects smooth muscle cell proliferation is unknown. A cytotoxic effect of the drug was ruled out since the cells excluded trypan blue and started growing again after picotamide removal. The fact that picotamide inhibited DNA synthesis induced by different mitogens, including peptide growth factors as well as a thromboxane A_2 mimetic, suggests that its mechanism of action is potentially related to a signaling pathway common to different mitogenic stimuli (Morinelli et al., 1994; Jones et al., 1995; Ko, 1997).

The effect of picotamide on migration and proliferation of arterial myocytes at the reported therapeutic concentrations (5–50 μM) (Fossati et al., 1992) may be of interest, given that interference with these processes has been suggested as a possible basis for antiatherosclerotic therapy (Ip et al., 1990; Ross, 1993). Although conflicting results have been reported in humans, smooth muscle cell proliferation occurs infrequently and may be episodic (Katsuda et al., 1993; O'Brien et al., 1993; Pickering et al., 1993). Since it is difficult to predict when such an event may occur, chronic therapy should probably be necessary to ensure suppression of replication at the critical times (Jackson and Schwartz, 1992). On the other hand, accelerated

smooth muscle cell proliferation appears to be a cause of early coronary occlusion in patients undergoing heart transplantation, coronary artery bypass grafting, and percutaneous transluminal coronary angioplasty, accounting for significant morbidity and mortality (Ip et al., 1990). Finally, recent findings support the notion that in-stent restenosis results from smooth muscle cell hyperplasia in patients with peripheral artery disease (Kearney et al., 1997). Thus, picotamide may decrease atherosclerosis progression by reducing platelet activation and preventing smooth muscle cell proliferation. Studies aimed at addressing the mechanisms of action of picotamide as well as its effects in an in vivo model are planned to gain insights into the clinical relevance of the antiatherosclerotic properties of the drug.

In summary, our data indicate that picotamide inhibits myocyte proliferation and migration in vitro in a concentration-dependent manner. These effects confer a pharmacological interest to the compound in the process of atherogenesis.

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References

Akopov, S.E., Orekhov, A.N., Tertov, V.V., Kashimov, K.A., Gabrielyan, E.S., Smirnov, V.N., 1988. Stable analogues of prostacyclin and thromboxane A_2 display contradictory influences on atherosclerotic properties of cells cultured from human aorta: the effect of calcium antagonists. Atherosclerosis 72, 245–248.

Ali, S.M., Davis, G., Becker, M.W., Dorn, G.W., 1993. Thromboxane A_2 stimulates vascular smooth muscle hypertrophy by up-regulating the synthesis and release of endogenous basic fibroblast growth factor. J. Biol. Chem. 268, 17397–17403.

Assoian, R.K., Grotendorst, G.R., Miller, D.M., Spora, M.B., 1984. Cellular transformation by coordinated action of three peptide growth factors from human platelets. Nature 309, 804–806.

Cocozza, M., Picano, T., Oliviero, U., Russo, N., Coto, V., Milani, M., 1995. Effects of picotamide, an antithromboxane agent, on carotid atherosclerotic evolution. A two-year, double-blind, placebo-controlled study in diabetic patients. Stroke 26, 597–601.

Corsini, A., Mazzotti, M., Raiteri, M., Soma, M.R., Gabbiani, G., Fumagalli, R., Paoletti, R., 1993. Relationship between mevalonate pathway and arterial myocyte proliferation: in vitro studies with inhibitors of HMG-CoA reductase. Atherosclerosis 101, 117–125.

Corsini, A., Verri, D., Raiteri, M., Quarato, P., Paoletti, R., Fumagalli, R., 1995. Effect of 26-aminocholesterol, 27-hydroxycholesterol and 25-hydroxycholesterol on proliferation and cholesterol homeostasis in arterial myocytes. Arterioscler. Thromb. Vasc. Biol. 15, 420–428.

Corsini, A., Bonfatti, M., Quarato, P., Accomazzo, M.R., Raiteri, M., Sartani, A., Testa, R., Nicosia, S., Paoletti, R., Fumagalli, R., 1996. Effect of the new calcium antagonist lercanidipine and its enantiomers on the migration and proliferation of arterial myocytes. J. Cardiovasc. Pharmacol. 28, 687–694.

DeClerck, F., Beetens, J., de Chaffoy Courcelles, D., Freyne, E., Janssen, P.A., 1989. R68070: thromboxane A_2 synthase inhibition and thromboxane A_2/prostaglandin endoperoxide receptor blockade, combined in one molecule: I. Biochemical profile in vitro. Thromb. Haemost. 61, 35–42.

Elmore, E., Swift, M., 1976. Growth of cultured cells from patients with Fanconi anemia. J. Cell Physiol. 87, 229–234.

Fossati, T., Parisi, S., Abbiati, G., Castiglioni, C., 1992. Determination of picotamide in human plasma and urine by high performance liquid chromatography. J. Chromatogr. 577, 382–386.

Freshney, R.I., 1986. Animal cell culture: a practical approach. In: Rickwood, D., Hames, B.D. (Eds), Practical Approach Series. IRL Press, Oxford, p. 14.

Friedman, R.J., Stemerman, M., Wenz, B., Moore, S., Gauldie, J., Gent, M., Tiell, M.L., Spaet, T.H., 1977. The effect of thrombocytopenia on experimental atherosclerotic lesion formation in rabbits: smooth muscle cell proliferation and re-endothelization. J. Clin. Invest. 60, 1191–1201.

Giustina, A., Bossoni, S., Cimino, A., Comini, M.T., Gazzoli, N., Leproux, G.B., Wehrenberg, W.B., Romanelli, G., Giustina, G., 1993. Picotamide, a dual TXB synthetase inhibitor and TXB receptor antagonist, reduces exercise-induced albuminuria in microalbuminuric patients with NIDDM. Diabetes 42, 178–182.

Gresele, P., Deckmyn, H., Arnout, J., Nenci, G.G., Vermylen, J., 1989. Characterization of *N,N′*-bis(3-picolyl)-4-methoxy-isophtalamide (picotamide) as a dual thromboxane synthase inhibitor/thromboxane A_2 receptor antagonist in human platelets. Thromb. Haemost. 61, 479–484.

Grosser, T., Zucker, T.-P., Weber, A.-A., Schulte, K., Sachinidis, A., Vetter, H., Schror, K., 1997. Thromboxane A_2 induces cell signaling but requires platelet-derived growth factor to act as a mitogen. Eur. J. Pharmacol. 319, 327–332.

Hanasaki, K., Nakano, T., Arita, H., 1990. Receptor-mediated mitogenic effect of thromboxane A_2 in vascular smooth muscle cells. Biochem. Pharmacol. 40, 2535–2542.

Ip, J.H., Fuster, V., Badimon, L., Badimon, J.J., Taubman, M.B., Chesebro, J.H., 1990. Syndromes of accelerated atherosclerosis: role of vascular injury and smooth muscle cell proliferation. J. Am. Coll. Cardiol. 15, 1667–1687.

Isner, J.M., Kearney, M., Bauters, C., Leclerc, G., Nikol, S., Pickering, J.G., Riessen, R., Weir, L., 1994. Use of human tissue specimens obtained by directional atherectomy to study restenosis. Trends Cardiovasc. Med. 4, 213–221.

Jackson, C.L., Schwartz, S.M., 1992. Pharmacology of smooth muscle cell replication. Hypertension 20, 713–736.

Jones, D.A., Benjamin, C.W., Linseman, D.A., 1995. Activation of thromboxane and prostacyclin receptors elicits opposing effects on vascular smooth muscle cell growth and mitogen-activated protein kinase signaling cascades. Mol. Pharmacol. 48, 890–896.

Katsuda, S., Coltrera, M.D., Ross, R., Gown, A.M., 1993. Human atherosclerosis: IV. Immunocytochemical analysis of cell activation and proliferation in lesions of young adults. Am. J. Pathol. 142, 1787–1793.

Kearney, M., Pieczeck, A., Haley, L., Losordo, D.W., Andres, V., Schainfeld, R., Rosenfield, K., Isner, J.M., 1997. Histopathology of in-stent restenosis in patients with peripheral artery disease. Circulation 95, 1998–2002.

Ko, F.N., 1997. Low-affinity thromboxane receptor mediates proliferation in cultured vascular smooth muscle cells of rats. Arterioscler. Thromb. Vasc. Biol. 17, 1274–1282.

Le Breton, H., Plow, E.F., Topol, E.J., 1996. Role of platelets in restenosis after percutaneous coronary revascularization. J. Am. Coll. Cardiol. 28, 1643–1651.

Modesti, P.A., Colella, A., Abbate, R., Gensini, G.F., Neri Serneri, G.G., 1989. Competitive inhibition of platelet thromboxane A_2 receptor binding by picotamide. Eur. J. Pharmacol. 169, 85–93.

Modesti, P., Cecioni, I., Colella, A., Costoli, A., Paniccia, R., Neri Serneri, G.G., 1994. Binding kinetics and antiplatelet activity of picotamide, a thromboxane A_2 receptor antagonist. J. Pharmacol. 112, 81–86.

Moncada, S., Vane, J.R., 1979. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostaglandin I_2. Pharmacol. Rev. 30, 293–331.

Morinelli, T.A., Zhang, L.-M., Newman, W.H., Meier, K.E., 1994. Thromboxane A_2 /prostaglandin H_2-stimulated mitogenesis of coronary artery smooth muscle cells involves activation of mitogen-activated protein kinase and S6 kinase. J. Biol. Chem. 269, 5693–5698.

Morisaki, N., Kanzaki, T., Motoyama, N., Saito, Y., Yoshida, S., 1988. Cell cycle-dependent inhibition of DNA synthesis by prostaglandin I_2 in cultured rabbit aortic smooth muscle cells. Atherosclerosis 71, 165–171.

Nagata, T., Uehara, Y., Numabe, A., Ishimitsu, T., Hirawa, N., Ikeda, T., Matsuoka, H., Sugimoto, T., 1992. Regulatory effect of thromboxane A_2 on proliferation of vascular smooth muscle cells from rats. Am. J. Physiol. 263, H1331–H1338.

Naito, M., Hayashi, T., Kuzuya, M., Funaki, C., Asai, K., Kuzuya, F., 1990. Effects of fibrinogen and fibrin on the migration of vascular smooth muscle cells in vitro. Atherosclerosis 83, 9–14.

Nakao, J., Ooyama, T., Ito, H., Chang, W.-C., Murota, S., 1982. Comparative effect of lipoxygenase products of arachidonic acid on rat aortic smooth muscle cell migration. Atherosclerosis 44, 339–342.

O'Brien, E.R., Alpers, C.E., Stewart, D.K., Ferguson, M., Tran, N., Gordon, D., Benditt, E.P., Hinohara, T., Simpson, J.B., Schwartz, S.M., 1993. Proliferation in primary and restenotic coronary atherectomy tissue. Implications for antiproliferative therapy. Circ. Res. 73, 223–231.

Oka, Y., Orth, D.N., 1983. Human plasma epidermal growth factor/β-urogastrone is associated with blood platelets. J. Clin. Invest. 72, 249–259.

Pakala, R., Willerson, J.T., Benedict, C.R., Phil, D., 1997. Effect of serotonin, thromboxane A_2, and specific receptor antagonists on vascular smooth muscle cell proliferation. Circulation 96, 2280–2286.

Pickering, J.G., Weir, L., Jekanowski, J., Kearney, M.A., Isner, J.M., 1993. Proliferative activity in peripheral and coronary atherosclerotic plaque among patients undergoing percutaneous revascularization. J. Clin. Invest. 91, 1469–1480.

Ross, R., 1971. The smooth muscle cell: II. Growth of smooth muscle in culture and formation of elastic fibers. J. Cell. Biol. 50, 172–186.

Ross, R., 1986. The pathogenesis of atherosclerosis—an update. New Engl. J. Med. 314, 488–500.

Ross, R., 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362, 801–809.

Skalli, O., Ropraz, P., Trezciak, A., Benzonana, G., Gillessen, D., Gabbiani, G., 1986. A monoclonal antibody against alfa-smooth muscle actin: a new probe for smooth muscle differentiation. J. Cell Biol. 103, 2787–2796.

Uehara, Y., Ishimitsu, T., Kimura, K., Ishii, M., Ikesa, T., Sugimoto, T., 1988. Regulatory effects of eicosanoids on thymidine uptake by vascular smooth muscle cells of rats. Prostaglandins 36, 847–857.

Willerson, J.T., Yao, S.K., McNatt, J., Benedict, C.R., Anderson, H.V., Golino, P., Murphree, S.S., Buja, L.M., 1991. Frequency and severity of cyclic flow alterations and platelet aggregation predict the severity of neointimal proliferation following experimental coronary stenosis and endothetiel injury. Proc. Natl. Acad. Sci. USA 88, 10624–10628.

Zucker, T.-P., Grosser, T., Morinelli, T., Halushka, P.V., Sachinidis, A., Vetter, H., Schror, K., 1995. Potentiation of PDGF-induced growth responses in coronary artery smooth muscle cells by thromboxane. Mediators in the Cardiovascular System: Regional Ischemia. Basel, Birkhauser Verlag, pp. 53–58.