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Picotamide: An Inhibitor of the Formation and Effects of TxA_2

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Key Words: Picotamide— TxA_2 antagonists— TxA_2 synthase inhibitors—Peripheral vascular diseases—Renal diseases—Myocardial ischemia—Inflammatory bowel disease.

INTRODUCTION

After the discovery of thromboxane A_2 (TxA_2) by Hamberg in 1975 (31), considerable efforts have been made to find a drug therapy to control its biological effects. Platelets produce a considerable amount of TxA_2 during aggregation and are involved in thromboembolic diseases. Early antithrombotic and anti TxA_2 therapies were viewed as antiplatelet treatments, and the cyclooxygenase inhibitors, particularly aspirin, became the drugs of choice for antithrombotic therapy. The usefulness of these drugs was limited because they do not specifically block TxA_2 . Thus, a more specific and effective drug to inhibit TxA_2 was sought. Another compelling reason to develop new specific and effective TxA_2 inhibitors was the discovery that many cells in addition to platelets, such as monocyte-macrophages, endothelial, and vascular muscle cells (44,55,63), synthesize and release TxA_2 . Even cells that do not synthesize TxA_2 at rest may express cyclooxygenase and synthesize TxA_2 when stimulated by inflammatory cytokines (67). Thus, TxA_2 may represent an important mediator of different inflammatory processes: it has diverse biological effects, including contraction of vascular and pulmonary smooth muscle, lysis of cell membranes, and promotion of leucocyte adhesion. As a consequence, TxA_2 may play a role not only in platelet aggregation but also in renal disease, chronic bowel disease and various cardiovascular disorders, including coronary artery disease.

Because of the possible wide-ranging effects of TxA_2 , the search for effective inhibitors of TxA_2 has been expanded. These inhibitors are being investigated as part of a general treatment strategy to be used in several diseases where increased TxA_2 formation plays a pathophysiological role.

Two specific anti TxA_2 approaches were initially proposed: inhibition of TxA_2 synthase and inhibition of TxA_2 receptors, but in clinical trials neither approach offered any

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advantage (21). Indeed, from a pharmacological point of view, the problems with both of these approaches were evident. TXA_2 synthase inhibitors lead to accumulation of endoperoxides prostaglandin H_2 /prostaglandin G_2 ($\text{PGH}_2/\text{PGG}_2$), which possess the main biological activity of TXA_2 and share a common receptor with TXA_2 (68). Antagonists at the $\text{PGH}_2/\text{TXA}_2$ receptor can be displaced by native TXA_2 if it is present at sufficiently high concentrations in the vicinity of aggregating platelets or TXA_2 -forming cells (i.e. at the sites where receptor blockade is mostly required).

A newly developed class of dual TXA_2 inhibitors, composing drugs that combine TXA_2 synthase inhibitory activity with $\text{TXA}_2/\text{PGH}_2$ receptor antagonist activity, seems to represent the ideal solution for effective TXA_2 blockade. The TXA_2 synthase inhibitor property may augment the synthesis of vasodilatory prostaglandins at the expense of TXA_2 , whereas the receptor antagonist activity prevents the accumulating PGH_2 from stimulating the $\text{TXA}_2/\text{PGH}_2$ receptor. A balance between these two activities is the most important requirement for dual inhibitors. Picotamide satisfies these requirements.

CHEMISTRY

Picotamide is a methoxy derivative of the 4-hydroxy-isophthalic acid [N,N'-bis(3-picolyl)-4-methoxy-isophthalamide] (CAS 80503-63-8). Its chemical structure is shown in Fig. 1. Its synthesis and that of some analogs were published initially by Orzalesi *et al.* (49). Picotamide is a white, odorless, crystalline powder that melts at 95–97°C. Its molecular formula is $\text{C}_{21}\text{H}_{20}\text{N}_4\text{O}_3 \cdot \text{H}_2\text{O}$, and its molecular weight is 394.4. Its solubility in water is low but it is soluble in organic solvents and acidic solutions.

PHARMACOKINETICS

Picotamide capsules gradually dissolve in the gastrointestinal tract and the drug is rapidly absorbed. After oral administration (p.o.) of 300 mg of the drug, the observed time to maximum concentrations was 0.5–0.75 h, with a mean of 0.69 ± 0.13 h, indicating rapid absorption from the gastrointestinal tract (23). The peak plasma concentration was 2.02 ± 0.37 $\mu\text{g/ml}$ (23).

Recent animal studies showed that picotamide diffuses from the plasma into the arterial wall and can inhibit the biological activities of TXA_2 formed outside platelets. After the intravenous injection of [^{14}C] picotamide to rabbits, radioactivity reached the arterial wall

FIG. 1. Chemical structure of picotamide.

with a peak time of 116 min for the aorta and 112 min for the femoral artery, with a $t_{1/2}$ of radioactivity disappearance of 124 and 111 min, respectively (38).

Previous studies performed with [^{14}C]-picotamide showed that urinary and fecal excretion accounted for 46% and 43% of administered dose, respectively (51). More recently it was shown that about 30% of the dose (300 mg p.o.) was recovered as unchanged drug in the 24 h urine, mostly (about 25%) during the first 4 h after treatment (23). In rats and monkeys an important biliary elimination was found (51). The relatively low amount of the unchanged drug excreted in the urine by humans suggests that in humans the drug is also excreted in the bile.

PHARMACOLOGY

Antiaggregating Properties

In Vitro Studies

Picotamide was first observed to have antiaggregating properties in rabbit platelets (50), and then also in human platelets (2,3,7,15,16,28,70). In those studies picotamide was incubated with platelets for 2–3 min. It appeared to be a weak inhibitor of both TXA_2 -mediated platelet aggregation (IC_{50} on platelet aggregation induced by a TXA_2 analogue, U46619, was 1.4×10^{-4} M) and platelet TXA_2 production (IC_{50} of 1.5×10^{-4} M), without any inhibitory effect on PGI_2 synthesis (7,16,28,71). Recent studies have demonstrated that the *in vitro* antiaggregating activity of picotamide increases time-dependently. The IC_{50} for U46619 was reduced from 6.1×10^{-4} M at 2 min, to 7×10^{-6} M when picotamide was incubated for 20 min with platelets (Fig. 2). Supporting these findings, the inhibition of TXA_2 formation by platelets during collagen-induced aggregation ($7.5 \mu\text{g/ml}$) appeared to be also time-dependent (41). Thus, the increase in the incubation time from 2 to 20 min resulted in a 2 orders of magnitude increase in the antiaggregating and anti TXA_2 activities of picotamide.

FIG. 2. Platelet aggregation induced by U46619 ($0.75 \mu\text{mol/L}$) with increasing concentrations of picotamide after different incubation times (empty squares = 2 minutes incubation, empty triangles = 10 minutes, filled squares = 20 minutes). Points represent mean values (from ref. 41 with permission).

Ex Vivo Studies

In a pioneer *ex vivo* study on the antiaggregating activity of picotamide, Schmutzler *et al.* (62) used Breddin's method (5) to demonstrate a significant decrease in platelet aggregation after a first dose of 1500 mg/day. The antiaggregating effect further increased during the following 30 days of picotamide treatment. The *ex vivo* antiaggregating activity of picotamide was confirmed by more recent studies. Violi *et al.* reported 55% inhibition of platelet aggregation induced by collagen (4 µg/ml) after administration of picotamide to healthy subjects 600 mg twice daily (b.i.d.) for 3 days (71). TXA₂ formation by aggregating platelets was similarly inhibited, whereas 6-keto-PGF₁α in blood samples remained unaffected (71).

The inhibitory activity of picotamide is reversible and lasts 8–12 hours after drug administration. In healthy subjects platelet aggregation induced by arachidonic acid (1 mM) or U46619 (0.5 µM) was inhibited by a single oral dose of 1000 mg picotamide from the fourth to the tenth hour (3). No effect on ADP-induced aggregation, which follows a TXA₂ independent pathway, was noted (3). A reduction of TXA₂ produced by aggregating platelets both 2 (–52%) and 8 hours (–38%) following drug administration was reported by Minuz *et al.* after administration of picotamide 300 mg three times a day (t.i.d.) for one week (36).

No correlation exists between the plasma concentration of the drug, which peaks after 2 hours (23), the inhibition of platelet aggregation, and TXA₂ production, which are detectable between 4 and 8–10 hours (3,36). These findings prompted initially the hypothesis that the effects of picotamide were achieved in part by a metabolite rather than the drug itself (3). Recent knowledge of the mechanism of action of picotamide indicates that picotamide *in vivo* may act as a functional noncompetitive antagonist.

Mechanism of Action

Picotamide was able to inhibit dose-dependently the aggregation of aspirin-pretreated platelets (unable to produce TXA₂) induced by the TXA₂ analogue U46619 (16,28). These results suggest that picotamide has a TXA₂ receptor inhibitory activity. Moreover, the decreased TXA₂ platelet aggregation induced by picotamide was associated with increased PGE₂ (28) and PGI₂ formation (3,28), which indicates a selective inhibitory activity of TXA₂ synthase (7).

Pulcinelli *et al.* (57) recently showed that picotamide inhibited both TXA₂ receptor effector systems. The first system is linked to phospholipase C (PLC) activation, which causes platelet aggregation and secretion, and the second mediates an increase in cytosolic Ca⁺⁺ and platelet shape change. In fact, at 10^{–4} M picotamide inhibited PLC activation and caused an 80% reduction in Ca⁺⁺ increase caused by U46619 in platelets (57).

The direct demonstration that picotamide is a TXA₂/PGH₂ receptor inhibitor was obtained by binding studies that showed that picotamide inhibited the binding of both agonist- ([³H]U46619) and antagonist- ([¹²⁵I]PTA-OH; 9,11-dimethylmethano-11, 12-methano-16-(3[¹²⁵I]-4-hydroxyphenyl)-13,14-dihydro-13-aza-15-tetranor-TXA₂) TXA₂ analogs (37).

The binding of picotamide to a single class of TXA₂ receptors (with a K_d of 325 nM) was recently confirmed by using radiolabelled picotamide (Fig. 3) (41). The binding of [³H]methylene-picotamide was inhibited not only by unlabelled picotamide but also by

TXA₂ stable analogs (Fig. 4) (41). Most importantly, picotamide can bind to the TXA₂ receptors in the vascular wall of human artery with the same characteristics as for binding to platelets (40). Picotamide binding to TXA₂/PGH₂ receptors reached equilibrium rather slowly (30 min) so that after 2–3 min of incubation (the usual time of incubation for platelets in aggregation studies) only 15–20% of the total TXA₂ receptors per platelet at equilibrium (about 2066 per platelet) were blocked (41). This pattern agrees with the findings obtained in the studies of platelet aggregation and may explain the reported weak antiaggregating activity when platelet aggregation was assessed after 2–5 minutes of incubation (3,28).

Another relevant aspect arising from the binding studies is that picotamide behaves, at least in part, as a functionally noncompetitive receptor antagonist. Indeed, [³H]-picotamide was almost completely displaceable from the TXA₂ receptor during the first 30 min of incubation. After this time the binding became progressively more stable so that after 2 hours only 50% of picotamide molecules bound to TXA₂ receptors were displaceable. This time-dependent stable interaction of picotamide with TXA₂ platelet receptor mimics a noncompetitive pattern of inhibition (54). *Ex vivo* studies have confirmed the noncompetitive blockade: the *ex vivo* binding of a labelled TXA₂ analog remained inhibited for up to 6 hours after a single administration of 300 mg of picotamide (39). This functionally noncompetitive antagonism at the TXA₂/PGH₂ receptor may explain the above reported discrepancy between the short half-life of picotamide in plasma (1 h) and its long-lasting (4–10 h) inhibitory effects on platelets. TXA₂ receptors remained occupied even when the plasma concentration of picotamide was reduced.

ANIMAL STUDIES

Several animal studies have shown that the inhibitory effects of picotamide on either TXA₂ synthase or on TXA₂ receptor lead to reduction of mortality in animal models with enhanced TXA₂ formation.

In rabbits picotamide significantly reduced mortality due to endotoxin (19). Gresele *et al.* (29) showed that picotamide and aspirin were equally effective in reducing the LD₅₀

FIG. 3. Saturation curve of [³H]methylene-picotamide binding to washed human platelets at 22°C and Scatchard analysis of the binding (from ref. 41 with permission).

FIG. 4. Displacement of the specific [³H]methylene-picotamide binding by increasing concentrations of different compounds. Solid squares = U46619 (9,11-dideoxy-11 alpha, 9 alpha-epoxymethano-prostaglandin F2); empty triangles = ONO11120 (9,11-dimethylmethano-11,12-methane-16-phenyl-13,14-dihydro-13-aza-15-tetranor-TXA₂); empty circles = picotamide; empty squares = TXB₂; solid triangles = PGE₂; solid circles = PGI₂ (from ref. 41 with permission).

of an injected collagen plus epinephrine combination. Picotamide and BM 13.505 (a TXA₂ receptor inhibitor), but not aspirin, reduced mortality in rats injected intravenously (i.v.) with U46619. Picotamide, but not aspirin, inhibited lethal effects of an i.v. injection of a suspension of hardened rat red blood cells, a model in which PGI₂ synthesis by platelets is not involved (29).

Golino *et al.* (27) tested the effectiveness of different agents known to interfere with the synthesis and/or the receptor of TXA₂ in abolishing the spontaneous or epinephrine-induced carotid flow variations in rabbits with experimental carotid artery stenosis and endothelial injury. A pure TXA₂/PGH₂ receptor antagonist (SQ29548) and a selective TXA₂ synthase inhibitor (dazoxiben) were equally effective, in inhibiting carotid flow reduction in about 50% of treated animals. Picotamide inhibited the flow reduction in over 90% of animals, suggesting that simultaneous blockade of TXA₂/PGH₂ receptor and inhibition of TXA₂ synthase are more effective than the separate inhibition of TXA₂ synthesis or TXA₂ receptor blockade (27).

CLINICAL STUDIES

The primary criterion for testing a TXA₂ inhibitor in a particular disease is evidence of involvement of TXA₂ in the pathogenesis of the disease. In early clinical studies picotamide was used as an antiaggregating agent under clinical conditions where TXA₂ plays only a minor role. After initial studies were performed in patients with either peripheral vascular disease or stable effort angina, and after the specific anti-TXA₂ activities of the drug were identified, picotamide was used in human diseases where formation of extra-

platelet TXA₂ might play a definite pathophysiological role, such as unstable angina, renal failure, or inflammatory bowel disease. In these latter conditions, where a specific anti-TXA₂ activity is particularly important, encouraging results were obtained with picotamide.

Peripheral Vascular Disease

Increased TXA₂ production in platelets has often been reported in peripheral vascular disease and picotamide has been used essentially as an antiplatelet agent. Eighteen studies on the effect of picotamide in peripheral vascular disease have been performed (1,6,8,11,12,13,14,18,20,24,30,42,50,52,56,61,64,66). Seven of these were double-blind, placebo-controlled (1,6,8,12,18,42,56) (Table 1). All studies reported an improvement in the Windsor Index and in the clinical course of the disease. The most important one was the ADEP study (1), a multicenter, randomized, double-blind, placebo-controlled study, aimed at assessing the clinical efficacy of picotamide in intermittent claudication. After a 1-month run-in period, 2304 patients were enrolled and randomly allocated to either picotamide (300 mg t.i.d.) or placebo. The occurrence of major and minor events was then recorded during an 18-month follow-up period. At the "intention to treat" analysis there was a borderline statistical difference between the two groups with respect to the cumulative major and the minor events (risk reduction 18.9%, $p = 0.056$). When patients who continued treatment during the follow-up period were considered, thus performing an "on-treatment analysis," events were observed in 106 patients in the picotamide group (10.1%) compared with 140 in the placebo group (13.1%), with an overall risk reduction of 23% ($p < 0.03$). A subanalysis (intention to treat analysis) of 438 diabetic patients enrolled in the ADEP study showed that picotamide significantly reduces total cardiovascular events in diabetic patients with peripheral obstructive arterial disease ($p < 0.02$) (71).

Clinical efficacy of picotamide was assessed also in other small studies. An improvement in the clinical course of macro- and microangiopathic lesions and the photoplethysmographic findings at 3 and 6 months follow-up was reported by Pibiri *et al.* (56) in a

TABLE 1. Controlled clinical studies on the effects of picotamide in cardiovascular diseases

Author	Year	Patients enrolled	Follow-up	Study design	Parameters investigated	ANOVA test
Coto <i>et al.</i> ¹²	1989	40	6 m	R, DB, PG, vs PL	Windsor Index	$p < 0.05$
					Walking ability	$p < 0.05$
Pibiri <i>et al.</i> ⁵⁶	1990	51	6 m	R, DB, PF, vs PL	Windsor Index	$p < 0.05$
					Walking ability	$p < 0.05$
Canonica <i>et al.</i> ⁶	1991	25	3 m	R, DB, CO, vs Pl	Windsor Index	$p < 0.05$
					Walking ability	$p < 0.05$
De Falco <i>et al.</i> ¹⁸	1991	87	24 m	O, PG, vsASA	Cardiovascular events	—
Balsano <i>et al.</i> ¹	1993	2304	18 m	R, DB, PG, vs PL	Cardiovascular events	$p < 0.029^*$
Neirotti <i>et al.</i> ⁴²	1994	20	18 m	R, DB, PG, vs PL	Pletismographic wave	$p < 0.05$
Neri Sermeri <i>et al.</i> ⁴⁷	1994	40	1 w	R, Db, PG, vsASA	Anginal attacks	$p < 0.05$
					Silent ischemia	$p < 0.01$
Cocozza <i>et al.</i> ⁸	1995	50	24 m	R, DB, PG vs PL	Carotid lesion number	$p < 0.03$
					Percent stenosis	$p < 0.01$

DB = double blinded, PG = parallel groups, CO = cross over, PL = placebo, ASA = aspirin, R = randomized, O = open.

* log-rank test for risk reduction (23%).

group of 51 diabetic patients treated with picotamide 600 mg/day in comparison to the placebo group. These results were confirmed in a group of 20 non-diabetic atherosclerotic patients (stage II according to Fontaine) treated with picotamide 300 mg t.i.d. compared with the placebo group (42). Three double-blind, placebo-controlled studies (6,12,56) reported a significant improvement in the walking distance in patients who received picotamide (600–900 mg/day) compared with placebo (+ 23% and – 40%, respectively).

A recent double-blind, placebo-controlled study showed that long-term treatment (24 months) with picotamide slows the evolution of carotid atherosclerotic lesions (8). After 24 months, the number of carotid lesions ($p < 0.03$) and percent stenosis ($p < 0.01$) were significantly lower in the picotamide than in the randomized placebo group (8).

Myocardial Ischemia and Unstable Angina

Recent studies provided evidence that unstable angina may be sustained by an acute immune-mediated inflammatory reaction (33,46). Lymphocytes and monocytes were activated (46), with increased TXA_2 formation even in the presence of aspirin-blocked platelets (43,45), suggesting an extraplatelet source of TXA_2 . Moreover, in patients with unstable angina no relationship was found between the amount of TXA_2 produced by platelets (measured by assessing 1–3 dinor TXB_2 , a metabolite of TXB_2 of platelet origin) and anginal episodes (69), indirectly suggesting that platelet activation is not necessarily a primary event in myocardial ischemia. Monocytes from patients with active unstable angina produce an increased amount of TXA_2 (47) when compared with monocytes from patients in the inactive phase. The effects of picotamide were compared with those of aspirin in a pilot study in unstable angina (47). Picotamide, but not aspirin, controlled myocardial ischemia (anginal attacks – 84.8%, silent ischemic episodes – 64.2% and overall duration of ischemia – 69.8%), although aspirin inhibited platelet TXA_2 production more than picotamide. The results of this study indicate that picotamide can control myocardial ischemia that is mainly due to TXA_2 formed by monocytes in the atherosclerotic plaque, whereas aspirin at the usual therapeutic dose (324 mg/day) is unlikely to reach the extravascular sites in acetylated form. Indeed, aspirin is rapidly deacetylated by the esterases in plasma and the liver. In an open study performed on 19 patients with stable effort angina, picotamide (900 mg/day for 10 months) significantly reduced the mean nitrate consumption (48).

Renal Disease

Increased TXA_2 generation has been reported in different renal diseases and may contribute to glomerular damage (4,53,65). In patients with type II diabetes with microalbuminuria at rest, short term administration of picotamide (900 mg/day for 10 days) was associated with a reduction in albuminuria after exercise (25). In a small prospective study performed on 6 diabetic patients (9-month treatment), picotamide reduced proteinuria at the 3, 6, and 9-month follow-up tests (26).

In renal transplantation TXA_2 may play a role, as suggested by the increased urinary excretion of TXA_2 metabolites reported in patients with acute graft rejection (22). In a randomized, double-blind, placebo-controlled study performed on 36 renal transplant patients, prolonged picotamide treatment (600 mg/day for 1 year) significantly reduced urinary excretion of TXB_2 and improved renal function. After one year of picotamide

treatment, the mean plasma concentration of creatinine decreased in the picotamide group ($-14.2 \pm 3.9 \mu\text{mol/l}$ vs baseline) but not in the placebo group ($+13.5 \pm 5.2 \mu\text{mol/l}$ vs baseline) (58,59).

Inflammatory Bowel Disease

Although the etiology of inflammatory bowel disease remains obscure, there is a growing body of evidence suggesting a possible role of TXA_2 (32,34). In Crohn's disease TXA_2 is produced in excess not only in inflamed mucosa but also by normal bowel and by isolated intestinal and peripheral blood mononuclear cells (60). After the initial studies in animal models, where TXA_2 inhibitors showed beneficial effects, picotamide has been recently tested in inflammatory bowel disease in humans. In a clinical trial performed in 9 patients with Crohn's disease, picotamide (600 mg b.i.d.) inhibited colonic mucosal generation of TXB_2 and improved the clinical course of the disease (diarrhea and abdominal pain) (9,10).

SIDE EFFECTS

Picotamide was well tolerated even in studies with long follow-up periods. The side effects in the picotamide group of the ADEP study (1) were assessed by monitoring the adverse reactions (ADRs, classified according to the World Health Organization coding system) in terms of their severity, duration, relationship to treatment, evolution, and consequences, and were reported in a separate study (72). ADRs in the picotamide group did not differ from those in the placebo group. In particular, 165 out of 1150 patients (14.3%) on picotamide and 156 out of 1154 (13.5%) on placebo complained of at least one ADR. The total number of ADRs reported in the case report forms were 240 for picotamide and 239 for placebo. The most frequent ADRs were gastrointestinal disturbances, predominantly abdominal pain (8.2% for picotamide and 8.5% for placebo). Others were nausea (1.6% for picotamide and 1.0% for placebo), headache (1.4% and 0.8%), and pruritus (1.1% and 0.8%).

CONCLUSIONS

In conclusion, picotamide is a TXA_2 antagonist that inhibits the synthesis of TXA_2 at the synthase level and its effect at the receptors. Although its clinical efficacy appears promising in different human diseases, larger clinical studies are needed to definitively assess its efficacy in clinical conditions where platelet and extraplatelet TXA_2 play relevant roles. If large clinical trials confirm its clinical efficacy, picotamide might replace classical cyclooxygenase inhibitors in the treatment of vascular, renal, and inflammatory bowel diseases.

REFERENCES

1. Balsano F, Violi F, and the ADEP Group. Effect of picotamide on the clinical progression of peripheral vascular disease. *Circulation* 1993;87:1563-69.
2. Berettini M, De Cunto M, Romanelli A, Volpato I, Nenci GG. Studio in vitro sul meccanismo dell'azione antiaggregante piastrinica della picotamide. *Boll Soc Ital Biol Sperim* 1983;59:309.
3. Berettini M, De Cunto M, Parise P, Grasselli S, Nenci GG. "In vivo" and "ex vivo" effects of picotamide,

- a combined TXA₂-synthase inhibitor and receptor antagonist, on human platelets. *Eur J Clin Pharmacol* 1990;39:495-500.
4. Bonomini V. Thromboxane A₂ and renal pathology. In: Neri Serneri et al. eds. Thromboxane A₂ and other vasoconstrictors in clinical conditions. Florence: Scientific Press 1993.
 5. Breddin K. *Die Thrombozytenfunktion bei hämorrhagischen Diathesen, Thrombosen und Gefäßkrankheiten*. Stuttgart: F.K. Schattauer. 1968.
 6. Canonico V, Ammaturo V, Guarini P, et al. Valutazione clinico-strumentale della efficacia della picotamide nel trattamento delle arteriopatie croniche ostruttive degli arti inferiori. *Min Cardioangiol* 1991;39:75-80.
 7. Cattaneo M, Tenconi PM, Lecchi A, et al. In vitro effects of picotamide on human platelet aggregation, the release reaction and thromboxane A₂ production. *Thromb Res* 1991;62:717-724.
 8. Cocozza M, Picano T, Oliviero U, Russo N, Coto V, Milani M. Effects of picotamide, an antithromboxane agent, on carotid atherosclerotic evolution. *Stroke* 1995;26:597-601.
 9. Collins CE, Benson MJ, Burnham WM, Rampton DS. Selective thromboxane synthesis inhibition/receptor antagonism with picotamide: A new therapeutic approach in inflammatory bowel disease? *Gut* 1993;34:29.
 10. Collins CE. An open trial of antiplatelet therapy in active Crohn's disease using picotamide, a thromboxane antagonist. *Congr Brit Soc of Gastroenterology* (Abstract) 1994.
 11. Corsi M, Angelici AM. Considerazioni sull'evoluzione della malattia aterosclerotica dopo tre anni di terapia con un antiaggregante piastrinico selettivo per il trombossano A₂. *Min Angiol* 1991;16:95-97.
 12. Coto V, Cocozza M, Oliviero U, et al. Clinical efficacy of picotamide in long-term treatment of intermittent claudication. *Angiology* 1989;40:880-885.
 13. Craveri A, Colombo L, Cristina M, Coloru M, De Angeli B, Lanfredini M. Modificazioni dell'aggregazione piastrinica mattutina in soggetti affetti da arteriopatia obliterante periferica dopo somministrazione serale di picotamide. *Clin Ter* 1991;136:23-29.
 14. Danese C, Sergio G, Giunta G, et al. Effects of prolonged picotamide therapy on platelet activity in patients with peripheral arterial disease. *Curr Med Res Opinion* 1988;11:221-226.
 15. Davi G, Belvedere M, Catalano I, et al. Platelet function changes induced by picotamide administration in type II diabetic patients. *Thromb. Halm. Disord.* 1991;3:17.
 16. Deckmyn BOH, Gresele P, Van Outte E, Nenci GG, Vermynen JJ. Synergism of a thromboxane synthetase inhibitor and a receptor antagonist in reducing platelet aggregation. *Thromb Haemostas* 1985;54:677.
 17. De Cunto M, Berrettini M, Parise P, DeRegis M, Orzalesi G, Nenci GG. Studio in vitro dell'effetto di alcuni derivati dell'acido 4-idrossi-isoftalico sull'aggregazione di piastrine umane. *Boll Soc Ital Biol Sperim* 1983;59:302.
 18. de Falco FA, Montariello A, Mastroberto G, Visconti OS. Clinical efficacy of picotamide. *Clin Ter* 1991;138:21-6.
 19. Feldhoff CM, Gresele P, Pieters G, Vermynen J. Acetylsalicylic acid, BM 13.177 and picotamide improve the survival of endotoxin-infused rabbits. *Thromb Res* 1988;52:87-92.
 20. Ficara A. Revisione di 8 casi clinici di arteriopatia giunti alla nostra osservazione, in terapia con picotamide. *Giorn Ital Angiol* 1990;X (1/90).
 21. Fiddler GI, Lumley P. Preliminary clinical studies with thromboxane synthase inhibitors and thromboxane receptor blockers: a review. *Circulation* (Suppl 1) 1990;81:69-78.
 22. Foegh ML, Lim KL, Alijani MR, Helfrich GB, Ramwell PW. Thromboxane and inflammatory cell infiltration of the allograft of renal transplant patients. *Transplant Proc* 1987;19:3633-3636.
 23. Fossati T, Parisi S, Abbiati G, Castiglioni C. Determination of picotamide in human plasma and urine by high-performance liquid chromatography. *J Chromatography* 1992;577:382-386.
 24. Galati G, Terrinoni V, Rengo M, et al. La picotamide nel trattamento delle arteriopatie obliteranti degli arti inferiori. *Chirurgia* 1989;2:345-348.
 25. Giustina A, Bossoni S, Cimino A, et al. Picotamide, a dual TXB synthetase inhibitor and TXB receptor antagonist, reduces exercise-induced albuminuria in microalbuminuric patients with NIDDM. *Diabetes* 1993;42:178-183.
 26. Giustina A. Effect of long-term administration of picotamide on baseline and exercise-induced urinary albumin excretion in patients with type II diabetes mellitus and incipient nephropathy. *Clin Ther* 1994;16:191.
 27. Golino P, Ambrosio G, Gresele P, et al. The in vivo antiplatelet effects of thromboxane A₂ synthase inhibitors are potentiated by simultaneous thromboxane A₂/prostaglandin H₂ receptor blockade. *J Pharmacol Exp Ther* 1991;266:511-16.
 28. Gresele P, Deckmyn H, Arnout J, Nenci GG, Vermynen J. Characterization of N,N'-bis(3-picolyl)-4-methoxy-isophthalamide (picotamide) as a dual thromboxane synthase inhibitor/thromboxane A₂ receptor antagonist in human platelets. *Thromb Haemostas* 1989;61:479-485.
 29. Gresele P, Corona C, Alberti P, Nenci GG. Picotamide protects mice from death in a pulmonary embolism model by a mechanism independent from thromboxane suppression. *Thromb Haemostas* 1990;64:80-86.

30. Guarini P. Valutazione a medio termine dell'efficacia clinico-strumentale e della tollerabilità della picotamide in pazienti affetti da arteriopatia obliterante degli arti inferiori. *Minerva Cardioang* 1989;37:517.
31. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci USA* 1975;72:2994-2998.
32. Hawkey CJ, Rampton DS. Prostaglandins and the gastrointestinal mucosa: are they important in its functions, disease or treatment? *Gastroenterology* 1985;89:1462-1488.
33. Jude B, Agraou N, McFadden E, et al. Evidence for time-dependent activation of monocytes in the systemic circulation in unstable angina but not in acute myocardial infarction. *Circulation* 1994;90:1662-1668.
34. Lauritsen K, Laursen LS, Bukhave K, Rask-Madsen J. In vivo profiles of eicosanoids in ulcerative colitis, Crohn's colitis and C. difficile colitis. *Gastroenterology* 1988;95:11-17.
35. Matera G, Chisari M, Altavilla D, Foca A, Cook JA. Selective thromboxane synthetase inhibition by picotamide and effects on endotoxin induced lethality. *Proc Soc Exp Biol Med* 1988;187:58-61.
36. Minuz P, Lechi C, Arosio E, et al. Effect of Picotamide on platelet aggregation and on thromboxane A₂ production in vivo. *Thromb Haemostas* 1991;65:312-316.
37. Modesti PA, Colella A, Abbate R, Gensini GF, Neri Serneri GG. Competitive inhibition of platelet thromboxane A₂ receptor binding by picotamide. *Eur J Pharmacol* 1989;169:85-93.
38. Modesti PA, Colella A, Cecioni I, Arrigoni M, Abbiati G, Neri Serneri GG. Capacity of picotamide to diffuse into arterial wall and to bind to receptors of vascular muscle cells. *Thromb Haemostas* 1995;73:1323.
39. Modesti PA, Colella A, Cecioni I, Gensini GF, Abbate R, Neri Serneri GG. Acute reduction of TXA₂ platelet binding sites after TXA₂ receptor inhibitor in vivo administration. *Br J Clin Pharmacol* 1991;31:439-442.
40. Modesti PA, Colella A, Cecioni I, Costoli A, Neri Serneri GG. Picotamide inhibits TXA₂/PGH₂ receptors of vascular muscle cells from human arteries. *Thromb Haemostas* 1993;69:459.
41. Modesti PA, Cecioni I, Colella A, Costoli C, Paniccia R, Neri Serneri GG. Binding kinetics and antiplatelet activities of picotamide, a thromboxane A₂ receptor antagonist. *Br J Pharmacol* 1994;112:81-86.
42. Neirotti M, Molaschi M, Ponzetto M, et al. Hemodynamic, hemorheologic, and hemocoagulative changes after treatment with picotamide in patients affected by peripheral arterial disease of the lower limbs. *Angiology* 1994;45:137-41.
43. Neri Serneri GG. Prostaglandins in patients with ischemic heart disease. In: Herman AG, Vanhoutte PM, Demolin H, Goossen A, eds. *Cardiovascular pharmacology of prostaglandins*. New York: Raven Press 1982:361-374.
44. Neri Serneri GG, Abbate R, Gensini GF, Panetta A, Casolo GC, Carini M. TXA₂ production by human arteries and veins. *Prostaglandins* 1983;25:753-765.
45. Neri Serneri GG, Gensini GF, Abbate R, et al. Abnormal cardiocoronary thromboxane A₂ production in patients with unstable angina. *Am Heart J* 1985;109:732-38.
46. Neri Serneri GG, Abbate R, Gori AM, et al. A transient intermittent lymphocyte activation is responsible for the instability of angina. *Circulation* 1992;85:1200-1220.
47. Neri Serneri GG, Gensini G, Poggesi L, et al. The role of extraplatelet thromboxane A₂ in unstable angina investigated with a dual thromboxane A₂ inhibitor: Importance of activated monocytes. *Coronary Artery Dis* 1994;5:137-145.
48. Nico F, Biagi A. Impiego di un antiaggregante, la picotamide, nel trattamento della malattia coronarica: risultati preliminari. *Giorn Ital di Ricerche Cliniche e Terapeutiche* 1992;13:83.
49. Orzalesi G, Selleri R, Caldini O. Su alcuni nuovi derivati dell'acido 4-idrossiisofthalico con attività anti-coagulante e fibrinolitica. *Il Farmaco* 1969;24:522-532.
50. Orzalesi G, Selleri R, Volpato I. Fibrinolysis and inhibition of platelet aggregation by N,N'-bis(3-picolyl)-methoxyisophthalamide (G-137). In: Davidson JF, Samama MM, Desnoyes PC, eds. *Progress in Chemical Fibrinolysis and Thrombolysis*. New York: Raven Press, 1975:267-269.
51. Parisi S, Abbiati G. Picotamide. Single dose and steady state kinetic study in healthy volunteers. Integrated clinical, pharmacokinetic and statistical report. *Rapporto Interno Istituto Ricerche L.P.B.*, 1994.
52. Passariello N, Marrazzo G, Paolisso G, Fresa R, Sgambato S. Effetti di una somministrazione cronica di picotamide sull'assetto emoreologico in soggetti con diabete tipo II. *Giorn Ital Angiol* 1990; X (1/90).
53. Patrono C, Ciabattini G, Remuzzi G, et al. Functional significance of renal prostacyclin and thromboxane A₂ production in patients with systemic lupus erythematosus. *J Clin Invest* 1985;101:1-1018.
54. Patscheke H. Thromboxane A₂/Prostaglandin H₂ receptor antagonists. A new therapeutic principle. *Stroke* (Suppl 4). 1990;21:139-142.
55. Pawloski NA, Kaplan G, Hamill AL, Cohn ZA, Scott WA. Arachidonic acid metabolism by human monocytes. *J Exp Med* 1983;158:393-412.
56. Pibiri L, Petruzzo P, De Giudici A, Brotzu G. Picotamide: Prevenzione e terapia della vasculopatia diabetica. *Clin Ter* 1990;133:233-237.

57. Pulcinelli FM, Pignatelli P, Riondino S, Parisi S, Castiglioni C, Gazzaniga PP. Effect of picotamide on the calcium mobilization and phospholipase C activation in human platelets. *Thromb Res* 1994;74:453-61.
58. Quarto di Palo F, Elli A, Palazzi P, Parenti M, Rivolta R, Zanussi C. Role of a prostaglandin E₁ analogue, and picotamide, in the prevention of acute graft rejection by cyclosporine. *New Engl J Med* (letter) 1991;323:832.
59. Quarto di Palo F, Elli A, Rivolta R, Parenti M, Palazzi P, Zanussi C. Prevention of chronic cyclosporine nephrotoxicity in renal transplantation by picotamide. *Trans Proc* 1991;23:969-71.
60. Rampton DS, Collins E. Thromboxanes in inflammatory bowel disease. In: Neri Serneri GG, Bonomini V, Gensini GF, Pozzi E, Prisco D, eds. Florence: Scientific Press, 1993:163-171.
61. Saitta A, Bonaiuto M, Mileto A. Valutazione della attivita' arteriolare e di alcuni parametri emocoagulativi durante il trattamento con picotamide. *Clin Ter* 1991;139:9.
62. Schmutzler R, Hartmann H, Casale G, Magliano A. Picotamide: Effects on coagulation, fibrinolysis and platelet aggregation during prolonged administration in the aged. *Age and Ageing* 1978;7:246-250.
63. Smith EF. Thromboxane A₂ in cardiovascular and renal disorders: Is there a defined role for thromboxane receptor antagonist or thromboxane synthase inhibitors? *Eicosanoids* 1989;2:199-212.
64. Speciale F, Taurino M, Colella E, Rizzo L, Fiorani P. Studio clinico sulla picotamide in pazienti vasculopatici. *Medicina* 1989;80:5.
65. Stork JE, Dunn MJ. Hemodynamic roles of thromboxane A₂ and prostaglandin E₂ in glomerulonephritis. *J Pharmacol Exp Ther* 1985;233:672-678.
66. Tazza G, Tazza R, Valentini M, Leoni G, Avenia N, Ragusa M. Picotamide: Utilizzo nelle arteriopatie obliteranti croniche degli arti inferiori. *Clin Ter* 1991;137:399-402.
67. Ullrich V, Nusing R. Thromboxane synthase. From isolation to function. *Stroke* (Suppl 4) 1990;21:134-138.
68. Ushikubi F, Nakajima M, Hirata M, Okuma M, Fujiwara M, Narumiya S. Purification of the thromboxane A₂/prostaglandin H₂ receptor from human blood platelets. *J Biol Chem* 1989;264:16496-16501.
69. Vejar M, Fragasso G, Hackett D, et al. Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. *Thromb Haemostas* 1990;63:163-68.
70. Violi F, Ghiselli A, Iuliano L, Pratico' D, Alessandri C, Balsano F. Inhibition by picotamide of thromboxane production in vitro and ex vivo. *Eur J Clin Pharmacol* 1988;33:599-602.
71. Violi F, Longoni A, Castiglioni C, for the ADEP group. Effect of picotamide in preventing cardiovascular events in 438 diabetic patients: results of a multicentre, placebo controlled, 18-month study. *J Am Coll Cardiol* 1995;368A.
72. Violi F, Longoni A, Castiglioni C, Balsano F, for the ADEP group. Safety of picotamide, an antiplatelet agent, in an 18-month, double blind, placebo controlled, multicenter trial in 2304 patients with peripheral vascular disease. *Curr Ther Res* 1993;54:111-121.