Plasma and Saliva Levels of PGI_2 and TXA_2 in the Headache-Free Period of Classical Migraine Patients. The Effects of Nicardipine

R Puig-Parellada,* J,M. Planas,* J. Giménez,* J. Sánchez,* J. Gaya,** E. Tolosa*** and J. Obach***

SYNOPSIS

The levels of Prostacyclin (PGI_2) and Thromboxane A_2 (TXA_2) were assayed simultaneously (RIA) in the plasma and saliva of 9 patients suffering from classical migraine attacks. The assays were done during an attack-free period. In relation to the control group we observed a significant decrease in the plasma levels of PGI_2 together with a sharp increase in TXA_2 in saliva. When the patients were treated with nicardipine, a calcium antagonist, the TXA_2 increase in saliva did not occur. These results suggest both a systemic and local effect in the classical migraine attacks. We explain and discuss our results by referring to the PGI_2: TXA_2 equilibrium system. Nicardipine action might be related to its ability to reduce the calcium entry into the cell induced by thromboxane.

Key words: prostacyclin, thromboxane, eicosanoids, saliva.

(*Headache* 31:156-158, 1991)

INTRODUCTION

Among the different mediators implicated in the pathophysiology of migraine are arachidonic acid metabolites. A recent review[1] concluded that eicosanoids may have a role in migraine considerably more important than is generally assumed. Preliminary experiments done in our laboratory demonstrated in patients with common migraine a sharp increase in salivary Prostaglandin E_2 (PGE_2) and TXA_2 during a migraine attacks.[2]

We report here the results of a study on eicosanoid levels in classical migraine. Saliva and plasma TXA_2; and PGI_2 levels were determined[1-7] only during attack-free periods[3,4] because of the low frequency of attacks in the patients. Plasma levels reflect a systemic alteration but since migraine is a local disorder it seems reasonable to look for an alteration in saliva.

In the patients studied we also investigated the effect of a calcium antogonist, nicardipine, because it has been shown that this compound is effective in the treatment of migraine.[2,8]

MATERIALS AND METHODS

Nine patients, 6 women and 3 men (mean 23.7 years, range 13-40 years), suffering from classical migraine but otherwise healthy, were admitted for study. None of them was suffering from tension headache.

Samples of saliva and plasma were obtained, for the TXA_2 and PGI_2 determinations, on two occasions. The first samples were obtained during a migraine free period, at least 10 days after the last migraine attack. Patients were then treated with nicardipine (Ferrer Internacional, Barcelona, Spain) (20 mg three times a day) for 2 months. Nicardipine was then discontinued and a second sample of saliva and plasma was obtained 48 hours later.

Analgesic treatment of acute attacks was limited during the study to paracetamol (acetaminophen) and no patient took aspirin or non-steroidal antiinflammatory drugs in the fortnight prior to, or throughout, the assessment period.

Eicosanoid assay in plasma. The blood samples were withdrawn on EDTA and lysine acetylsalicylate. The plasma samples were extracted in micro-columns of octadecyl-silica (ODS) Sep-Pack, Watters) and the eicosanoids isolated by HPLC in ODS columns (Novapack Watters). The obtained fractions corresponding to the different eicosanoids were assayed by RIA.

The 6 Keto PGF_{1a} and the TXB_2 were assayed with an Amersham kit (Amersham U.K.)with an iodinated tracer. The results were expressed as picogrammes per ml of plasma of the stable metabolites TXB_2 (TXA_2) and 6-Keto PGF_{1a} (PGI_2). In our control group there were 15 healthy patients for TXA_2 and 18 for PGI_2.

Eicosanoid assay in saliva. Roughly 5 g. of saliva was obtained from each patient. The saliva was accurately weighed and then 5 ml of 10% TCA and 5 ml of a solution, pH 3.15, containing 90% 0.40M formic acid buffer and 10% ethanol solution were added. The mixture was stirred and centrifuged (3000 rpm, 10 min.) after leaving to stand for 10 minutes.

The precipitate was washed twice with 2.5 ml of 10% TCA and 2.5 ml of the pH 3.5 solution, and respun.

The supernatants were pooled and adjusted to pH 3.2-3.5 with triethylamine. The adjusted supernatants were passed through a Sep-Pack C-18 column (Watters).

Column activation and elution were carried out in accordance with the technique described by Powell et al.[9]

The volume of the samples obtained was reduced by 50% with N_2 and stored frozen (-20°C) until assayed for TXB_2 and PGI_2 by a radioimmunoassay kit (Advanced Magnetics Inc.) with a tritium tracer.

The results were expressed as picogrammes per gramme of saliva of the stable metabolites TXB_2 (TXA_2) and 6-Keto PGF_{1a} (PGI_2). The number of healthy patients in our control group was 9 for both metabolites.

Statistical analysis. The comparisons between the different situations were done with the Student t-test.

The results are expressed as mean ± standard error.

RESULTS

Eicosanoid levels in plasma. Our results show a significant decrease in the plasma levels of PGI_2 in the headache-free period, in relation to the control group (Table 1) ($P > 0.01$). No alteration in TXA_2 was observed.

Table 1
Eicosanoid levels in plasma

	TXB_2 (pg/ml plasma)	**6 Keto PGF_{1a}** (pg/ml plasma)
Control	19 ± 2.6	51 ± 4.7
Classical migraine	33 ± 4.6	10 ± 1.4*
Nicardipine	33 ± 7.1	11 ± 2.8*

* P>0.01 in relation to the control group.

Nicardipine treatment had no incidence on the levels of eicosanoids present in the headache-free period.

Eicosanoid levels in saliva. Our results show a significant increase in the TXA_2 levels in the head-ache-free period in relation to the control group ($P>0.001$) (Table 2). No alternation was observed in the levels of PGI_2.

Nicardipine reduced the increased levels of TXA_2 in the headache-free period down to the control levels ($P>0.01$).

DISCUSSION

Our results indicate that classical migraine patients show in the headache-free period a decrease in PGI_2 levels in plasma (a systemic effect) and an increase of TXA_2 in saliva (a local effect) suggesting an imbalance in the TXA_2: PGI_2 system.

Table 2
Eicosanoid levels in saliva

	TXB_2 (pg/g saliva)	**6 Keto PGF_{1a}** (pg/g saliva)
Control	39 ± 4.6	25 ± 3.2
Classical migraine	145 ± 23.1 *	40 ± 5.9
Nicardipine	51 ± 16.9#	34 ± 8.3

* P>0.01 in relation to the control group.
# P>0.01 in relation to the classical migraine group.

A deficiency in PGI_2 production has also been found in diabetes, atherosclerosis[10] and thrombocytopenia purpura[12] and has long term implications in the development of thromboembolic and atherosclerotic complications.[11] Such complications have been reported as being frequent in migraine families.[4] The decrease in PGI_2 synthesis could also offer an explanation for the hyperaggregation and other platelet disorders typically seen in migraine.[7]

We have not detected alterations of TXA_2 in plasma, an observation which coincides with that of other authors,[4,7] although occasionally a decrease in thromboxane production between attacks has also been observed.[3]

Experimental and clinical data suggest, in the pathogenesis of migraine,[13] an abnormality in the intracranial vascular system, probably secondary to a spreading cerebral oligemia.[14] Cerebral ischemia has been suggested as a possible ethiology. Ischemia induces a rapid and specific accumulation of arachidonic acid in the brain, which is subsequently peroxidized to eicosanoids and other products.[1]

We found in our patients a significant increase of TXA_2 in saliva. Such an alteration in salivary arachidonic acid metabolites could reflect an enhanced local production of these metabolites.

TXA_2 has a powerful constricting effect on cerebral blood vessels, and has been implicated in the etiology of stroke and cerebral vasospasm.[15] TXA_2 has also been defined as a calcium ionophore able to transport calcium through the cell membrane to the cytosol.[13,16] The cytosolic calcium plays an important role in eicosanoid synthesis and in the activation of the myofibrillary proteins.[17-20]

We have found that nicardipine reduces the increase of TXA_2 in saliva during the headache-free phase. These results can be explained by the ability of nicardipine to reduce calcium entry into the cells,[21-23] suggesting that TXA_2 could play a role in the pathophysiology of migraine attacks.

REFERENCES

1. Parantainen J, Vapaatalo H: Prostaglandins in the pathophysiology of migraine, in P.B. Curtis - Prior (ed): Porstaglandins: Biology and Chemistry of Prostaglandins and Related Eicosanoids. New York, Churchill Livingstone, 1988, pp 386-401.

2. Obach J, Planes JM, Puig Parellada P: Increase in PGE_2 and TXA_2 in the saliva of common migraine patients. Action of calcium channel blockers. *Headache* 29:498-501, 1989.

3. Keinänen-Kiukaanniemi S, Kääpä P, Saukkonen AL, Viinikka L, Ylikorkala O: Decreased thromboxane production in migraine patients during headache-free period. *Headache* 24:339-341, 1984.

4. Hedman C, Winther K, Knudsen JR: Platelet function in classic migraine during attack - free periods. *Acta Neurol Scand* 78:271-277, 1988.

5. Vardi J, Flechter S, Alguati A, Regev I, Ayalon D: Prostaglandin E_2 levels in the saliva of common migrainous women. *Headache* 23:59-61, 1983.

6. Joseph R, Steiner JT, Poole CJM, Littlewood J, Clifford Rose F: Thromboxane synthetase inhibition: Potential therapy in migraine. *Headache* 25:204-207, 1985.

7. Winther K, Hedman C: Platelet function and migraine, in J. Olesen and L. Edvinsson (eds): Basic Mechanisms of Headache, Elsevier Science Publishers B. V. (Biomedical Division), 1988, pp 301-312.

8. Diez Tejedor E, Frank A, Bescansa E, Viñals M, Barreiro P: Un nuevo antagonista del calcio (nicardipina) en la pro-filaxis de la migrafia. *Drugs of Today* 24(Supl. 3):215-220, 1988.

9. Powell WS: Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecyl-silyl-silica. *Prostaglandins* 20:947-957, 1980.

10. Whittle B JR, Moncada S: Pharmacological interactions between prostacyclin and thromboxanes. *Br Med Bull* 39:232-238, 1983.

11. Moncada S, Vane JR: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin, *Pharmacol Rev* 30:293-333, 1979.

12. Bunting S, Moncada S, Vane JR: The prostacyclin-thromboxane A_2 balance: pathophysiological and therapeutic implications. *Br Med Bull* 39:271-276, 1983.

13. Peroutka SJ: The pharmacology of calcium channel antagonists: a novel class of anti-migraine agents. *Headache* 23:278-283, 1983.

14. Olesen J, Lauritzen M, Tfelt-Hansen F, Henriksen L, Larsen B: Spreading cerebral oligemia in classical and normal cerebral blood flow in common migraine. *Headache* 22:242-248, 1982.

15. Towart R, Perzborn E: Nimodipine inhibits carbocyclic thromboxane-induced contractions of cerebral arteries. *Eur J Pharmacol* 69:213-215, 1981.

16. Gerrard JM, Peterson, Townsend-White ZG: Prostaglandins and platelet contraction. *Circulation* 54:Suppl II, 196-200, 1976.

17. Peters T: Principles of action of calcium entry blockers on cellular movements of calcium. *Inter Angio* 3:9-16, 1984.

18. Hassid A, Oudinet JP: Relationship between cellular calcium and prostaglandin synthesis in cultured vascular smooth muscle cells. *Prostaglandins* 32:457-478, 1986.

19. Knapp HR, Oelz O, Roberts L J, Sweetman BJ, Oates JA, Reed PW: Ionophores stimulate prostaglandin and thromboxane biosynthesis. *Proc Natl Acad Sci USA* 74:4251-4255, 1977.

20. Smith EF, Lefer AM, Nicolaou DC: Mechanism of coronary vasoconstriction induced by carbocyclic thromboxane A_2. *Am J Physiol* 240:493-497, 1981.

21. De Clerck F, Van Nueten JM: Platelet-mediated vascular contractions. Inhibition by flunarizine, a calcium-entry blocker. *Biochem Pharmacol* 32:765-771, 1983.

22. Okamatsu S, Peck RC, Lefer AM: Effects of calcium channel blockers on arachidonate-induced sudden death in rabbits. *Proc Soc Exp Biol Med* 166:551-555, 1981.

23. Pickard JD, Walker V, Vile J, Perry S, Smythe PS, Hunt R: Oral nimodipine reduces prostaglandins and thromboxane production by arteries chronically exposed to a periarterial haematoma and the antifibrinolytic agent tranexanic acid. *J Neurol Neurosurg Psych* 50:727-731, 1987.