ALTERATION OF HEMOSTASIS IN PATIENTS TREATED WITH SUBGINGIVAL NSAIDS DURING PERIODONTAL THERAPY

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ABSTRACT

The use of topical NSAIDs to complement periodontal therapy could help resolve the inflammatory process and clinical signs of the disease more rapidly.

A randomized clinical trial was performed on 59 patients, of whom 33 had chronic periodontitis and 26 were healthy controls. All diseased patients underwent scaling and root planing in one quadrant per week with subgingival application of gel 48 hours after each session. Gel was applied to healthy patients with the same frequency. Four types of gel were used, containing respectively placebo, acetylsalicylic acid (ASA) 1%, Ketoprofene (KTP) 1% and Ketoprofene 2%. The following clinical variables were studied: probing depth, attachment level, dental mobility, plaque index, gingival index and bleeding on probing; as well as the biochemical variables: bleeding time (Ivy), platelet count in whole blood and platelet-rich plasma, and platelet aggregation induced by different agonists.

Regarding clinical results, ASA was the most effective in reducing probing depth, gingival index and bleeding on probing ($p<0.05$). KTP 1% and 2% behaved similarly to each other and less effectively than ASA but differed significantly from placebo ($p<0.05$). Regarding biochemical tests, ASA had a highly significant inhibitory effect on platelet aggregation for all the agonists used. KTP 2% produced similar, though weaker responses. KTP 1% only showed alteration in the first aggregation phase at maximum ADP concentration of and none at the minimum concentration ($p<0.05$).

Key words : hemostasis; periodontal disease, drug therapy.

ALTERACIÓN DE LA HEMOSTASIA EN PACIENTES TRATADOS CON ANTIINFLAMATORIOS NO ESTEROIDEOS POR VÍA SUBGINGIVAL DURANTE LA TERAPIA PERIODONTAL

RESUMEN

El uso de AINEs en forma tópica como complemento de la terapia periodontal podría coadyuvar a resolver con más rapidez el proceso inflamatorio y los signos clínicos de la enfermedad. Se realizó un estudio clìnico randomizado en el que participaron 59 pacientes, 33 con periodontitis crónica y 26 controles sanos. A todos los pacientes enfermos se les realizó raspaje y alisado radicular en un cuadrante por semana, aplicándose subgingivalmente el gel a las 48 hs de cada sesión. Los pacientes sanos recibieron los geles en los mismos intervalos. Se utilizaron 4 tipos de geles conteniendo: placebo, ácido acetilsalicílico (AAS) 1%, Ketoprofeno (KTP) 1% y Ketoprofeno 2%. Se estudiaron las variables clínicas: profundidad de sondaje, nivel de inserción, movilidad dentaria, índice de placa, índice gingival y sangrado al sondaje; y bioquímicas: tiempo de sangría (Ivy), recuento plaquetario en sangre entera y plasma rico en plaquetas, y agregación plaquetaria frente a distintos agonistas.

En cuanto a los resultados clínicos, el AAS fue el más efectivo en la reducción de la profundidad de sondaje, el Índice gingival y el Sangrado al sondaje ($p<0,05$). Tanto KTP al 1% y al 2% se comportaron en forma similar y menos efectiva que AAS pero con diferencias significativas con respecto al placebo ($p<0,05$). Con respecto a las pruebas bioquímicas, el AAS mostró un efecto inhibitorio de la agregación plaquetaria altamente significativo con todos los agonistas utilizados. Respuestas similares pero con menor intensidad se hallaron con KTP 2%. El KTP 1% sólo mostró alterada la primer fase de agregación a la máxima concentración de ADP y ninguno a la mínima siendo el valor de $p<0.05$.

Palabras clave: hemostasia, enfermedad periodontal, terapia farmacológica.

INTRODUCTION

Anti-infectious therapy for periodontal disease includes mechanical, surgical and non-surgical procedures as well as the use of systemic[1-6] and topical antibiotics and anti-inflammatory[7-9] drugs. There is currently sufficient evidence to prove that arachidonic acid products, both those from cyclooxygenase (COX) and those from lipoxygenase (LOX) are involved in the pathogenesis of periodontal disease. PGs are associated to tissue destruction, metabolic

changes in the fibroblast and bone resorption[10]. High levels of prostaglandin E2 (PGE2) in GCF correlate positively to periodontal inflammation and tissue destruction in humans[11-18] and animals[19,20]. Salvi GE et al.[21] related PGE2 levels and their correlation to active periodontal disease, suggesting that PGE2 could be a good biomarker of disease activity. The treatment for chronic periodontitis, in contrast to other types of periodontitis requiring adjunct medication therapy, is conventional mechanical debridement for removing plaque and calculus[22]. The use of topical NSAIDs as a complement to therapy might help resolve the inflammatory process and clinical signs of the disease more rapidly.

Some papers[1-6] report systemic use of NSAIDs, and a few others report topical subgingival use of NSAIDs such as flurbiprofen[7] and ketorolac[8,9] in the treatment of periodontal diseases. It should be noted that the side effects of anti-inflammatory therapy make its conventional daily systemic use impossible. This is why topical use is a valid alternative for complementary treatment of periodontal disease. Topical forms used in dentistry include mouth rinses, supra and subgingival irrigation and subgingival devices. Subgingival or intracrevicular devices increase the time that the active principle remains within the periodontal pocket, thus ensuring a longer delivery time. Different subgingival devices as vehicles for delivering NSAIDs have been studied, such as the subgingival irrigations described Paolantonio et al.[23] and polyacrylate cement strips described by Corry[24]. Subgingival irrigations require the dentist to spend a long time on each periodontal pocket, and polyacrilate strips involve a highly complex handling technique. We therefore believe that subgingival gel is a better alternative, as it is easy to handle and its viscosity ensures that it will remain *in situ* for a suitable length of time.

An analysis of the literature shows that NSAIDs administered topically to the skin reach significant plasma concentrations[25], therefore we wish to establish whether topical application in a periodontal pocket, considering its anatomical, physiological and pathological conditions (irregularities in epithelial integrity, large contact surface and altered microcirculation, among others), could produce plasma concentrations that would affect the hemostatic parameters in patients with periodontal disease, meaning that we would be dealing with a patient at risk of hemorrhage during dental procedures involving bleeding.

MATERIALS AND METHODS

Patients

This study was approved by the bioethics committee of Rosario National University, Argentina. It included 59 patients (21 to 40 years old) of whom 33 were diagnosed with chronic periodontitis (group A) and 26 had good periodontal health (group B).

Inclusion Criteria: The patients in group A are systemically healthy, with a diagnosis of chronic periodontitis with periodontal pockets ≥ 4 millimeters deep in at least three teeth per quadrant. The patients in group B are undergoing periodontal maintenance therapy. The systemically healthy condition was determined from the clinical history, physical examination and laboratory studies.

Exclusion Criteria: patients with chronic diseases, smokers, patients who received any kind of medication in the past thirty days.

Preparation of the intracrevicular gel

The placebo gel and gels containing 1% AAS, and 1% and 2% KTP were prepared using 4 g sodium carboxymethyl cellulose in 200 ml of distilled water[9].

Pharmacological treatment protocols

Patients in group A underwent non-surgical periodontal treatment as follows: one session of scaling and root planing in one dental quadrant at seven-day intervals (4 consecutive weeks). Intracrevicular gel was applied 48 hours after each session. Patients in group B underwent application of the assigned intracrevicular gel with the same frequency. Both groups were divided into 4 experimental treatments. Intracrevicular gel was applied using syringes with 21 Gauge x 11/2 0.80 x 40 needles after drying the area with an air jet.

Treatment 1: placebo gel (n=6+4)

Treatment 2: gel with ASA 1% (n=9+7)

Treatment 3: gel with ketoprofene 1% (n=8+7)

Treatment 4: gel with ketoprofene 2% (n=10+8)

Variables studied

- ***Clinical***

Clinical indices were taken before scaling and planing (baseline) and 30 days after starting the treatment (one week after the last gel application). The parameters recorded were: probing depth, attachment level, dental mobility, plaque index, gingival index and bleeding on probing.

• ***Biochemical***

Biochemical indices were taken at baseline, 15 and 30 days. The following were determined: bleeding time (Ivy), platelet count in whole blood and platelet rich plasma and platelet aggregation induced by different agonists.

Platelet aggregation induced by different agonists

We used the turbidimetric procedure following Born[26], which involves measuring the difference in optical density between platelet-rich plasma (PRP) and platelet-poor plasma (PPP). When the agonist agents or inducers are added, the platelets aggregate, which translates into an increase in the light transmitted through the aggregometer cuvette and recorded in the form of a chart. The results are expressed as percentage of aggregation at 5 minutes. The blood vessel is substituted by the reaction cuvette with a magnet that stirs the sample constantly, producing collisions among the platelets, simulating blood turbulence. The agents used were: ADP (Sigma) 1 µg/ml and 2 µg/ml, arachidonic acid (Sigma) 0.05 Mm and tendon collagen (Medi-Tech) 2 µg/ml. The reaction was performed at 37ºC. It is thus considered that the responses obtained *in vitro* correlate to what happens *in vivo*. The aggregometer was adjusted to 100% optical density with PRP and to 0% density with PPP. The aggregation curve was recorded using a special printer connected to the aggregometer, which records the changes in optical density of the platelet suspension as they aggregate and allow greater light transmission. The device used was a single channel Chrono-Log aggregometer with software (Aggro-Link) and a one-pen printer. The percentage of inhibition of the NSAIDs on platelet aggregation was calculated by measuring the maximum range of the aggregation curve at baseline and comparing it to that obtained post-treatment.

Statistical analysis

Individual statistical analysis to compare each protocol to its control group was done using Student's t-test. The results of all protocols were compared using ANOVA with subsequent comparisons using Dunnet's test. A level of $p<0.05$ was considered significant in each case.

RESULTS

A comparison of the differences in the clinical variables of the patients in group A at baseline and thirty days shows that there were statistically significant differences in probing depth, gingival index and bleeding on probing between each active drug group and the placebo group, whereas the plaque index showed no statistically significant difference for any of the groups (Table 1).

The platelet function in group B showed no significant difference for any of the study variables. However, in group A, a comparison of platelet function at 30 days for all treatment protocols in the group of patients with chronic periodontitis shows that the group that received placebo had no alteration in the platelet stimulations, whereas aggregation for all the inducers was significantly altered in the groups that received ASA 1% and Ketoprofene 2%. Ketoprofene 1% only altered the first phase of aggregation at the highest ADP concentration (Table 2).

Table 1: Values of reduction coefficient (baseline/30 days) (Group A).

	Clinical variables			
	Probing depth	*Plaque index*	*Gingival index*	*Bleeding on probing*
Placebo	0.76 ± 0.24	0.99 ± 0.61	0.92 ± 0.64	0.29 ± 0.18
ASA 1%	1.26 ± 0.13*	1.04 ± 0.84	1.42 ± 0.76*	0.61 ± 0.49*
Ketoprofene 1 %	1.02 ± 0.21*	0.93 ± 0.80	1.21 ± 0.83*	0.52 ± 0.34*
Ketoprofene 2 %	1.08 ± 0.28*	1.09 ± 0.74	1.23 ± 0.77*	0.59 ± 0.38*

*: statistically significant difference between tested groups and placebo group.
(ANOVA followed by Dunnet's test)

Table 2: Platelet aggregation at 30 days (Group A).

	Platelet function			
	ADP 1 µg/ml	*ADP 2 µg/ml*	*Arachidonic acid 0.05 Mm*	*Collagen 2 µg/m*
Placebo	normal	normal	normal	normal
AAS 1%	66.67% 2f*	66.67% 2f*	66.67% alt*	66.67% alt*
Ketoprofene 1 %	normal	37.50% 1f*	12.50% alt	12.50% alt
Ketoprofene 2 %	40% 1f*	40% 2f*	40% alt*	40% alt*

Data refer to the percent of affected patients regard the total sample
1f: first phase; 2f: second phase; alt: alteration; * significant difference respect to placebo group ($p<0.05$)

DISCUSSION

Evidence that NSAIDs are effective in reducing the progression of periodontal disease has been clearly established in studies on animals. Little research was available on humans until the 1990s. Paolantonio et al.[23] used a solution of 1% acetylsalicylic acid to perform subgingival irrigations in patients with periodontitis, demonstrating its usefulness in reducing sub-clinical inflammation of periodontal pockets evaluated by the quantity of PMNs in the gingival fluid. Corry[24] suggests that polymethacrylate cement strips as a vehicle for sustained NSAID release (the study used indomethacin, tolmetin and mefenamic acid) might be an important tool in the treatment of periodontal diseases.

Our studies showed that intracrevicular administration of NSAIDs to patients with chronic periodontitis can reduce probing depth, plaque index, gingival index and bleeding on probing. We propose the use of ASA 1% and KTP 1% and 2% in gels, which have good bioadhesion, as a vehicle[27], in contrast to other studies such as Paolantonio et al.[23], Corry[24] or others such as Preshaw et al.[28] or Okuda et al.[29], which used mouth rinses. Other studies such as Paquette et al.[30] administered KTP systemically to patients with adult periodontitis. For the treatment of inflammatory lesions, finding an effective pathway which is circumscribed to periodontal tissues would have the advantage of reducing the adverse effects of NSAIDs, which often affect the gastrointestinal tract and kidneys, as reported in a review published Nguyen et al.[31].

A comparison of the final clinical results for all groups shows that those treated with ASA 1% and KTP 1% and 2% compared to placebo showed significant reductions in probing depth, gingival index and bleeding on probing, but not in plaque index. Our results differ from those of Heasman and Seymour[32], who found no influence of NSAIDs on probing depth and gingival index, but agree with them in that NSAIDs do not alter the quantity of bacterial plaque (Table 2).

Finally, we consider that ASA was the most effective protocol in the reduction of probing depth, gingival index and bleeding on probing. The two KTP concentrations behaved similarly to each other; they were less effective than ASA but differed significantly from placebo.

Laboratory values were determined three times: at baseline, fifteen days and thirty days after the beginning of the experiment. One of the questions we asked ourselves was whether periodontal disease was itself able to alter any of the hemostatic parameters compared to standard parameters. All the baseline determinations for patients with chronic periodontitis were within normal values; therefore we dismissed the possibility that periodontal disease might perturb hemostasis.

For all groups and treatment protocols we found similar results in the middle and at the end of the experiment, therefore, when hemostatic parameters are affected at the second week of treatment, they remain stable until the end of the study. We attributed this to the acetylation of platelet cyclooxygenase, which remains inhibited throughout the lifetime of the platelet (approximately 12 days) because platelets have no nucleus enabling them to synthesize new enzyme, and no thromboxane A2 (a potent platelet aggregator) is produced.

The group of patients undergoing periodontal maintenance, who therefore did not have active periodontal disease, showed no alteration in any of the parameters studied, either in the placebo group or in the groups on which ASA and KTP were used. This is explained by the conservation of epithelial integrity. In contrast, when we analyzed the results for patients with chronic periodontitis, no alteration was found when the placebo gel was used, but there were alterations in the ASA and KTP groups. Epithelial alterations related to inflammatory lesion of periodontal disease have been documented. They include the formation of epithelial buds, like extensive outgrowths on the epithelium of the sulcus and the junction, which increase in the total amount of epithelium present. Paradoxically, the areas of epithelial proliferation alternate with others where the lining is very thin or even discontinuous. The basal membranes of the epithelium of the junction and sulcus usually remain intact but show localized alterations including interruptions, thinning or thickening, duplication and presence of basal lamina material detectable in the underlying connective tissue[33-36]. These changes appear in other epithelia at different stages of the disease, and seem to be a nonspecific response of the inflammatory process. The basal lamina that surrounds capillaries and venules in the infiltrated region has similar alterations[33,37].

Among the platelet agonists that have been studied *in vitro*, the most relevant physiologically seem to be thrombin, ADP, adrenalin, collagen and arachi-

donic acid. The platelet surface has specific receptors for each of these agonists and these receptors are linked to intracellular structures, whose alteration by the receptor-agonist complexes leads to intracellular changes that characterize the activated platelet by activating three metabolic pathways [38]: one that depends on ADP and serotonin, another that depends on the release of TxA2 and is through cyclooxygenase and thromboxane-synthetase when they act on arachidonic acids and cyclic endoperoxides, respectively, and a third pathway mediated by collagen and thrombin.

When ADP was used as an inducer, in the group of patients with chronic periodontitis on which we used ASA 1%, six patients (66.67%) showed alteration in the second phase of platelet aggregation at both ADP concentrations, while in the group on which we used KTP 1% only three patients (37.5%) showed alteration in the first phase of aggregation at the higher concentration and none at the lower concentration. When we used KTP 2%, four patients (40%) showed alteration in the first phase at the lower concentration and in the second phase at the higher concentration (Table 2). The same trend is observed for aggregation with arachidonic acid and collagen.

ASA had a highly significant inhibitory effect with all the agonists used. Similar, though weaker responses were found with KTP 2%. KTP 1% only showed alteration in the first phase of aggregation at the higher ADP concentration, and no alteration at the lower concentration, with $p<0.05$ (Table 2).

Further studies are needed to determine the clinical safety of other NSAIDs. In an upcoming study we plan to test the administration of other agents of this kind and other specific inhibitors of inflammatory mediators, such as the FNT- α antagonists. At the University of Messina (Italy), Di Paola et al. [39] used an experimental periodontitis model in rats to determine the results of an FNT-α antagonist, etanercept, administered subcutaneously.

The results are encouraging because they show a reduction in the development of the inflammatory process and tissue injury associated to periodontitis. Etanercept is delivered subcutaneously to patients with early and late rheumatoid arthritis, and was found to be efficacious and well tolerated in several trials and in clinical practice in adult patients [40]. Considering its clinical efficacy in chronic inflammatory processes and its capacity to act on the experimental inflammation model in periodontal tissues, it probably provides similar or better clinical results as those we obtained, without altering patient hemostasis. Further work will be required on the pharmaceutical form of the gel, and its efficacy when administered intracrevicularly will need to be tested.

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