# COMPARATIVE STUDY OF THE EFFECTS OF ADMINISTRATION OF $PGE_1$, $PGE_2$, AND ENZYMATIC PREPARATION WITH PROSTAGLANDIN H-SYNTHASE ACTIVITY ON ORTHODONTIC TOOTH MOVEMENT

**A. Ilyiná[*], G. D Alvarado[**], R. E. Trimmer[***], L. M. G. García[*], S. J. A. Villarreal[*], H. J. L. Martínez[*], L. J. A. Villaviscencio[**], and M. J. Rodríguez[*]**

**In this study the effect of prostaglandins $E_1$ and $E_2$ ($PGE_1$ and $PGE_2$) administration was compared with the effect of administration of enzymatic preparation with prostaglandin H-synthase (PGHS) activity. The preparation of solubilized microsomes with PGHS activity was obtained from bovine vesicular glands in the presence of calcium ions. All orthodontic treatments were carried out on cats divided into 3 groups (3 cats in each group). The mechanic force was effectuated by using of continuous power-chains on both sides of the maxilla. The first premolares were extracted and the canine movements were evaluated for up to 3 weeks (every third day). One side received submucosal local injections of $PGE_1$ (50 $\mu$g/injection), $PGE_2$ (6 $\mu$g/injection) or PGHS (0.006–0.015 U/injection) and the other received vehicle injections. It was demonstrated that the distal canine movement was faster (approximately double) on the side receiving prostaglandins E (PGEs) or PGHS administrations as compared to the vehicle-injected side. Throughout this study, no side effects were observed macroscopically in the gingiva and roentgenographically in the alveolar bone.**

## Introduction

Tooth movement during orthodontic treatment requires remodeling of periodontal tissues, especially in alveolar bone. The role of local bone metabolism associated with orthodontic tooth movement has been considered to be related to the biologic response of periodontal tissues to applied mechanical force [1].

The role of prostaglandins (PGs) as biochemical mediators of bone resorption induced by orthodontic tooth movement in rats and cats, was previously reported [2, 3]. It was suggested that orthodontic mechanical stress induced synthesis and secretion of PGs by localized cells, which stimulated bone resorption. Yamasaki and associates [4] showed that local administration of $PGE_1$ y $PGE_2$ in gingiva near the distal area of canines caused almost double the rate of monkey canine tooth movement seen in the vehicle-injected side and that no side effects were observed in the gingiva macroscopically. Then they applied the $PGE_1$ clinically and the same results were observed [5]. Chymbley and Tuncay [6] supported the histologic data by showing that indomithacin, a specific inhibitor of prostaglandin synthesis, reduced the rate of orthodontic tooth movements.

These data indirectly indicate the important role of prostaglandin H synthase (PGHS, EC 1.14.99.1) in the bone resorption associated with orthodontic tooth movement. PGHS is the first and rate-limiting enzyme in the transformation of polyunsaturated fatty acids into prostaglandins [7, 8]. Moreover, it is well known that the PGHS is therapeutically important because it is selectively inhibited by indomithacin, aspirin and related nonsteroidal antiinflammatory drugs [9, 10].

PGHS is an integral protein located in cells, mainly in the membrane of the endoplasmic reticulum [7, 8]. The enzyme exhibits two enzymatic activities (cyclo-oxygenase and peroxidase) and requires participation of heme and four molecules of substrates: polyunsaturated fatty acid, two oxygen molecules and an electron donor [11]. This enzyme is undoubtedly of biotechnological interest, since it is applied for the biosynthetic production of a wide array of prostaglandins [7, 12].

A crucial factor and disadvantage of PGHS application is the fast and irreversible inactivation of the enzyme in the course of catalysis [8, 11–13]. The enzyme inactivation is accompanied by formation of hemoprotein radicals and a relatively slow destruction of the heme-enzyme complex [10–13]. It has been demonstrated that fast and dramatic changes in the protein structure occur in the course of the substrate conversion [14, 15]. The kinetic experiments and theoretical analysis [16] demonstrated that the inactivation proceeds via enzyme-substrate intermediates involved in the mechanism of AA conversion into $PGH_2$ [7, 8].

We supposed that the inactivation of PGHS takes place during prostaglandin synthesis as the biologic response to applied mechanical forces in the orthodontic tooth treatment. So, in this work we proposed to increase the insuficient level of enzyme in tissue by injection of the solution of solubilized microsomes from bovine vesicular glands and

[*] Departamento de Biotecnología de la Facultad de Ciencias Químicas de la Universidad Autónoma de Coahuila.
[**] Asociación Odontológica Mexicana para la Enseñanza y la Investigación, México, D. F.
[***] Clínica Veterinaria "La Florida", Saltillo, Coahuila.
Blvd. V. Carranza e Ing. J. Cárdenas V., Col. República, Saltillo, C.P. 25280, Coahuila, Correo electrónico: ailina@alpha1.sal.uadec.mx.

compare the response with the effects observed under the prostgalndin $E_1$ and $E_2$ (PGEs) injections. To obtain the $PGE_1$ and $E_2$ the biosynthesis from polyunsaturated fatty (eicosatrienoic and arachidonic, respectively) acids was carried out using homogenized bovine vesicular glands as biocatalyst.

### Materials and Methods

Preparations of PGHS were isolated from bovine vesicular glands according to method applying the capacity of calcium ions as described previously [17]. The Beckman J2-HS centrifuge with JA-10 rotor was used for centrifugation. The following reagents were purchased from Sigma Chemical Company (USA): Trizma, diethyldithiocarbamate sodium salt (DEDTC), Tween-20, $CaCl_2$, HCl, ethyl-acetate, formic acid, eicosatrienoic and arachidonic acids, L-adrenaline, hemine. EDTA disodium salt and ethanol were from Baker Analyzed (USA).

The protein concentration was determined according to the method of Lowry [18]. Spectrophotometric assay [17] of PGHS cyclo-oxygenase plus peroxidase activities was performed in 2 ml of 50 mM Tris-HCl buffer, pH 8, containing 0.05% Tween-20. The reaction mixture contained an aliquot of solubilized microsomes, 1.4 mM L-adrenaline as an electron donor and 0.2 M hemine as a prosthetic group. To begin the reaction 0.35-mM arachidonic acid was added. The kinetics of L-adrenaline oxidation in the PGHS catalyzed reaction was monitored at 480 nm (Beckman DU-50) at 22 °C.

Prostaglandins $E_1$ and $E_2$ were synthesized from eicosatrienoic and arachidonic acids (10 mg), respectively, using homogenized bovine vesicular glands as biocatalyst as described early [19]. To purify the PGEs the TLC technique was applied using Silicagel plates (Merk) and ethyl-acetate: formic acid (400:5) solution as eluent. TLC applied standards of PGEs were colored by iodine vapor and the substances were extracted by ethyl-acetate (pH 3). After ethyl-acetate evaporation the stock solutions of PGEs were prepared in ethanol and kept at −15 °C before its using in the experiments. To determine the PGEs concentration in the stock solutions and their stability in the buffer of 50 mM Tris-HCl, pH 8, containing 20 mM of $CaCl_2$ and 1.0% Tween-20 used as vehicle to prepare the injection in orthodontic part of study, the spectrophotometric assay based on the alkaline isomerization of PGEs was performed as described early [19].

All orthodontic treatments were carried out on cats (4 female and 5 male animals of indefinite ages and quality) weighting 2.2–4.2 kg divided into 3 groups (3 cats in each group): the first group of cats received the injections of $PGE_1$, the second group was treated by $PGE_2$ y the third—by enzymatic preparation with PGHS activity. The cats were kept under standard conditions. They were anesthetized with ketamine (20 mg/kg) every time when injections and measurements were realized. The mechanic force was effectuated by using of continuous power-chains on both sides of the maxilla. The first premolars were extracted and the canine movements were evaluated for up to 3 weeks (every third day). One side received submucosal local injections of $PGE_1$ (50 $\mu$g/injection), $PGE_2$ (6 $\mu$g/injection) or PGHS (0.006–0.015 U/injection) and the other received vehicle injections. Injections were made with a 1ml syringe and a 26-gauge, 1/2 inch needle. Then the injections were discontinued whereas the canine movements were evaluated for some days more. The side effects were monitored macroscopically and roentgenographically in the gingiva and in the alveolar bone, respectively, during all time of study.

### Results and Discussion

The mechanism of action of prostaglandins (PGs) in promoting bone resorption has received wide attention. PGs are used in orthodontics to shorten the period of tooth movement. In this study the effect of $PGE_1$ y $PGE_2$ administration was compared with the effect of administration of enzymatic preparation with prostaglandin H-synthase (PGHS) activity.

The preparations of $PGE_1$ and $E_2$ were synthesized from eicosatrienoic and arachidonic acid, respectively. So, 2.03 mg of purified $PGE_1$ and 0.25 mg of purified $PGE_2$ were obtained that corresponds to 20 and 2.5% of yield, respectively. The difference in the yields is probably due to the distinct activity of enzymes on homogenized bovine vesicular glands obtained from different animals and applied in the biosynthesis as biocatalyst.

Prostaglandins are a family of fatty acids and do not dissolve in water. Solutions for injection are usually made from a concentrated stock solution of PGs, which is prepared by adding ethanol to PGs. So, the $PGE_1$ and $E_2$ were dissolved in ethanol and aliquots (0.02 ml) containing 0.05 and 0.006 mg of prostaglandin, respectively, were kept at −15 °C to its using in the orthodontic treatment. It is known that most natural prostaglandins are unstable in the water solution and it is preferable to keep them in organic solvents [5, 10]. The concentration of PGE solution was determined by spectrophotometric assay at first and twentieth days of storage. It was observed that after twenty days only 64% and 49% of $PGE_1$ and $PGE_2$, respectively, were conserved. It was the reason to increase at twice the quantity of PGE solution applied in the orthodontic treatment. The dose of PGs used in the present study is almost 1/10 to 1/30 of the biosynthesis levels of $PGE_1$ + $PGE_2$ and 1/1000 to 1/2000 of the per diem biosynthesis amount of all PGs in a physiologic state of human beings [20].

The stability of prostaglandins in buffer was checked spectrophotometrically prior to the application of $PGE_1$ and $E_2$ solutions for orthodontic tooth movement. Buffer solution of 50 mM Tris-HCl, pH 8, containing 20 mM of $CaCl_2$ and 1.0% Tween-20 used as vehicle to prepare the injection for orthodontic treatment, was the same that was applied to obtain the solubilized microsomes containing the PGHS, which were used as preparation with enzyme activity in the present study. Buffer (0.5 ml) was added to aliquot of PGE solution to decrease the ethanol concentration in injection to 3.8/% and discard the possible side effects of ethanol. The concentration of $PGE_1$ and $E_2$ in

this buffer was determined after different time of incubation of them at 37 °C. There was a very slight fluctuation in the $PGE_1$ and $E_2$ concentration at 0 and 30 min of incubation. These results suggest that $PGE_1$ and $E_2$ are stable in buffer solution up to at least 30 min, which is more than enough for application in the present study and for general clinical using.

As it was mentioned above the preparation of solubilized microsomes with PGHS activity was obtained from bovine vesicular glands in the presence of calcium ions. The aliquots of enzymatic preparation (0.5 ml) were kept at −15 °C during various days until to be used in the orthodontic treatment. Prior to the application of enzyme, the activity of PGHS was determined. Table 1 shows the characteristics of PGHS preparation during orthodontic treatment effectuated in the present study. It was detected the decrease of PGHS activity at ninth and twelfth days of the application. At sixteenth day the enzyme preparation obtained in other extraction was used. The PGHS activity of this preparation was greater than at twelfth day but it was smaller than the activity of previous preparation at first day of its application (Table 1).

Table 1

**Characteristics of enzymatic preparation with PGHS activity during its application in orthodontic treatment**

| Day of treatment | Volume of applied preparation, ml | Quantity of applied protein, mg | Specific activity, μmol/(mg min) | Total activity, U/injection |
|---|---|---|---|---|
| 0 | 0.5 | 0.96 | 0.016 | 0.015 |
| 4 | 0.5 | 0.96 | 0.016 | 0.015 |
| 9 | 0.5 | 0.96 | 0.009 | 0.009 |
| 12 | 0.5 | 0.96 | 0.006 | 0.006 |
| 16 | 0.5 | 0.99 | 0.009 | 0.009 |

Table 2

**Comparison of distal canine movement in the different groups of cats participated in the orthodontic treatment**

| I-1 (male cat weighting 3.15 kg) | | | I-2 (female cat weighting 2.00 kg) | | | I-3 (female cat weighting 2.40 kg) | | |
|---|---|---|---|---|---|---|---|---|
| Day of treatment | Distal canine movement, mm | | Day of treatment | Distal canine movement, mm | | Day of treatment | Distal canine movement, mm | |
| | $PGE_1$-treated side | Control side | | $PGE_1$-treated side | Control side | | $PGE_1$-treated side | Control side |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 3 | 0 | 6 | 1 | 0 | 3 | 0 | 0 |
| 9 | - | 0 | 9 | 1 | 0 | 6 | 0 | 0 |
| 12 | 4 | 0 | 13 | 2 | 0 | 10 | 1 | 0 |
| 16 | 5 | 1 | 16 | 2 | 0 | 13 | 1 | 0 |
| 19 | 5 | 2 | 27* | 3 | 2 | 24* | 3 | 3 |
| 30* | 6 | 4 | 30* | 4 | 3 | 27 | 4 | 3 |
| II-1 (male cat weighting 2.25 kg) | | | II-2 (female cat weighting 2.23 kg) | | | II-3 (male cat weighting 4.20 kg) | | |
| Day of treatment | Distal canine movement, mm | | Day of treatment | Distal canine movement, mm | | Day of treatment | Distal canine movement, mm | |
| | $PGE_2$-treated side | Control side | | $PGE_2$-treated side | Control side | | $PGE_2$-treated side | Control side |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 3 | 2 | 6 | 5 | 2 | 3 | 2 | 0 |
| 9 | 4 | 3 | 9 | 5 | 2 | 6 | 2 | 1 |
| 12 | 7 | 6 | 13 | 6 | 3 | 10 | 2 | 1 |
| 16 | 10 | 6 | 16 | 6 | 3 | 13 | 4 | 1 |
| 19 | 10 | 6 | 27* | 8 | 6 | 24* | 5 | 3 |
| II-1 (male cat weighting 2.25 kg) | | | II-2 (male cat weighting 2.40 kg) | | | II-3 (female cat weighting 2.20 kg) | | |
| Day of treatment | Distal canine movement, mm | | Day of treatment | Distal canine movement, mm | | Day of treatment | Distal canine movement, mm | |
| | PGHS-treated side | Control side | | PGHS-treated side | Control side | | PGHS-treated side | Control side |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 1 | 0 | 3 | 1 | 0 | 3 | 1 | 0 |
| 9 | 3 | 0 | 6 | 3 | 2 | 6 | 1 | 0 |
| 12 | 5 | 1 | 10 | 4 | 2 | 10 | 2 | 0 |
| 16 | 5 | 1 | 13 | 5 | 2 | 13 | 3 | 2 |
| 19 | 5 | 1 | 24* | 5 | 5 | 24* | 5 | 4 |
| 30* | 7 | 4 | 27* | 5 | 5 | 27* | 7 | 4 |

*Notation.* I,—the group received the injections of $PGE_1$, II,—the group that was treated by $PGE_2$, and III,—by enzymatic preparation with PGHS activity. All cats received the injection of placebo (buffer mentioned in text) in the control side. The days when the injections have been discontinued are labeled by (*). The data corresponded to change of preparation doses are underlined.

The results presented in the Table 2 show that local administration of PGHS as well as $PGE_1$ or $E_2$ combined with mechanical tooth movements accelerates the rate of tooth movement in the cats. The distal canine movement was faster (approximately double) on the side receiving PGEs or PGHS administrations as compared to the vehicle-injected side. The results obtained with $PGE_1$ agree with data obtained in experiments on monkeys and in clinical application [4, 5]. Interruption of injections (Table 2) provoked the decreasing in rate of tooth movement in the treated side. The ration of treated: control movement was decrease when the injections were discontinued. Throughout this study, no side effects were observed macroscopically in the gingiva and roentgenographically in the alveolar bone.

The mechanism of acceleration of the rate of tooth movement in PGHS-treated cases may be related to evidence that local PGHS application increase PGs biosynthesis that stimulate bone resorption *in vivo*. It has been demonstrated that injections of 10 $\mu$g of $PGE_1$ or $E_2$ in gingiva cause the appearance of osteoclasts and bone resorption [2]. The role of cyclic AMP and calcium in the induction of osteoclasts incident to experimental tooth movement in rats was reported [4, 5]. PGs cause a significant increasing in the content of cyclic AMP and intracelular calcium in different tissues of organisms [10]. These effects of PGs on periodontal tissues are probably related to the acceleration of the rate of tooth movement by PGHS and PGEs injections that was shown in the present study. Whatever the exact mechanism may be, the results show that tooth movement was enhanced in the cats by injections of preparation with PGHS activity as well as of $PGE_1$ or $E_2$.

So, the results of this study show that local injection of preparation with PGHS activity may be effective method of accelerating orthodontic tooth movement in the same manner as it is accepted for the treatments with PGEs applied clinically.

**Acknowledgements**

The present research was supported by intern project of CGEPI of University Autonomous of Coahuila, Mexico.

**References**

1. King G.J. and Thiems S. *Arch. Oral Biol.* 1979. **24**. P. 811–815.
2. Yamasaki K., Miura F., abd Suda T. *J. Dent. Res.* 1980. **59**. P. 1635–1642.
3. Davidovitch Z. and Shanfeld J.L. *J. Dent. Res.* 1980. **59B**. P. 977.
4. Yamasaki K., Shibata Y., and Fukuhara T. *J. Dent. Res.* 1982. **61**. P. 1444–1446.
5. Yamasaki K., Shibata Y., Imai S., Tani Y., Shibasaki Y., and Fukuhara T. *Am. J. Orthod.* 1984. **81**. P. 508–518.
6. Chumbley A.B. and Tuncay O.C. *J. Dent. Res.* 1981. **60**[a]. P. 596.
7. Ilyina A.D., Pineda M.G., Garza Y., Cerda F.R., and Rodríguez M.J. *Bioquimia (de Asociación Mexicana de Bioquímica Clínica)*. 1995. **20**. P. 243–248.
8. Ilyina A.D., Pineda M.G., Garza Y., Cerda F.R., and Rodríguez M.J. *Bioquimia (de Asociación Mexicana de Bioquímica Clínica)*. 1995. **20**. P. 290–293.
9. Vane J.R. *Nature*. 1971. **231**. P. 232–235.
10. Varfolomeev S.D. and Mevkh A.T. *Prostaglandins as Molecular Bioregulators*. 1985. M. V. Lomonosov Moscow University P. H., Moscow.
11. Varfolomeyev S.D. and Mevkh A.T. *Biotechnol. Appl. Biochem.* 1993. **17**. P. 291–304.
12. Wallach D.P. and Daniels E.G. *Biochim. Biophys. Acta*, 1971. **231**. P. 445–457.
13. Smith W.L., Thomas E.E., Kulmacz R.J., Lawrence J.M., and Tsai A.-L. *Biochemistry*. 1992. **31**. P. 3–7.
14. Tsai A.-L., Kulmacz R.J., Wang J.-S., Wang Y., Van Wart H.E., and Palmer G. *J. Biol. Chem.* 1993. **268**. P. 8554–8563.
15. Mevkh A.T., Miroshnikov K.A., Igumnova N.D., and Varfolomeev S.D. *FEBS Lett.* 1993. **321**. P. 205–208.
16. Mevkh A.T., Basevich V.V., Yarving I., and Varfolomeev S.D. *Biokhimiya*. 1982. **47**. P. 1852–1858.
17. Ilyina A.D., Pineda M.G., Martínez H.J.L., Martínez V.M., and Rodríguez M.J. *Rev. Soc. Quim. Mex.* 1997. **41**. P. 99–103.
18. Lowry O.H., Rosebrough N.J., Farr A.L., and Randall R.J. *J. Biol. Chem.* 1951. **193**. P. 265–275.
19. Mevkh A.T., Sud'ina G.F., Yakusheva L.A., Myagkova G.I., and Evstigneeva R.P. *Biokhimiya*. 1985. **50**. P. 974–979.
20. Samuelson B. *Adv. Biosci.* 1973. **9**. P. 7.