Effect of Ibuprofen on IL-1β, TNF-α and PGE2 Levels in Periapical Exudates: A Double Blinded Clinical Trial

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ABSTRACT

Background: Bone resorption is one of the main features of inflammatory periapical lesions and is mainly mediated by interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and prostaglandin-E2 (PGE2). Recent investigations of these lesions revealed that pharmacological modulation may be possible. **Objective:** The aim of this study was to evaluate the effect of Ibuprofen on IL-1β, TNF-α and PGE2 levels in periapical exudates and compare the results with a group of placebo control. **Methods:** Thirty patients with non vital teeth and radiographic lesions were divided into two groups of case and control according to their entrance to the study. Periapical exudates were taken from root canals using absorbent paper points and followed by 400 mg Ibuprofen and placebo prescribed one tablet every 6 hour for three days and in the fourth day second samples were taken, then final cleaning, shaping and obturation of the canals were completed. IL-1β, TNF-α and PGE2 levels were determined by enzyme-linked immunosorbent assays (ELISA). Data were analyzed using paired t-test and student's t-test. **Results:** The results showed that PGE2 levels were decreased significantly in the case group to 86.92 ± 72.42 Pg/ml following Ibuprofen treatment comparing with the pre-treatment (164.96 ± 12.255 Pg/ml) (p=0.02) and placebo group (154.2 ± 97.13 Pg/ml) (p=0.001). But there were no significant differences in IL-1β and TNF-α level between the two groups and in each group before and after treatment. **Conclusion:** The data indicate that Ibuprofen, as a non-steroidal anti-inflammatory drug (NSAID), can be used to block PGE2 release, enhance healing of inflammatory periapical lesions and possibly to inhibit bone resorption.

Keywords: Ibuprofen, Interleukin-1β, Lesion, Periapical, Prostaglandin-E2, Tumor Necrosis Factor-α

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INTRODUCTION

Periradicular lesions are commonly the result of an inflammatory process that evoked by the interaction between the invading bacteria to the pulp and the host immune system (1). This interaction induces the immunocompotent cells to release active mediators and cytokines capable to resist the invading factors and destruction of periradicular tissues, such as periradicular bone which is the main feature of formation of periapical lesions (2,3). Interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) are proinflamatory cytokines and release from the immunocompotent cells like macrophages, T and B lymphocytes and plasma cells. These cells have been shown to be present in human periapical lesions and stimulate bone resorption, prostaglandins (PG) synthesis, protease production, leukocyte adhesion to endothelial cells, enhance neutrophils and lymphocytes activities (4-6). In addition, PGE2 is a strong activator of osteoclasts and rapid bone loss during acute phase of apical periodontitis. Studies by Wang and Stashenko provided sufficient evidence that osteoclast activation may be mediated in part by de novo formation of cyclooxygenase pathway products such as prostaglandins that can be significantly impeded by non-steroidal anti-inflammatory drugs (NSAIDs) (7,8).

Over the years, the treatment of these lesions is focused on stopping the host response to the bacterial stimulation at the apical foramen/foramina. Methods used to achieve this goal include the cleaning, shaping, eliminating bacteria from the root canal system and followed by obturation. With the elimination of the bacterial stimuli the periapical lesion should resolve and repair should take place but healing of the lesion may take many months.

Because macrophages can live for months in the region thus their state of activation may persist to inhibit the fibroblasts, maintain osteoclastic activity, and inhibit osteogenesis therefore preventing bone and soft tissue to repair (9,10). In a study by Kvist and Reit healing process of periapical lesions was compared following surgical and nonsurgical retreatment (11). At 12 months a significant difference was found in favor of surgical treatment that faded by 48 months to almost no difference between the two groups suggesting that early removal of the activated macrophages enhances the healing process in the apical area. Assuming that such mechanisms are involved in impaired healing of periapical lesions, pharmacological intervening in the process may be wise. Many drugs have been successfully used to achieve this goal e.g. glucocorticoid steroids (9,12) and NSAIDs (13). Ibuprofen as NSAIDs exerts its effects through the inhibition of cyclooxygenase enzymes COX1 and COX2 (14). In this regard our purpose was evaluation the effect of Ibuprofen on IL-1β, TNF-α and PGE2 level s in periapical exudates.

MATERIALS AND METHODS

Subjects. Patients who referred to the department of endodontics affiliated to Hamadan University of medical sciences, Hamadan, to receive root canal treatment because of pulp necrosis and periapical lesions were selected for the study. The aim of the study and possible side effects of the drug were explained to the patients and written consents were obtained. Thirty patients participated in the study were randomly assigned into two equal groups according to their entrance to the study. Ibuprofen (400 mg 1 tablet q6h) was prescribed for the case group and placebo pills for control group in the same

interval. The patients with gastrointestinal problems and renal failure were excluded from the study. None of the lesions were related to marginal periodontitis and none extended to sinuses (6,15).

Sampling of Periapical Exudates. The involved teeth were isolated with rubber dams and penetration to the pulp chamber and unroofing were made using a low speed round bur for entering the chamber. Following the measurement of the working length the root canal was enlarged at least to size 30 in apex. After the root canal was dried with sterile paper points, a size 30 paper point (Ariadent Co. Tehran, Iran) was inserted to the root canal close to the established working length and held for 30 seconds. In the teeth with active draining of exudate paper point was taken out and 3milimeters of its tip was cut in to a tube containing 250 micro liters of Potassium phosphate buffer, vortexed for 1 min and stored in -70 $^{\circ}$C until the time of assay (6). Routine cleaning and shaping was then completed and the access openings were sealed with Cavit (Ariadent Co. Tehran, Iran) 12 tablets/pills of ibuprofen / placebo for three days were prescribed, and in the fourth day the whole procedure were repeated and second samples were obtained. Cleaning, shaping and obturation were completed in this session (3).

Table 1. Main demographic and clinical characteristics of the study population.

	Ibuprofen treated (n=15)	placebo control (n=15)	Total (n=30)	P value*
Sex (M/F)	6/9	3/12	9/12	0.44
Age (years)	26.8 ± 9.8	27.3 ± 11.8	27. 1 ± 10.8**	0.9
Radiolucent area				
>5 mm	53.3%	50%	51.65%	0.85
≤5 mm	46.7%	50%	48.35%	
Pus discharge				
Present	20%	25%	23%	0.55
Absent	80%	75%	77%	
Sinus tract				
Present	20%	25%	23%	0.55
Absent	80%	75%	77%	
Pain (palpation/percussion)				
Present	20%	17%	18.5%	0.64
Absent	80%	83%	81.5%	

*- *t-test* to compare of two group
**- Values are means± standard deviations
Two groups were not characterized by any significant differences.

Measurement of $IL_1\beta$, TNF-α and PGE2. IL-1β, TNF-α and PGE2 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer instructions. IL-1β and TNF-α kits were sandwich-ELISA (Bender Medsystem, Vienna, Austria) and PGE2 was a competitive-ELISA (Cayman Chemical, USA). The results were expressed as picograms per milliliter (pg/ml) for each mediator.

Statistical Analysis. IL-1β, TNF-α and PGE2 levels of pre/post treatment in each patient were compared by using paired t-test and to compare the data between two groups t-test were used.

RESULTS

The main demographic and clinical characteristics of the study population are illustrated in Table1 (previous page).
Compared to the control group, patients treated with Ibuprofen were not characterized by any significant differences. PGE2 and IL-1β were detected in all periapical exudate samples, whereas TNF-α was undetectable in about 10% of samples that were equally distributed between pre/post-treatment of two groups.

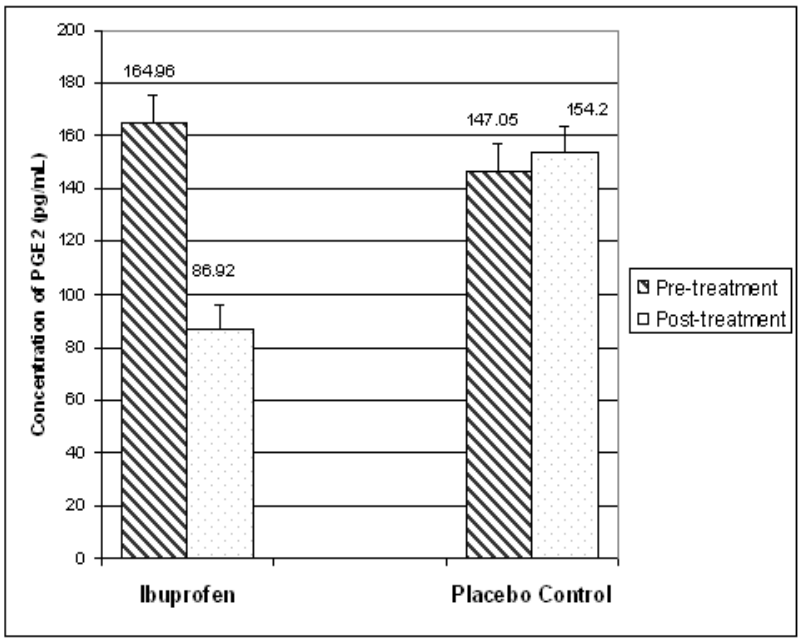

Figure 1. Comparison periapical exudates PGE2 levels at pre/post-treatments in the Ibuprofen treated and placebo controlled groups.

Data analysis showed that the mean level of PGE2 was decreased from 164.9 ± 121.25 Pg/ml to 86.92 ± 72.42 Pg/ml significantly (p=0.02) in patients undergoing root canal treatment after taking Ibuprofen whereas in the control group the mean level of PGE2 did not decrease significantly (p=0.94) after placebo consumption (Table 2, Figure 1).
Comparing PGE2 level in Ibuprofen group (86.92 ± 72.42 Pg/ml) and placebo treated group (154.2 ± 97.13 Pg/ml), reduction in PGE2 levels were also significant (p=0.001) (Table 2, Figure 1).

Table 2. Concentration of periapical exudates mediators' levels at pre/post-treatment in the Ibuprofen treated and placebo controlled groups.

Mediators (pg/ml)	Ibuprofen treated (n=15)	placebo control (n=15)	P value*
PGE2			
Pre-treatment	164.96 ± 121.25**	147.05 ± 104.02	NS***
Post-treatment	86.92 ± 72.42	154.2 ± 97.13	0.001
P value****	0.02	NS	
TFN-α			
Pre-treatment	2.6 ± 2.25	3.96 ± 2.17	NS
Post-treatment	2.61 ± 2.51	1.66 ± 1.33	NS
P value	NS	NS	
IL-1β			
Pre-treatment	66.80 ± 49.64	55.5 ± 24.86	NS
Post-treatment	63.73 ± 30.49	54.5 ± 22.46	NS
P value	NS	NS	

* *t-test* to compare of two group
** Values are means± standard deviations
*** Not significant
**** Pair *t-test* to compare pre/post treatment of each group

Changes in IL-1β and TNF-α levels before and after Ibuprofen treatment and versus placebo group were not statistically significant (Table 2, Figure 2, 3).

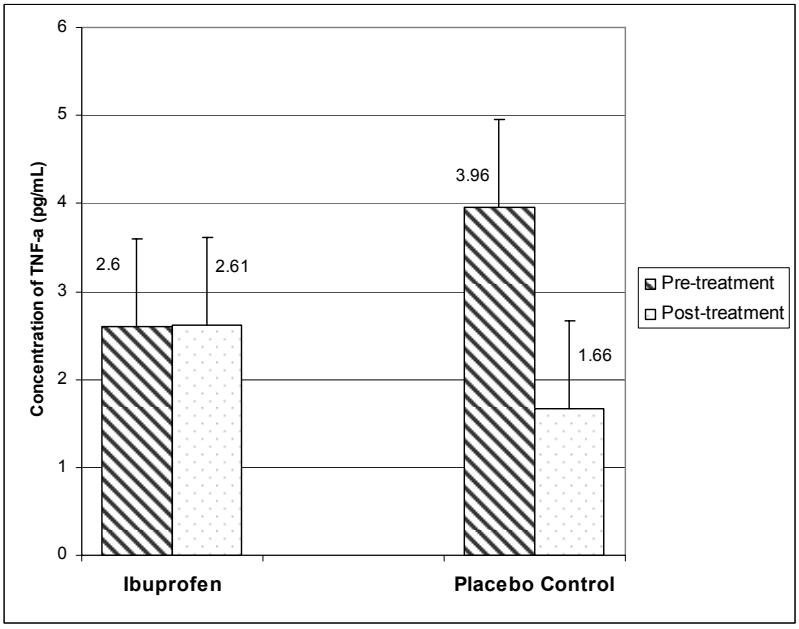

Figure 2. Comparison of periapical exudates TNF-α levels at pre/post-treatment in the Ibuprofen treated and placebo control groups.

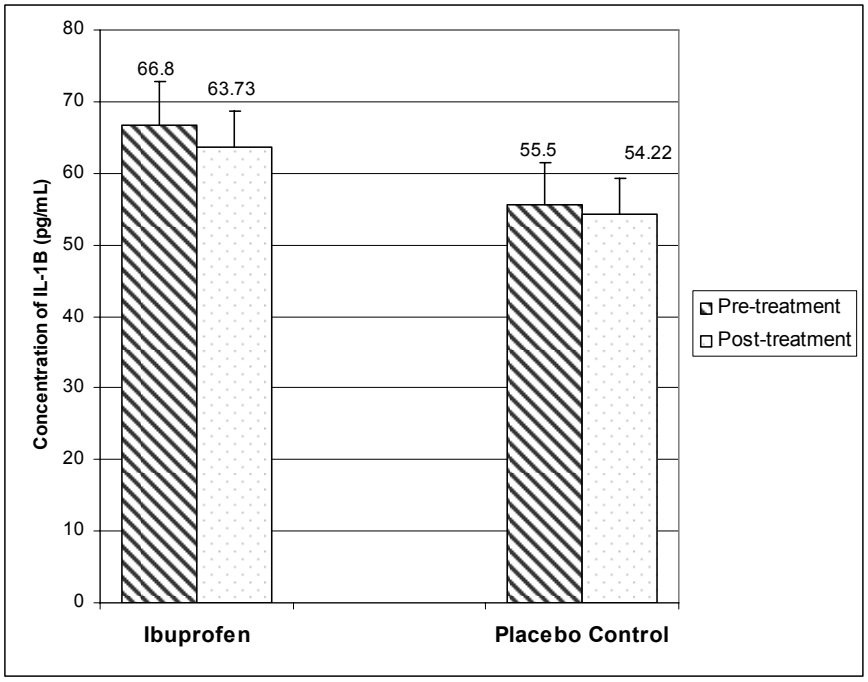

Figure 3. Comparison periapical exudates IL-1β levels at pre/post-treatment in the Ibuprofen treated and placebo controlled groups.

DISSCUSION

This study was a clinical trial dealing with the effect of Ibuprofen on proinflamatory mediators IL-1β, TNF-α and PGE2 in the periapicular exudates. We observed no statistical changes in the proinflamatory cytokines levels in control placebo group after endodontics treatment and IL-1β and TNF-α in the ibuprofen group after endodontic and ibuprofen therapy which is in consistency with Alptekin et al. work (3). Nevertheless these results were in contrast with Shimauchi et al. who showed that the mean PGE2 levels significantly decreased following the endodontic therapy (16). The mean levels of PGE2 in periapical exudates decreased significantly in Ibuprofen group after treatment with ibuprofen. These changes were also significant when Ibuprofen treated group compared against control placebo group. Ibuprofen as a non steroidal anti-inflammatory drug exerts its effects through the inhibition of cyclooxygenase enzymes COX1, COX2 (14).

Ibuprofen blocks both isotypes with clinical doses (17). Study by Wang and Stashenko (18) provided convincing evidence that among the long list of mediators capable to activate osteoclasts and promote periapical bone resorption the main factors are IL-1β and TNF-α. They have also suggested that osteoclastic activation by these cytokines is mediated by cyclooxygenase pathway products such as prostaglandins. Wang and stashenko (7) in another study demonstrated approximately 60% of bone resorbing activity related to IL-1β is inhibited by indomethacin. These finding shows the fact that

IL-1β induced bone resorption is partly dependent on PGE2 production. IL-1β and TNF-α did not change considerably after ibuprofen treatment and in comparison with control placebo group. These findings are not consistent with Matsuo and et al. in their study IL-1β demonstrated to decline in periapical exudates after root canal treatment. The differences can be due to different teeth studied and the time period between the two samplings. Our study demonstrated the effect of Ibuprofen on periapical exudate PGE2 levels, but IL-1β and TNF-α did change in the time period of the study therefore due to the prominent role of PGE2 in apical bone resorption Ibuprofen seems to be beneficial to control bone destruction and promote healing. In Conclusion, our study demonstrated the effect of Ibuprofen on periapical exudate PGE2 levels, but IL-1β and TNF-α did change in the time period of the study.

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