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ORIGINAL

Effects of using different biological products on IL-1ß TNF-α, PGE_2 concentrations in rabbits with periodontitis

Efectos del uso de diferentes productos biológicos sobre las concentraciones de IL-1ß TNF-α, PGE_2 en conejos con periodontitis

Samer Salim Jaafer[1] ✉, Faten Ben Amor[1] ✉

[1]Department of Peridontics, College of Dentisry, Al-Muthanna University.
[2]Anatomy, Research Laboratory of Oral, Health and Oro Facial Rehabilitation, Faculty of Dental Medicine, University of Monastir, Monastir, Tunisia.

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ABSTRACT

Introduction: platelet-rich plasma (PRP) is a plasma fraction of autologous blood with a high platelet concentration, containing key cytokines and growth factors.
Method: this study, conducted in the Clinical Surgery Department of the College of Dentistry, University of Al-Muthanna, involved 20 New Zealand white rabbits divided into four groups. A 1mm gingival wound was created on each rabbit, followed by immediate injections:

- 10µl saline
- 10µl PRP
- 10µl Platelet-poor plasma (PPP)
- 3ng hepatocyte growth factor (HGF) in 10µl engineered tendon matrix

Rabbits were sacrificed on days 0, 1, 3, 5, and 12 for tissue harvesting.
Results: interleukin 1 beta Increased in PPP group compared to HGF, PRP, and control groups. Tumor Necrosis Factor alpha: Increased in PPP group.While,PRP significantly reduced PGE2 levels on days 1, 3, and 5. On day 12, levels were low in both PRP and control groups. PPP slightly reduced PGE2 on days 1, 3, and 5. HGF reduced PGE2 on days 1, 3, and 12, with a transient response on day 0.
Conclusion: PRP sustains the up-regulation of proinflammatory factors like Interleukin 1 beta and Tumor Necrosis Factor alpha. Its anti-inflammatory function is partially mediated through HGF, producing similar anti-inflammatory effects.

Keywords: PRP; IL-1ß TNF-α; PGE2; Rabbits; Periodontitis.

RESUMEN

Introducción: el plasma rico en plaquetas (PRP) es una fracción de plasma de sangre autóloga con una alta concentración de plaquetas, que contiene citocinas y factores de crecimiento clave.
Método: este estudio, realizado en el Departamento de Cirugía Clínica de la Facultad de Odontología de la Universidad de Al-Muthanna, involucró a 20 conejos blancos de Nueva Zelanda divididos en cuatro grupos. Se creó una herida gingival de 1 mm en cada conejo, seguida de inyecciones inmediatas:

- 10µl de solución salina
- 10µl de PRP
- 10µl de plasma pobre en plaquetas (PPP)
- 3ng de factor de crecimiento hepatocitario (HGF) en 10µl de matriz tendinosa diseñada

Los conejos fueron sacrificados en los días 0, 1, 3, 5 y 12 para la recolección de tejidos.
Resultados: Interleucina 1 beta: Aumentó en el grupo PPP en comparación con los grupos HGF, PRP y

control. Factor de Necrosis Tumoral alfa: Aumentó en el grupo PPP. Prostaglandina E2 (PGE2): El PRP redujo significativamente los niveles de PGE2 en los días 1, 3 y 5. En el día 12, los niveles fueron bajos tanto en los grupos PRP como en los grupos de control. El PPP redujo ligeramente la PGE2 en los días 1, 3 y 5. El HGF redujo la PGE2 en los días 1, 3 y 12, con una respuesta transitoria en el día 0.
Conclusión: el PRP sostiene la regulación al alza de factores proinflamatorios como la Interleucina 1 beta y el Factor de Necrosis Tumoral alfa. Su función antiinflamatoria está mediada parcialmente a través del HGF, produciendo efectos antiinflamatorios similares.

Palabras clave: PRP; IL-1ß TNF-α; PGE2; Conejos; Periodontitis.

INTRODUCTION

PRP has been utilized in dental implantology for stimulating new bone formation or peripheral nerve regeneration. Several animal and human studies have assessed the effect of PRP in implantology. Many of these studies have reported the beneficial effects of PRP on hard and soft tissue healing. For instance, increased bone activity and faster bone regeneration after using PRP were explored by scintigraphy in dogs.[1,2]

Song *et al.*, (2019)[3] transferred autologous PRP into the canine implant bed to study the effect of PRP on nerve innervation in the peri-implant bone. They demonstrated that PRP exhibited a significant effect on the diameter of the myelinated nerve fibres and might help to improve regeneration of nerve fibres in peri-implant bone, more specifically 6 months after healing.

Taschieri[4] reported that the use of PRP in association with implants immediately placed into fresh extraction sockets proved beneficial effects on soft tissue healing in the clinical studies of post-extraction implants.

Based on the current findings, local application of PRP may provide accelerated healing of hard and soft tissues in the proximity of dental implants during routine implant surgery. However, characterization of the healing with autologous PRP in physiological osseointegration of implants remains poorly documented or even controversial. Moreover, the effect of the concentrations of PRP on the assumed development of peri-implant bone microstructures in a longer observation time has hardly been investigated.[1]

Autologous plasma fractions, such as platelet-rich plasma (PRP) and platelet-poor plasma (PPP) have been utilized in dental implantology for stimulating new bone formation,[5] angiogenesis,[6] and peripheral nerve regeneration.[7]

Activated platelet-derived factors serve as messengers and regulators that influence a variety of cell–cell and cell–extracellular matrix (ECM) interactions and serve to modify the pericellular microenvironment. The most important growth factors released by platelets in PRP include vascular endothelial growth factor (VEGF), transforming growth factor-ß (TGF-ß), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), matrix metalloproteinases 2 and 9, and interleukin-8.[8]

This investigation was designed to study the effects of using different biological products on IL-1ß TNF-α, PGE2 concentrations in rabbits with periodontitis.

METHOD

In this study, 20 New Zealand white strains of rabbits were used, with ages ranged between 5-10 months and weights ranged between (3,5-5kg), these rabbits were putted underwent laboratory conditions, such as suitable temperatures from (18 - 20 ▫C), 12 hrs., under light and 12 hrs. under darkness to allowed animals to acclimation for 20 days before start the experiment.[9] Adult rabbits should be kept in individual mesh cages (0,90 × 0,60 × 0,45 m) hung at a height of 0,8 cm from the ground so that excrement can fall out into collecting trays. 12 to 14 hours of light are necessary for the colony's circadian biorhythms and animals should be routinely observed for food consumption and fecal characteristics.

Study Groups

The present study was completed in the laboratory of Clinical Surgery Department in College of Dentistry, University of Al-Muthanna. During the period from (1st of November 2021) until (1st of February 2022).

The study groups of the present study were (20) New Zealand white strains of rabbits, which were divided randomly into four groups according to variation between groups, each group include 5 rabbits. A wound with a 1 mm diameter was created on the gingival of each rabbit using a biopsy punch. The wounded rabbits were divided into four groups and the following injections were given to the rabbits immediately after wounding:

- First group rabbits (only wound group) were injected of 10µl saline.
- Second group (PRP group) were injected of 10µl PRP.
- Third group (PPP group) were injected of 10µl PPP.

- Fourth group (HGF group) were injected of 3ng HGF in 10µl of an engineered tendon matrix (ETM) prepared from rabbit according to the published protocol.

One rabbit in each group were sacrificed on days 0, 1, 3, 5, 12, and the sample were harvested. Day 0 tissue were removed from rabbits immediately after injections.

Platelet rich plasma preparation

This was prepared according to Dhurat & Sukesh[10]

Gingival tissue preparation

Gingival tissues were weighed, minced, placed in buffer (100 µl buffer for each 1mg tissue) provided by the ELISA kit manufacturer, and homogenized. The tissue samples were centrifuged at 3 000 g for 30 min at 4°C, and the supernatant was collected to measure PGE2 and HGF. The measured values were normalized with respect to tissue weight.[11]

Determination of HGF in PRP and rabbit whole blood

Rabbit whole blood (4 ml) was withdrawn from an ear vein of each rabbit and mixed with 0,5ml 3,8 % sodium citrate (SC); 1ml of blood-SC mixture was used as a whole blood sample.[12] The concentrations of HGF in rabbit whole blood and PRP were measured using an ELISA kit.

Rabbit Prostaglandin E2 ELISA Kit

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rabbit PGE2 antibody. PGE2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Rabbit PGE2 Antibody is added and binds to PGE2 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated PGE2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rabbit PGE2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Rabbit TNF-α ELISA Kit

Rabbit TNF-α ELISA Kit was used in this study for quantitative determination of TNF-α concentration and done according to company instruction (Elabscience/ China).

Rabbit IL-1-β ELISA Kit

Rabbit IL-Iβ ELISA Kit was used in this study for quantitative determination of IL-1β concentration and done according to company instruction (Elabscience/ China).

Statistical Analysis

Statistical analysis was carried out using SPSS version 26. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. ANOVA test was used to compare means between three groups or more. Correlation coefficient (r) was used to assess the relationship between two continuous variables. A p-value of ≤ 0,05 was considered as significant.[13]

RESULTS AND DISCUSSIONS

Effect of PRP, PPP, HGF and control groups on IL-1β and TNF-α

The effect of PRP, PPP, HGF and control groups on IL-1β were investigated, the results showed that, the mean difference of IL-1β was increase in PPP groups (72,57 ± 14,75) than in HGF (0,65 ± 0,52), PRP (1,34 ± 1,48) and control group (55,02 ± 6,36) with no significant difference. However, the effect of these factors on TNF-α were showed increase of this cytokine in PPP (71,78 ± 9,33) group more than HGF (0,61 ± 0,45), PRP (2,55 ± 2,73) and control group (54,75 ± 5,99) no significant difference. These results were shown in table 1.

Table 1. Effect of PRP, PPP, HGF and control groups on IL-1β and TNF-α

Study groups	IL-1β (mean ± SD)	TNF-α (mean ± SD)	P value
PRP	1,34 ± 1,48	2,55 ± 2,73	0,338
HGF	0,65 ± 0,52	0,61 ± 0,45	0,921
PPP	72,57 ± 14,75	71,78 ± 9,33	0,142
Control	55,02 ± 6,36	54,75 ± 5,99	0,798

An inflammatory response is the initial step in the process of wound healing, and a moderate inflammatory reaction is helpful to normal wound healing.[14] In the results of Qing[15] found that, the cytokines were significantly

lower with significantly in PRP group than in PPP group and control group ($P < 0,05$). Li[16] demonstrated that, PRP may suppress cytokine release, limit inflammation, and promote tissue regeneration. Tavassoli-Hojjati[17] concluded that the growth-stimulating effect of PRP is dose dependent with the best results in low concentrations. Andia[18] found IL-1ß, IL-8 and TNF-α were significantly induced by PRP compared to both HGF and PPP groups. Lee[19] demonstrated that, IL-1ß and TNF-α concentrations were similar in PPP and control group, and both lower than those in the PRP preparation.

PRP could have beneficial therapeutic effects on hard and soft tissue healing, due to growth factors stored in the platelets.[20] Moreover, previous studies reported an anti-inflammatory role of activated PRP.[21,22]

Blandford[23] known that, PRP may contain variable levels of IL-1ß receptor antagonist (IL-1ra), as shown in human serum. IL-1ra may block the induction of cellular inflammation in culture by exogenous IL-1ß.

Additionally, HGF could up regulate IL-1ra expression, thus exerting its anti-inflammatory effects on the tissue cells. However, this potential effect of serum IL-1ra on tissue cell inflammation must be much smaller than the effects of HGF, because the combined use of PRP and HGF antibody restored more than 60–70 % of COX-1 and COX-2 expressions and PGE_2 production, which was induced by IL-1ß treatment of tissue cells to levels comparable with PRP treatment alone.[24]

Therefore, Zhao[25] suspect that, in normal serum derived from healthy rabbits, IL-1ra levels are either low, or may be degraded during the process of PRP preparation, or a combination of the two.

Administration of an IL-1 receptor antagonist to rabbit decreased the nociceptive response to an incisional wound, and reduced the production of multiple inflammatory mediators, including keratinocyte-derived chemokine (KC) and macrophage inhibitory protein (MIP)-1α, within the wounds.[26] IL-1α and IL-1ß stimulated IL-8 and GRO-α production by primary keratinocytes. IL-1ß levels were highly correlated with IL-8 in surgical wounds.[27]

The effects of PRP, PPP, HGF and control groups on PGE_2 production

PRP injections did not significantly reduce PGE_2 levels immediately after injection on day 0, but they significantly decreased PGE_2 levels in rabbits gingival wounded on days 1, 3, and 5 post-treatment. On day 12, PGE_2 levels in both PRP injected and un-injected groups were lower than on day 0 with no apparent effect of the PRP treatment remaining. On the other hand, PPP injections did not significantly reduce the levels of PGE_2 in rabbits gingival wounded at any of the above time points.

However, as revealed by a trend analysis, PPP slightly reduced PGE_2 production in the rabbits gingival wounded on days 1, 3, and 5. This is more likely due to the low abundance of platelets in PPP. Furthermore, HGF injections reduced PGE_2 levels similar to PRP injections. Specifically, HGF significantly decreased PGE_2 levels on days 1, 3 and 12, albeit to varying degrees. On day 5, a slight reduction in PGE_2 levels was observed which was not statistically significant. Note that on day 0 the treatment groups exhibited a transient response to HGF treatment, as they had lower PGE_2 levels than the control group. This was likely due to the lag time (~1 hr) from injection of HGF to harvesting and processing of rabbits gingival tissues for PGE_2 measurement. In these experiments, the results from saline injections (wound only group) not appear any changes; therefore, the results from the saline injected group only are presented here as the control. These results were shown in figure 1.

An interesting finding *in vivo* model is that while PPP injection did not significantly reduce PGE_2 levels, it apparently caused a small reduction of PGE_2 production in wounded rabbit on days 0, 1, 3, and 5 post-treatment. This effect is similar to PRP, although much smaller, probably due to the much lower number of platelets in PPP than in PRP. In addition, PPP could possibly contain certain serum factors exerting an inhibitory effect on PGE_2 production in the wounded tissue.

This finding suggests that, PRP's effects on the inflammation of injured tissues are platelet concentration-dependent, and that blood clots, which contain fewer concentrated platelets than PRP, but a higher number of platelets than PPP, can exert anti-inflammatory effects at injured tissue sites.[28]

Now a further explanation of the cell tissue may be necessary to enable a better understanding of experimental results. First, when tissue are injured, inflammation ensues, which is characterized by an initial up regulation of IL-1ß production in injured sites. IL-1ß, which is secreted mainly by macrophages, is a potent inflammatory cytokine that up regulates the expression of other inflammatory mediators, including COX and matrix metallopeptidase. Second, tissue cells are known to respond to IL-1ß treatment by up regulating COX expression and increasing production of PGE_2.[29] Therefore, the use of IL-1ß and tissue cells may serve as an appropriate *in vitro* model to simulate tissue inflammation *in vivo*.

However, a limitation of the current *in vitro* model is that mechanical loading was not incorporated. Because patients resume daily activities after PRP injection, mechanical loading placed on injured tissue should be an important factor in assessing the efficacy of PRP treatment on injured tissue[30]. In clinics, training regiments are often prescribed, which may have additive effects on tissue inflammation. Based on this, Tang[31] suggest that, appropriate exercise after PRP treatment is likely beneficial, as small mechanical loading on suppresses cellular inflammation.

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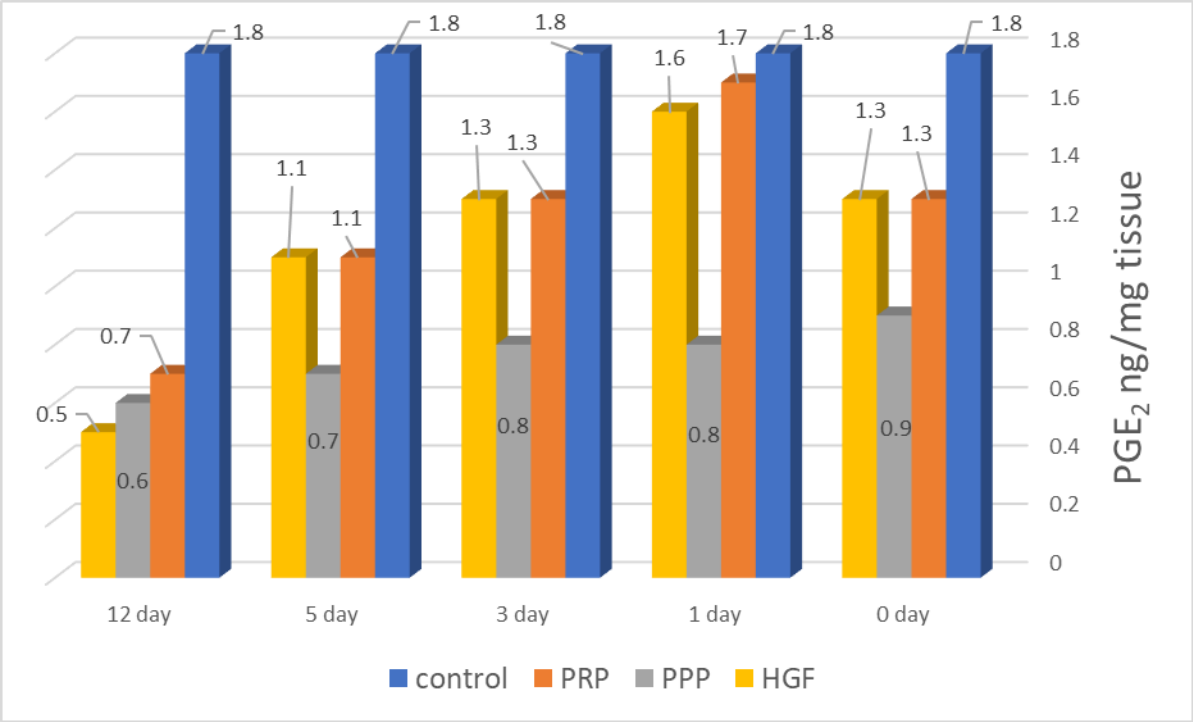

Figure 1. The effects of PRP, PPP, HGF and control groups on PGE_2 **production**

However, intensive exercise should be avoided because large mechanical loading may further worsen cellular inflammation by increasing the production of PGE_2.[32] PGE_2 may play an important role in tissue remodeling.[33]

CONCLUSION

Platelet-rich plasma is able to sustain the up-regulation of proinflammatory factors, (Interleukin 1 beta, tumor necrosis factor alpha). Anti-inflammatory function of in Platelet-rich plasma is at least partially mediated through hepatocyte growth factor, a major growth factor in Platelet-rich plasma, which produces anti-inflammation results similar to Platelet-rich plasma

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CONFLICT OF INTEREST

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AUTHORSHIP CONTRIBUTION

Conceptualization: Samer Salim Jaafer, Faten Ben Amor.
Data curation: Samer Salim Jaafer, Faten Ben Amor.
Formal analysis: Samer Salim Jaafer, Faten Ben Amor.
Research: Samer Salim Jaafer, Faten Ben Amor.
Methodology: Samer Salim Jaafer, Faten Ben Amor.
Project management: Samer Salim Jaafer, Faten Ben Amor.
Resources: Samer Salim Jaafer, Faten Ben Amor.
Software: Samer Salim Jaafer, Faten Ben Amor.
Supervision: Samer Salim Jaafer, Faten Ben Amor.
Validation: Samer Salim Jaafer, Faten Ben Amor.
Display: Samer Salim Jaafer, Faten Ben Amor.
Drafting - original draft: Samer Salim Jaafer, Faten Ben Amor.
Writing - proofreading and editing: Samer Salim Jaafer, Faten Ben Amor.