J Musculoskelet Neuronal Interact 2009; 9(3):167-172

Original Article

Hylonome

Effect of homologous PRP on proliferation of human periodontally affected osteoblasts. *In vitro* preliminary study. Report of a case

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Abstract

Objective: The purpose of this *in vitro* study was to evaluate the effect of two concentrations of homologous platelet-rich plasma (PRP) on the proliferative response of osteoblasts derived from a patient with aggressive periodontitis. **Methods:** 8.5ml of venous blood were taken from 1 healthy and non-smoker volunteer. PRP was prepared following the protocol of Curasan. Osteoblasts were derived from alveolar bone chips obtained from a patient with aggressive periodontitis during conventional periodontal surgery and a clinically healthy person during crown lengthening surgical procedure. Cells were grown in 24-well dishes and on day 2 of quiescence were treated with 1% and 5% (v/v) of PRP. The effect on cell proliferation was estimated by measuring [^{3}H] thymidine incorporation. After 48h of incubation, cells were processed to subject to scintillation counting. Counts per minute were determined for each sample. **Results:** The addition of 1% and 5% of PRP provoked a statistical significant ($p<0.05$) increase in cell growth. **Conclusions:** Data revealed significant enhancement of proliferative response of osteoblasts in the presence of PRP, which might serve as a source of growth factors promoting periodontal repair by modulating cell response and activities.

Keywords: Platelet-rich Plasma, Periodontitis, Growth Factors, Osteoblasts

Introduction

New bone formation is a prerequisite for the regeneration of tissues lost through periodontal disease and for the osseointegration of implants used in restorative dentistry[1]. Thus, the interest of the researchers has been focused on therapeutic procedures stimulating the formation and supporting the growth of the new bone into osseous defects. A variety of regenerative techniques has been proposed including bone replacement grafts, membranes and proteins like growth factors. The investigation into the wound healing mechanisms pointed out the participation of several growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factors (IGF-I, II), transforming growth factors (TGFs), etc. Growth factors exert regulatory effects on the homeostasis of the periodontal tissues and they also have the ability of modifying the response of periodontal soft and hard tissues during the healing processes after their exogenous application[2,3].

In previous *in vitro* studies, we have shown that PDGF-BB and bFGF exert mitogenic effect on human PDL cells alone or in combination with bone allografts[4,5]. Furthermore, we demonstrated that PDGF-BB and TGF-β1 as well promote DNA synthesis by human PDL cells and human gingival fibroblasts[6].

Platelet-rich plasma (PRP) which constitutes a concentrated suspension of growth factors has been fractionated from blood plasma of patients. Growth factors including PDGF-BB, TGF-β1, IGF-I, PDEGF, PDAF and PF-4, released from the platelets are involved in wound healing and are considered as promoters of tissue regeneration[7-9]. PRP has also been found to stimulate blood vessel formation[10] and umbilical endothelial cell proliferation[11,12].

The preparation and application of PRP can be accomplished in dental clinic and the beneficial outcomes of the product could be suggested as a suitable agent in the treatment of periodontal defects.

The authors have no conflict of interest.

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Accepted 6 June 2009

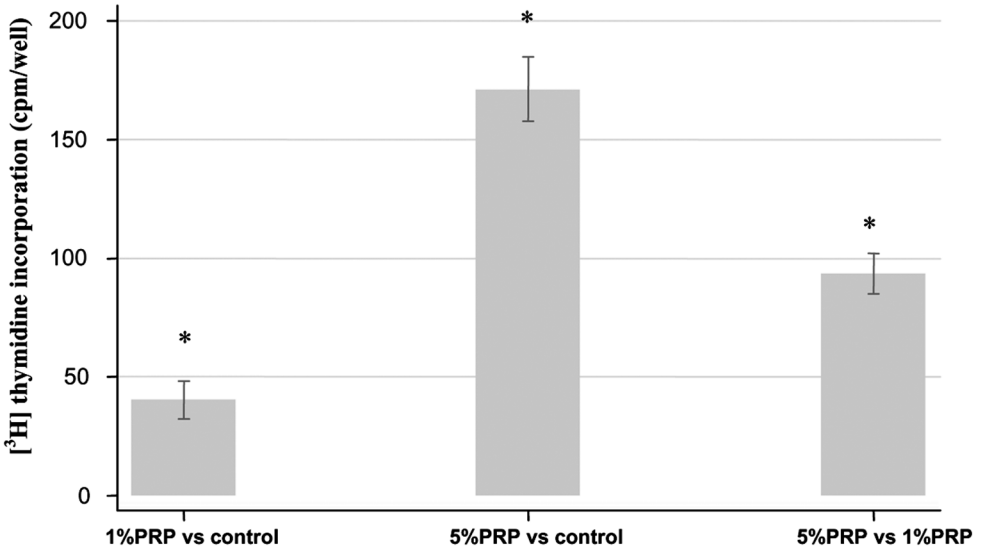

Figure 1. Effect of 1% and 5% v/v of PRP on the DNA synthesis by osteoblasts derived from a clinically healthy subject (HOB). Proliferation was determined by [^{3}H] thymidine incorporation (cpm/well) at 48hrs. Results are represented as mean (95% Confidence Interval) % estimated relative change of [^{3}H] thymidine incorporation of five individual experiments, each conducted in triplicate. Statistical significance was determined at a 0.05 level ($p<0.05$).

Considering that a limited number of studies has been published evaluating the mitogenic effect of PRP on human osteoblast cells, the purpose of this *in vitro* preliminary study was to evaluate the effect of homologous PRP on the proliferative response of osteoblasts derived from a patient with aggressive periodontitis.

Material and methods

Collection and PRP preparation

8.5 ml of venous blood were taken from 1 healthy and non-smoker volunteer (38 years of age) member of our research group. PRP was prepared following the protocol of Curasan (PRP kit, Curasan, Kleinostheim, Germany)[13]. 8.5 ml of whole blood was initially centrifuged at 2.400 rpm for 10 min to separate platelet-rich plasma (PRP) and platelet-poor plasma (PPP) portions from the red blood cell (RBC) fraction. A second cycle of centrifugation followed at 3.600 rpm for 15 min to separate PRP from PPP. The result of the two cycles was 0.6 ml of PRP, which were stored at -80°C until use.

Cells and cell culture conditions

Osteoblasts (OB) were derived from alveolar bone chips obtained from a patient (female, 35 years old) with aggressive periodontitis (AgOB) during conventional periodontal surgery and a clinically healthy person during crown lengthening surgical procedure (HOB).

Patients were informed about the purpose of the study and signed consent form. The Faculty of Dentistry, University of Athens, approved the protocol of the study.

Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal bovine Serum (FBS) (cell culture media were from Gilbo-BRL, Palsey, UK) in an environment of 5% CO_2, 85% humidity and 37°C and subcultured once a week at 1:2 split ration using a trypsin-citrate solution (0.25%-0.3% respectively)[14].

Cells were grown in 24-well dishes and on day 2 of quiescence were treated with 1% and 5% (v/v) of PRP.

The cells were used at passages 3 to 5 in all experiments.

DNA synthesis was estimated by measuring [^{3}H] thymidine incorporation to determine the effect on cell proliferation.

DNA synthesis assay

Cells were plated at a density of $2x10^4$ cells/cm^2 in Minimal Eagle's Medium (MEM) containing 10% FBS allowed to grow until confluence and then synchronized in MEM containing 0.1% human Fetal Calf Serum (FCS). After two days of serum deprivation fresh medium was added to the quiescent cultures along with the indicated concentrations of human sera and methyl-[^{3}H] thymidine (Amersham, Buckingamshire UK) at 0.15 μCi/ml, 25 Ci/mmol. After 48 hrs of incubation the culture medium was decanted the cells were washed with phosphate-buffered saline (PBS), fixed with 10% ice-cold trichloroacetic acid, washed extensively under running tap water and air-dried. DNA was solubilized by addition of 0.3 N NaOH/1% Sodium dodecyl sulfate (SDS) and the lysates were subjected to scintillation counting[14]. Counts per minute (cpm) were determined for each sample.

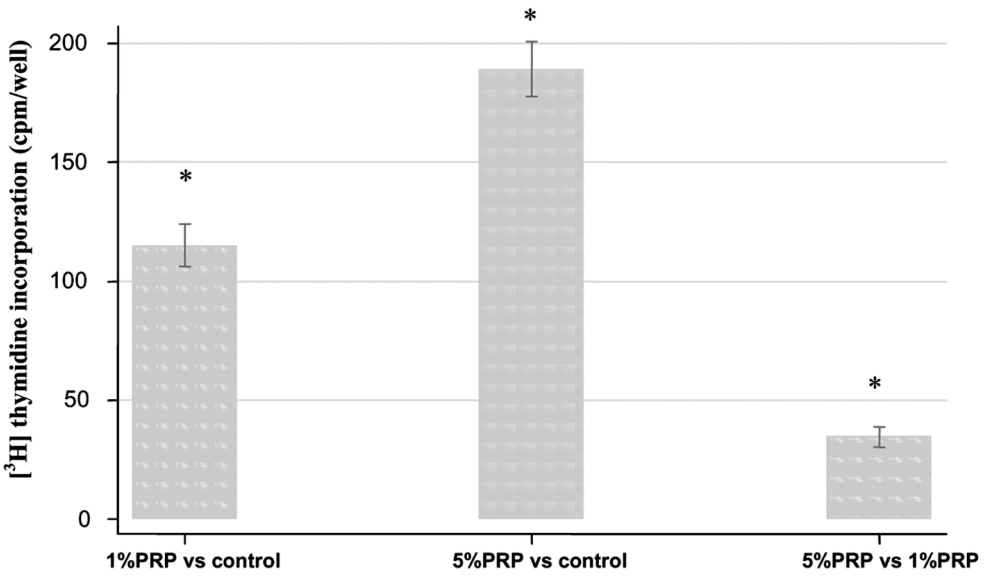

Figure 2. Effect of 1% and 5% v/v of PRP on the DNA synthesis by osteoblasts derived from a patient with aggressive periodontitis (AgOB). Proliferation was determined by [^{3}H] thymidine incorporation (cpm/well) at 48hrs. Results are represented as mean (95% Confidence Interval) % estimated relative change of [^{3}H] thymidine incorporation of five individual experiments, each conducted in triplicate. Statistical significance was determined at a 0.05 level ($p<0.05$).

Statistical analysis

Data were analyzed using the Poisson regression models. Comparison of the [^{3}H] thymidine incorporation differences was performed at 48hrs between HOB and AgOB treated with 1% and 5% (v/v) of PRP. Values represent the mean (95% CI) % relative change of cpm/well from five individual experiments, each conducted in triplicate. Statistical significance was determined at a 0.05 level ($p<0.05$).

Results

Effect of PRP on OB proliferation

This study demonstrates the effect of PRP on the proliferation of OB derived from a healthy person (HOB) and from a patient suffered from aggressive periodontitis (AgOB). The cell response was determined by measuring the [^{3}H] thymidine incorporation. Two doses, 1% and 5% (v/v) of PRP were tested.

The results of the study are shown in Figures 1, 2 and 3. The addition of 1% and 5% PRP in HOB for 48hrs provoked a statistical significant ($p<0.05$) increase in cell growth compared to control. A statistical significant ($p<0.05$) difference between 1 and 5% PRP was also observed with 5% PRP stimulating a more pronounced cell response. Furthermore, the treatment of AgOB with 1 and 5% PRP also revealed a statistical significant ($p<0.05$) enhancement of [^{3}H] thymidine incorporation compared to control with the dose of 5% PRP showing again a more intense result. A comparison of the results of DNA synthesis by HOB and AgOB in the presence of 1 and 5% PRP showed that cell proliferation increases in a statistical significant ($p<0.05$) dose-dependent manner.

Discussion

PRP is widely used in treatment of periodontal defects and in implantology as well. The therapeutic outcomes are characterized as encouraging due to growth factors included in PRP at significant levels.

In a recent *in vitro* studies, we have demonstrated that growth factors like PDGF-BB, bFGF and TGF-β1 have a significant mitogenic effect on human osteoblast-like and periodontal ligament (PDL) cells[4-6,15].

In this *in vitro* pilot study, we evaluated the possible role of PRP in the treatment of periodontal osseous defects; homologous PRP was used on cell cultures derived from alveolar bone chips obtained from a patient with aggressive periodontitis and a clinically healthy person. The effect of PRP two doses (1 and 5% v/v) on cell proliferation was estimated. The results demonstrated that the addition of PRP led to a significant increase in cell growth of HOB and AgOB. Both concentrations promoted cell proliferation, while 5% (v/v) of PRP showed a tendency for further increase compared to 1% (v/v) of PRP. Furthermore, in this study PRP was prepared from 1 healthy and non-smoker volunteer (female, 38 years of age). Research data demonstrated that gender and sex of the donor have no influence in platelet-count or growth factor concentrations in PRP[15a,15b].

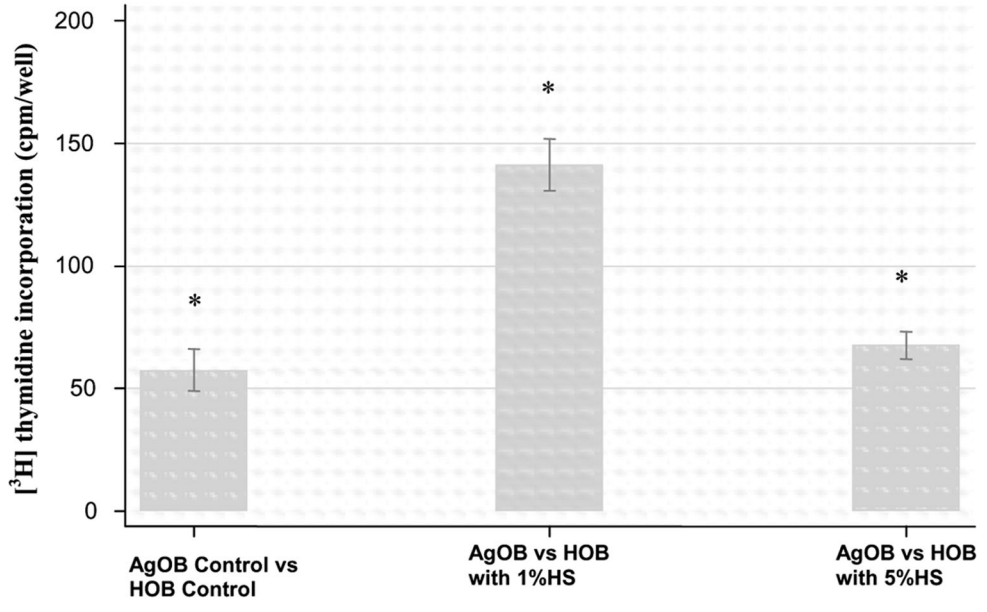

Figure 3. Comparison of the effect of 1% and 5% v/v PRP on DNA synthesis by HOB and AgOB. Proliferation was determined by [^{3}H] thymidine incorporation (cpm/well) at 48hrs. Results are represented as mean (95% Confidence Interval) % estimated relative change of [^{3}H] thymidine incorporation between HOB Control and AgOB Control, HOB and AgOB in the presence of 1% PRP and HOB and AgOB in the presence of 5% PRP. Statistical significance was determined at a 0.05 level ($p<0.05$).

Previous *in vitro* research studies investigating the effect of PRP on animal bone formation[16] showed that the viability and the proliferation of alveolar bone chips derived from dogs were suppressed by high PRP concentrations and stimulated by low concentrations (1-5% PRP) after a culture period of 7 days. Furthermore, the results of *in vitro* studies using human cell lines, revealed that PRP stimulates collagen synthesis by human PDL and osteoblastic MG63 cells[17]. Okuda et al. (2003)[7] treated osteoblast MG63, epithelial cells and rat osteoblast cells with PRP for 24hrs and found that DNA synthesis and cell division of osteoblastic cells were significantly increased but it was down-regulated the ALPase activity and proliferation of epithelial cells.

Other *in vitro* studies showed that PRP enhanced human osteoblast, human osteosarcoma cell and stromal cell proliferation in a dose-dependent manner. In particular, Lucarelli et al. (2003)[18] investigated the effect of PRP on stromal cell proliferation and differentiation and they proved that after 6 days of culture, 10% PRP promoted cell proliferation and cells expanded with 10% PRP and then exposed to dexamethasone can mineralize extracellular matrix. Ferreira et al. (2005)[19] evaluated the influence of various concentrations of PRP on human osteoblast growth and they concluded that 50% PRP added to growth medium showed the best proliferative results. Kanno et al. (2005)[20] used two different human osteosarcoma cell lines to assess the effect of PRP on cell proliferation and differentiation and they found that PRP increased dose-dependently the growth and ALPase activity of these cells suggesting that PRP could play a favorable role as an activator on bone regeneration and wound healing.

A recent systematic review was conducted to evaluate the efficacy and safety of PRP[21]. The authors concluded that in the treatment of severe form of chronic periodontitis, there is an improvement in the depth reduction of gingival recession and clinical attachment level. Furthermore, they noted that there are little data about PRP safety.

Moreover, PRP has also shown promising therapeutic outcomes when used in periodontal defects combined with bone allografts and guided tissue regeneration[22-24]. PRP combined with an anorganic bovine bone graft has been shown to promote maxillary sinus augmentation[25,26] and its topical application significantly increased bone regeneration at implants host sites during early healing in minipigs[27].

A limitation of this study is that we did not assess the levels of growth factors released by platelets. Data from previous studies indicate that PRP content in platelets is higher than blood and that increased levels of PDGF-AB and TGF-β1 and low levels of PDGF-BB and TGF-β2 were are detected in PRP[7,13]. In the present study, the Curasan method was used for the PRP preparation. There are two commercially available techniques for PRP preparation: Curasan and Platelet Concentrate Collection System (PCCS). Appel et al. (2002)[28], in an attempt to compare these two systems in terms of platelet concentrates for growth factor enrichment, found that the amount of growth factors correlated with the number of platelets within platelet concentrates and the platelet concentration could be increased between 11.7+/-2.4 times with Curasan method and 5.0+/-2.3 times with PCCS method.

Our data revealed statistically significant promotion of proliferative response of both healthy and periodontally affected cells in the presence of PRP. It might be suggested that PRP could be beneficial for periodontal regeneration in patients with severe periodontitis.

The research data demonstrate that PRP might serve as a source of growth factors that promote periodontal repair and regeneration by modulating cell response and activities. Considering that, PRP collection is simple, easy, and safe and its use does not involve any danger for the general health of the patient, PRP application could be a useful adjunct to the therapeutic approach of periodontal defects and dental implants.

Acknowledgements

The authors would like to express their thanks to Dr. Kletsas D. Laboratory for Enzyme Research, Institute of Biology, National Centre for Scientific Research (NCSR) 'Demokritos', Athens, 15310 Greece, for his technical support.

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