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PLATELET-RICH PLASMA AND FIBROBLASTS: HORMESIS EFFECTS

Andreea Tibeică[1], Norina Consuela Forna[2+], Silviu Cătălin Tibeică[+], Doriana Agop-Forna[3*], Marcel Costuleanu[4]

1"Grigore T. Popa" University of Medicine and Pharmacy - Iași, Romania, Faculty of Dental Medicine
[1]Ph. D. Student
2"Gr.T.Popa" U.M.Ph. - Iași, Romania, Faculty of Dentistry, Department of Implantology
3"Gr.T.Popa" U.M.Ph. - Iași, Romania, Faculty of Dentistry, Department of Oral Surgery
4"Grigore T. Popa" University of Medicine and Pharmacy - Iași, Romania
*Corresponding author: Doriana Agop-Forna - drdorianaforna@gmail.com

+ Author with the same contribution as the first author

ABSTRACT

Aim of the study The goal of our study was represented by the analysis of the effects of different concentrations of platelet-rich plasma extracts on the degree of senescence of dermal fibroblasts (by evaluating β-galactosidase) in the presence of metformin and rapamycin. **Material and methods** Rat dermal fibroblasts from storage were cultivated using conventional laboratory protocols and subsequently assessed for β-galactosidase expression. Then, we used PRP gel in a concentration of 1% and 5%, administered in the culture medium of fibroblasts for 5 cell passages. Moreover, the pharmacologic active substances we used for the various experiments and protocols were metformin (1 µM) and rapamycin (1 µM). **Results** The results obtained demonstrate that PRP 1% has the effect of transforming fibroblasts into younger cells, especially in the presence of metformin, a programmer of energy metabolism, and then rapamycin, an autophagy stimulator and mTOR pathway activator. It should be noted that a concentration of 5% PRP in the culture medium of fibroblasts for 5 passages has the opposite effect, of their biochemical aging. **Conclusions** The results obtained demonstrate that PRP 1% has the effect of transforming fibroblasts into younger cells, especially in the presence of metformin and then rapamycin. Moreover, the hormetic effects were demonstrated in the case of platelet-rich plasma concerning cultured fibroblasts.

Key words: platelet-rich plasma, fibroblast, hormesis, β-galactosidase, metformin, rapamycin

INTRODUCTION

Numerous researches are now centered on new progressions in the domain of bone grafting, wherein platelet-derived growth factor (PDGF) is recognized as a pivotal determinant in the process of bone regeneration. Some work provides an elucidation of the method by which PDGF may be utilized in surgical procedures involving bone grafting. This advancement holds promise for the future application of PDGF in facilitating accelerated bone regeneration or suppressing bone development, particularly in the context of osteosarcoma. The significant specific functions of platelet-derived growth factor (PDGF) encompass mitogenesis, which refers to the augmentation of healing cell populations, angiogenesis, which involves the transformation of endothelial cells into functional capillaries, and macrophage activation, which serves to debride the wound site and serve as a secondary source of growth factors for ongoing repair and bone regeneration. Therefore, PDGF can be employed in wounds including bone abnormalities to mask the wound while repairing the bones [1].

The prevailing method for managing bone abnormalities and persistent non-unions commonly entails the utilization of autogenous bone transplants. However, the

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acquisition of these grafts might present difficulties, and the surgery may result in substantial morbidity. Three main therapy approaches have demonstrated resistance to traditional therapies in the management of bone defects and non-unions. These approaches include the utilization of synthetic bone graft substitutes (BGS), a combination of BGS with bioactive compounds, and the integration of BGS with stem cells. Within the domain of synthetic bone growth substrates (BGS), a diverse array of biomaterials has surfaced, serving as scaffolds in the field of bone tissue engineering (TE). The aforementioned materials include biometals such as titanium, iron, magnesium, and zinc, together with bioceramics like hydroxyapatite (HA) and tricalcium phosphate (TCP). Bone tissue engineering (TE) scaffolds function as transient implants, facilitating the infiltration of tissue and the subsequent regeneration of fresh bone. These materials are carefully engineered to improve the process of bone mending by the optimization of geometric, mechanical, and biological characteristics. Continuous remodeling of these scaffolds is aided by bone cells such as osteoblasts and osteoclasts. Stem cells and bone cells collaborate through many signaling pathways to control bone regeneration in cases of bone injury or deformation. Bone TE may enhance bone deficiencies through efficacious therapy by specifically targeting signaling pathways. The present analysis offers valuable perspectives on the intricate relationship between cells and the contemporary status of bioceramics within the realm of bone regeneration [2].

An emerging area of medical practice known as regenerative dentistry encompasses the integration of stem cell technologies, tissue engineering, and dental science. The process utilizes biological processes to facilitate the regeneration of impaired oral tissues and reinstate their functional capabilities. The term "platelet-rich plasma" (PRP) refers to a biological substance that is characterized as the proportion of autologous blood plasma that contains a higher concentration of platelets compared to the original whole blood. Platelet granules contain a complex combination of essential cytokines and growth factors. The use of PRP has garnered significant interest within the field of regenerative medicine. The underlying justification for the use of PRP is its function as a biomaterial, facilitating the transportation of essential growth factors and cytokines from platelet granules to specific regions, hence facilitating tissue regeneration across diverse tissues. Researchers have recently started employing PRP therapy as an innovative approach to restore impaired tissues such as the liver, bone, cartilage, tendon, and dental pulp, owing to an improved comprehension of cell signaling and growth factor biology [3].

The wound healing process is frequently associated with hormetic dosage responses, which primarily involve the examination of cell survival, proliferation, migration, and collagen deposition in human and mouse fibroblasts through *in vitro* investigations. A diverse array of compounds, encompassing endogenous agents, pharmaceutical preparations, plant-derived extracts, and various dietary supplements, as well as physical stressor agents such as low-level laser treatments, were shown to elicit hormetic responses. Numerous comprehend-sive mechanistic investigations have successfully discovered prevalent signaling pathways and their inter-pathway connections that facilitate the hormetic dosage responses. The aforementioned findings serve to enhance and expand upon a comparable complete evaluation of the manifestation of hormetic dosage responses in keratinocytes. The results of some studies provide evidence

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for the widespread applicability of the hormetic dosage response in important wound healing outcomes. This suggests that the notion of hormesis plays a crucial role in wound healing, offering some valuable insights for both experimental assessment and therapeutic interventions [4].

The goal of our study was represented by the analysis of the effects of different concentrations of platelet-rich plasma extracts on the degree of senescence of dermal fibroblasts (by evaluating β-galactosidase) in the presence of metformin and rapamycin.

MATERIAL AND METHODS

Rat dermal fibroblasts from storage were cultivated using conventional laboratory protocols and subsequently assessed for β-galactosidase expression using known flow cytometry methods, as demonstrated in prior work [5].

The platelet-rich plasma gel was obtained as previously described [6]. The venous blood of 7 patients was obtained and subsequently centrifuged. Following this, 2 ml of conventional platelet-rich plasma was combined with 60 µl of a sterile 10% calcium chloride solution, containing a citrate inhibitor. The mixture was then left to coagulate. Next, the platelet-derived thrombin was released through squeezing and then we collected the plasma that is rich in such factors. Subsequently, autologous thrombin-rich plasma was combined with conventional platelet-rich plasma at a ratio of 1:4 and left to undergo coagulation. PRP gel is formed within a time frame of 5-10 minutes.

For hormetic effects, we used PRP gel in a concentration of 1% and 5%, administered in the culture medium of fibroblasts (in sterile conditions) for 5 cell passages.

Moreover, the pharmacologic active substances we used for the various experiments and protocols were metformin (1 µM) and rapamycin (1 µM). The gathered speculative data underwent statistical refinement utilizing many well-recognized and enhanced procedures often employed in medical research. One such way is the One WAY ANOVA test, which was supplemented where needed with the conventional Student-Newman-Keuls test.

All the experiments were approved by the Ethics Committee of the University of Medicine and Pharmacy Grigore T. Popa from Iaşi.

RESULTS AND DISCUSSIONS

In the presence of 1% PRP in the culture medium of rat fibroblasts, β-galactosidase was approximately 35% (on average) lower, as compared to the absence of PRP (value considered to be 100%), as well as being equal to a small "rejuvenation" (Fig. 1).

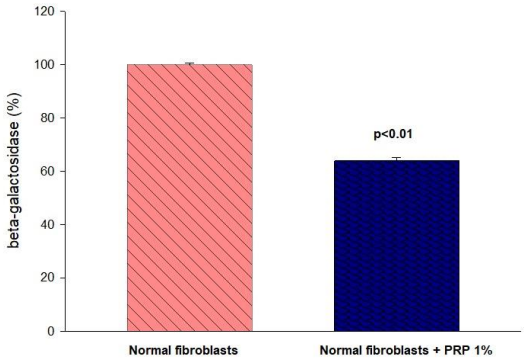

Figure 1. Flow cytometry (n=7) demonstrates reduced β-galactosidase values for fibroblasts in the presence of PRP 1% for 5 passages

Furthermore, we can find that PRP 5% in the culture medium of fibroblasts induces an increase of approximately 18% (on average) in β-galactosidase, as compared to control fibroblasts, which demonstrates a slight "aging" process of the treated cells, after 5 passages (Fig. 2). So, it might be evident that there exists a difference of approximatively 44,90% (on average) between the concentrations of 1% and 5% PRP on fibroblasts cultures.

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On the other hand, the association of metformin 1 µM with PRP 1% induced a β-galactosidase reduction effect of approximately 48% (on average), compared to fibroblasts treated with PRP 1% alone. Thus, an additional effect of reducing β-galactosidase of approximately 20% (on average) is demonstrated in the presence of metformin, a programmer of energy metabolism (Fig. 3).

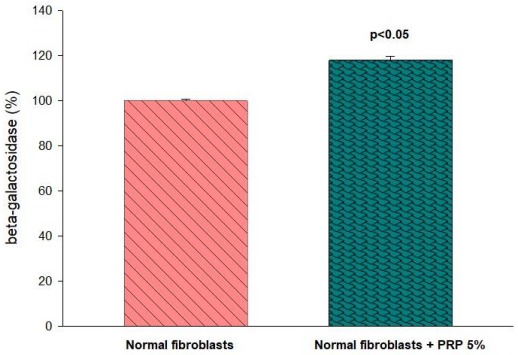

Figure 2. Increased β-galactosidase values for fibroblasts in the presence of PRP 5% for 5 passages

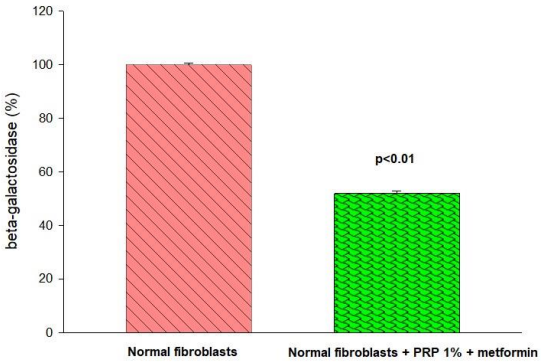

Figure 3. Decreased β-galactosidase values for fibroblasts in the presence of metformin 1 µM and PRP 1% for 5 passages

When speaking about the addition of rapamycin 1 µM on PRP 1%, there exists an induced β-galactosidase decrease effect of approximately 40% (on average), compared to fibroblasts treated with PRP 1% alone. Thus, there is an additional effect of reducing β-galactosidase of approximately 8% (on average) in the presence of rapamycin, an autophagy stimulator and mTOR pathway activator (Fig. 4). But these effects do not statistically significantly differ from those of metformin, which are somewhat stronger.

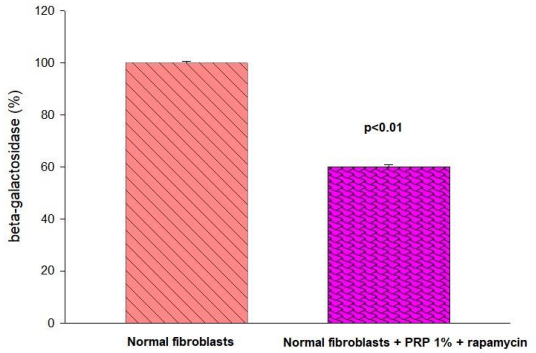

Figure 4. Decreased β-galactosidase values for fibroblasts in the presence of rapamycin 1 µM and PRP 1% for 5 passages

The results obtained demonstrate that PRP 1% has the effect of transforming fibroblasts into younger cells, especially in the presence of metformin and then rapamycin. It should be noted that a concentration of 5% PRP in the culture medium of fibroblasts for 5 passages has the opposite effect, of their biochemical aging. Thus, the hormetic effect of PRP can be described in this case.

Platelet-rich plasma has emerged as a well-recognized and versatile method for wound healing, exhibiting a broad spectrum of uses. Although there is significant variation in the composition of platelet-rich plasma products used for specific wound healing purposes, it is widely acknowledged that these diverse mixtures of platelet-rich plasma complexes consistently exhibit biphasic concentrations that resemble normal blood levels. These concentrations are not influenced by the type of tissue being treated or the specific endpoints being measured. The platelet-rich plasma region is distinctive due to its utilization of the hormetic idea in addressing the challenges posed by intricate biological combinations [7].

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Some study presents an evaluation of the hormetic dosage responses exhibited by stem cells of the apical papilla derived from human subjects. In vitro, a diverse array of agents, such as dietary supplements (e.g., berberine, EGCG, resveratrol), pharmaceutical/comercial substances (e.g., fluoride, platelet-rich plasma, lithium), and endogenous agents (e.g., insulin growth factor-2, transforming growth factor β, lipopolysaccharide), were found to induce hormetic dose responses. This work aims to elucidate the fundamental molecular principles that underlie the responsiveness of stem cells of the apical papilla hormetic doses, emphasize the necessity of conducting in vivo assessment studies, and explore the possible therapeutic implications of the current findings. Notably, almost 90% of recorded instances of hormetic effects with stem cells of the apical papilla have taken place during the last two years, indicating a significant recent surge in interest regarding their possible therapeutic uses [8].

Numerous pharmaceutical substances, including hazardous agents, as well as radiation therapy, have been documented to elicit bi-phasic hormetic effects on cultured cells. However, these effects are observed exclusively when the dose and duration of treatment are optimized. Prior research has predominantly utilized various laser modalities, except CO_2 laser, and has not yet conducted a comparative analysis of the hormetic response between normal and malignant cells. In this study, we examined the potential of CO_2 laser therapy to elicit hormesis in human gingival fibroblast (HGF) and oral squamous cell carcinoma (HSC-2) cells. Cell growth was enhanced by CO_2 laser irradiation at low power levels, whereas it was inhibited at high power levels. Out of the three dispatch modalities, super pulse (SP)2 had the most impact in stimulating growth in HGF, even at a somewhat lower irradiation dosage compared to the level that caused cytotoxicity. The cytotoxicity of higher irradiation dosages was shown to be comparable across normal (HGF) and tumor (HSC-2) cells, with a plateau of cytotoxicity seen within 24 hours. Given the limited and minimal range and size of the hormetic response in HGF cells, it is imperative to determine the ideal circumstances for inducing hormesis to apply it clinically in dentistry [9].

Activated platelets secrete a concentrated solution of growth factors that play a crucial role in the process of wound healing. An effective method for administering activated platelets to wounds involves the utilization of platelet-rich plasma obtained through the centrifugation of the patient's venous blood. This process involves activating the platelets using collagen or calcium chloride, as well as autologous thrombin. Subsequently, the supernatant, known as platelet-poor plasma (PPP), is carefully extracted. Platelet-rich plasma (PRP) is commonly administered either injection into the lesion or topically, followed by the application of a moisture-retentive dressing to seal the wound within or over it. PRP, frequently administered alongside platelet-rich plasma (PPP), has been utilized in the treatment of chronic and acute wounds at varying intervals, depths, and frequencies. Different dosages and carriers of PRP have been employed, with diverse outcomes. Multiple meta-analytic studies have indicated that platelet-rich plasma (PRP) therapy enhances the healing process of open diabetic foot ulcers and venous ulcers. Additionally, PRP has the potential to decrease pain and the occurrence of surgical site infections (SSIs) in both open and closed acute surgical wounds. Nevertheless, the lack of uniformity in research methodologies and outcome assessments hindered the

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consistency of pain and SSI findings. The addition of 1 intraoperative dose of PRP at the surgical site before sealing elective foot and ankle surgery incisions did not have a consistent influence on healing or deep SSI rates in 250 patients, compared to 250 identical patients who received the same procedure without PRP. The optimal parameters for delivering and using PRP on different types of wounds, to enhance SSI, acute wound pain, and healing results, have yet to be determined after extensive study spanning many decades [10].

CONCLUSIONS

The goal of our study was represented by the analysis of the effects of different concentrations of platelet-rich plasma extracts on the degree of senescence of dermal fibroblasts (by evaluating β-galactosidase) in the presence of metformin and rapamycin.

The results obtained demonstrate that PRP 1% has the effect of transforming fibroblasts into younger cells, especially in the presence of metformin and then rapamycin.

It should be noted that a concentration of 5% PRP in the culture medium of fibroblasts for 5 passages has the opposite effect, of their biochemical aging. Thus, the hormetic effect of PRP can be described in this case.

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