Effects of platelet rich plasma on fascial healing in rats with fecal peritonitis[1]

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ABSTRACT

PURPOSE: To evaluate the effects of platelet rich plasma (PRP) on the healing of fascia wherein peritonitis has been created.

METHODS: Twenty eight Wistar Albino rats were divided into four groups. Only a primary fascial repair following laparotomy was performed on Group 1, a primary fascial repair performed and PRP treatment applied following laparotomy on Group 2, and a fecal peritonitis created following laparotomy and a primary fascial repair carried out on Group 3. A fecal peritonitis was created following laparotomy and primary fascial repair and PRP treatment on the fascia was carried out on Group 4.

RESULTS: TNF-α was found to be significantly lower in the control group (Group 1). It was detected at the highest level in the group in which fecal peritonitis was created and PRP applied (Group 4). TGF-β was determined as being significantly higher only in Group 4. Histopathologically, the differences between the groups in terms of cell infiltration and collagen deposition were not found to be significant.

CONCLUSION: When platelet rich plasma was given histologically and biochemicaly as wound healing parameters cellular infiltration, collagen accumulation, and tissue hydroxyiproline levels were not increased but neovascularization, fibroblast activation and TNF Alfa levels were increased and PRP accelerated wound healing.

Key words: Platelet-Rich Plasma. Peritonitis. Wound Healing. Rats.

Introduction

Wound healing is one of the oldest issues in the literature. Ventral hernia remains one of the most common surgical problems in the USA today, approximately 350,000 cases[1]. Greater problem is the 30–60 % recurrence rate after hernia repair[2]. Despite advance research and prosthesis materials in repair strategies, techniques, and technologies, recurrent herniation continues to be a major issue on patients and the healthcare system[3].The biggest reason is that they have complications and are the disadvantages of this prosthesis material[4]. Some of these are mesh infection, contraction, erosion, extrusion, and fistula formation. Therefore the rate of hernia formation after laparotomy remains high[5]. Biologic prostheses such as porcine or human acellular dermal matrices are reviled for their high mesh failure rates due to unsuccessful tissue incorporation or enzymatic- immunologic reaction[6].

Autologous Platelet-rich plasma (PRP), recently, has become popular as a treatment modality in the areas of orthopedic and trauma surgery, spinal surgery, plastic-reconstructive surgery, oral and maxillofacial- dental surgery, ophthalmological surgery, heart by-pass surgery and burns[7-9]. It contains the growth factors; platelet-derived growth factor (PDGF), epidermal growth factor,fibroblast growth factor(FGF), transforming growth factor–β(TGF-β), insulin-like growth factor (IGF-I, IGF-II), endothelial cell growth factor and vascular endothelial growth factor (VEGF). Besides, it includes bioactivefactors or non-growth factors which normal wound healingagents; serotonin, histamine, dopamine, calcium, adenosine, fibronectin, fibrin, and vitronectin[7]. They have been found to expedite epidermal, epithelial, and endothelialregeneration, aggravating angiogenesis, stimulate soft tissue healing, enhance the hemostatic response, decrease dermal scarring, increase collagen synthesis and assist cell migration[10].

In this study, we aimed a unique experimental technique on rats. After performing fecal peritonitis, PRP was applied locally to see effects on fascia healing with the consideration of positive effect on wound healing.

Methods

Our study was conducted at Fırat University, Faculty of Medicine, Laboratory Animal Breeding and Experimental Research Center with permission No. 09 dated 01.10.2012 from the Firat University Rectorate, Laboratory Animals Ethics Board Chair.

In order to reduce genetic differences, twenty eight 5 months-old Wistar Albino rats, ranged 223-264g in weight were obtained from the same animal laboratory to be used in the experiments. The animals were fed with standard laboratory feed and tap water and kept in appropriate cages at 22±2°C temperature where a 12 hour light/12 hour dark environment was provided.

The rats were divided randomly into four groups (n=7 in each group). Only a primary fascial repair following laparotomy was performed on Group 1 (Control Group), a primary fascial repair following laparotomy and PRP treatment on the fascia was carried out in Group 2 (PRP group), and a fecal peritonitis was created following laparotomy and primary fascial repair was implemented in Group 3 (Fecal peritonitis group). A fecal peritonitis was created following laparotomy and primary fascial repair implementation and PRP applied on the fascia in Group 4 (Fecal peritonitis + PRP group). All groups were taken to a room allocated to them, placed into cages and monitored under expert veterinary supervision.

Preparation of platelet rich plasma

1.5 ml of blood drawn from the renal level of the inferior vena cava was taken from each rat for use in themselves, put into EDTA containing tubes and subjected to centrifugation at 5600 rpm (Figure 1).At the end of the process, the blood was divided into three layers, namely a platelet poor plasma, erythrocytes and a yellowish portion containing platelets. The platelet poor plasma part was discarded and the remaining part was centrifuged again at 2400 rpm. At the end of this process, the erythrocytes was separated, yielding PRP.

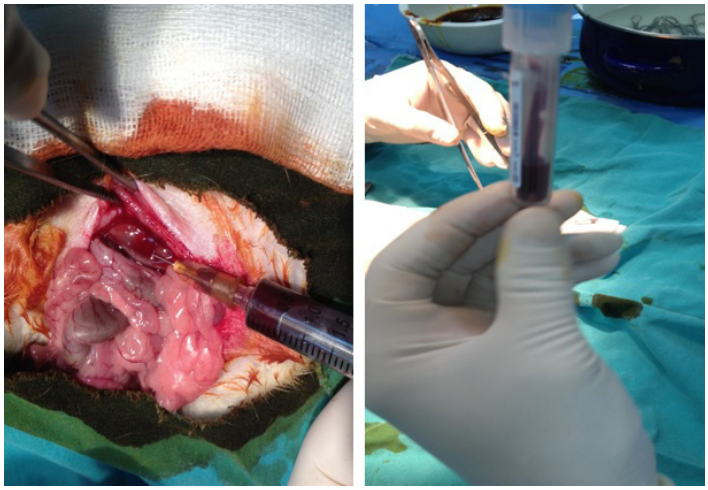

FIGURE 1 - Blood drawn from the renal level of the inferior vena kava was taken, put into EDTA containing tubes.

Surgical procedure

Ketamine hydrochloride 50 mg/kg, Xylazine hydrochloride 5 mg/kg was delivered intramuscularly, achieving appropriate depth of anesthesia and the abdominal wall was shaved, cleansing it with 10% Iodine povidone solution and covered with a sterile drape. With a 4 cm abdominal midline incision on the skin, subcutaneous tissues and the fascia was incised, opening the abdomen. After drawing blood for the PRP, the fascia was closed with a 3-0 polyglactin suture and the skin with a 3-0 silk suture in continuation (Figure 2). After 1 week, a total of 5 ml blood was drawn from each of the rats who were decapitated under anesthesia in order to study the biochemical parameters. The skin incision was opened and the fascia incision line excised, including 2 cm of healthy tissue, with the purpose of histopathological examination and investigation of the tissue hydroxyproline level.

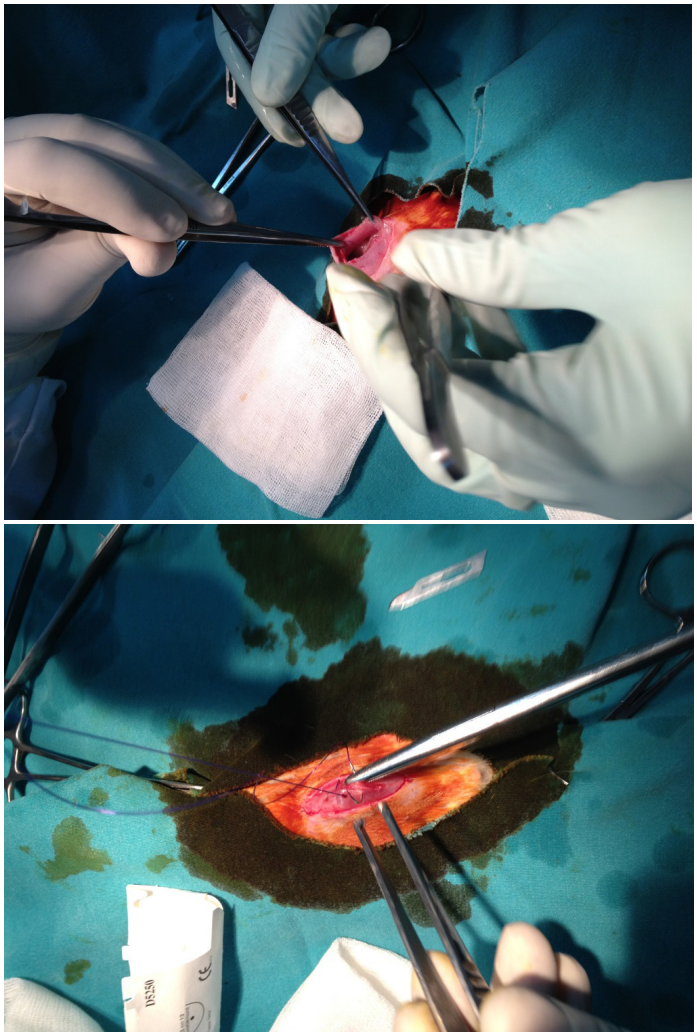

FIGURE 2 - The fascia was closed with a polyglactin suture.

Biochemical examination

TNF-α (Cat No: CK-E30635) and TGF-β levels (Catalogue No: CK-E30636) were measured by the using EastBioPharm(China) ELISA kits. (Cat No: CK-E30191) was used for tissue hydroxyproline levels (Figure 3).

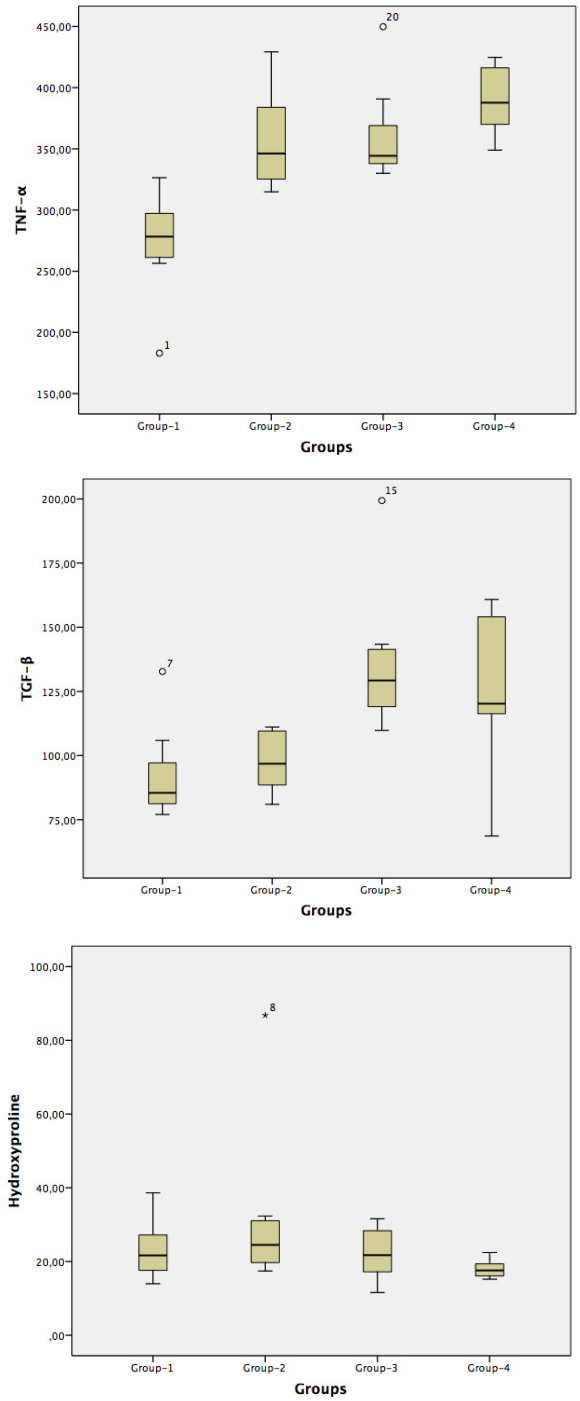

FİGURE 3 - Comparison of TNF-α, TGF-β and Hydroxyproline levels between groups.
TNF-α (pg/mL*), tumor necrosis factor alpha;* TGF-β (ng/mL), *transforming growth factor beta; Hydroxyproline* (µg/g).

Histopathological examination

At the end of the study, tissue samples covering the central area and wound edges of rats were obtained, and fixed in 10% neutral buffered formalin solution. The samples were routinely processed, cut about 5 µm thick, and stained with hematoxylin-Eosin (H-E) and Crossman's trichrome staining methods. Histological changes in the wound areas such as inflammatory cell infiltration, neovascularization, fibroblast activation were scored as follows: 1-no change, 2-mild, 3- moderate, 4- severe. Collagen deposition was classified as: 1- no evidence, 2- light scattering, 3 -abundant fibres, 4- confluent fibres. The rate of the healing process was determined statistically by considering the scores of the inflammatory cell infiltration as negative value, and the scores of neovascularization, fibroblast activation and collagen deposition as positive values.

Statistical analysis was conducted using the SPSS (v.16) software. The Kruskal-Wallis Variance analysis was used in comparing the groups, while the Mann Whitney U test was used in the paired comparison of the data for which the difference was significant according to this test ($p<0.05$ was considered statistically significant).

Results

One rat in the Group 4 was excluded from the study because it died. TNF-**α and** TGF-β levels were measured from the blood drawn on the 7th day of the study. Biopsy specimens taken from the incision wounds were subjected to biochemical examination and their hydroxyproline amounts determined. The resulting data are shown in the table (Table 1). TNF-α levels turned out to be significantly lower in the control group (Group 1) than the other groups ($p<0.001$). The highest levels were detected in the group which fecal peritonitis was created and PRP applied (Group 4). TGF-β levels were detected significantly higher only in Group 4 ($p<0.001$). Although hydroxyproline levels were numerically the highest in Group 2, the differences between them were not observed to be significant ($p<0.05$).

TABLE 1 - Comparison of biochemical and histochemical parameters between groups.

Parameters/Groups	Group-1	Group-2	Group-3	Group-4	p
TNF-α	272.10±45.77	358.41±42.03	362.60±43.36	389.23±28.70	<0.001
TGF-β	93.13±19.86	97.85±12.65	123.37±32.92	137.04±30.17	<0.001
Hydroxyproline	23.41±8.59	32.90±24.41	22.29±7.49	18.04±2.61	0.186
Inflammatory cell infiltration	2.29±1.254	2.00±1.00	3.29±0.488	2.86±0.900	0.427
Neovascularization	2.57±0.535	2.71±0.488	1.86±0.378	2.57±0.535	<0.05
Fibroblast activation	2.71±0.488	3.29±0.488	2.29±0.488	2.71±0.488	<0.05
Collagen accumulation	2.43±0.535	2.86±0.378	2.29±0.488	2.57±0.535	0.523

TNF-α (pg/mL), *tumor necrosis factor alpha;* TGF-β (ng/mL), *transforming growth factor beta; Hydroxyproline* (µg/g).

Histopathological evaluation revealed that inflammatory cell infiltration was high in Group 3 compared to the other groups. Although, PRP decreased the inflammatory cell infiltration in Group 2 compared to Group 1, and in Group 4 compared to Group 3, the difference was not statistically significant. Similarly, PRP increased the neovascularization, fibroblast activation and collagen deposition, but the difference was not significant statistically for collagen.

Discussion

PRP was used for the first time in 1987 by Ferrari *et al*.[11] with the purpose of reducing the transfusion of homologous blood products following open heart surgeries. Today, in the field of general surgery, its benefits have been shown in pathologies such as perianal fistulas, hidradenitissuppurativa, liver injury, anastomotic leak and acute abdomen[7,12]. The conviction in literature with respect to PRP, in general, is of the same leaning. The reason for the small number of negative outcomes may be the equipment used, the protocol used to activate the platelet gel, specific characteristics of the patient and the lesion, the platelet concentration used, the way of applying-obtaining it and different storage times[13].

There are a small number of PRP extraction and collection systems that are approved by the World Health Organization (WHO). Smart Pre P (Harvest Technologies Corp., Norwell, MA) and Platelet Concentrating Collection Systems (3 /Implant Innovations, Palm Beach Gardens, FL.), Sorin Angel, Arteriocyte Magellan (Medtronic, Minneapolis, MN), Biomet GPSII, Depuy Symphony. These systems work to achieve between 2-8 fold increased concentration of platelets[14,15]. PRP may also be obtained using a standard laboratory centrifuge device. But two spins and

more than one transfer process are needed in this procedure and in the end it might be difficult to preserve the sterility. Additionally, it might pose a problem in terms of the platelets it contains and the key protein amounts contained within them[16]. We were led to obtain PRP using the laboratory centrifuge technique in our study both in terms of cost and also against the possibility of not being able to draw adequate amounts of blood due to the fact that it is a laboratory animal study. PRP obtained in this way is of more limited effectiveness compared to that obtained with special devices by drawing about 50-60 cc of blood. A consensus was reached that the platelet concentration required in order for PRP to achieve tissue healing should be 1,000,000 /Ml [17]. In the analysis conducted in our study, the average platelet concentration was measured as 1,000,000 /mL. There are authors who claim that higher concentrations adversely affect wound healing[18].

Despite medical advances today, in cases with peritonitis - particularly fecal peritonitis- the risk of infections that might develop after closing up the abdomen and subsequent increased incisional hernia is still a major cause of morbidity, especially in emergency surgical procedures[19]. Incisional hernias lead to significant loss of labor, morbidity and affect the quality of life negatively. The only treatment option is surgery. Fascia healing is based on the same principle as wound healing and is closely related to tissue regeneration[20]. The tissue level of hydroxyproline one of the main ingredients of collagen and a good indicator of wound healing objectively reflects the amount of collagen synthesis in the wound. In our study, it was determined that the hydroxyproline level increased in Group 2 specimens (treated with PRP), demonstrating the positive contribution of PRP to wound healing. Heffner *et al*.[21] went one step further, determining that PRP has a positive contribution to myocyte degeneration, collagen organization and fascia tensile strength when used together with collagen products. PRP is said by some authors to cause cutaneous wounds in particular to heal more quickly due to its positive contributions in the dermal matrix stimulation, revascularization and proliferation phases[22,23]. There are also authors who claim that it contributes to wound healing by increasing direct fibroblast proliferation, leukocyte migration and angiogenesis[24]. Histological findings in this study indicated a useful effect on wound healing of PRP by increasing neovascularization, fibroblast activation and collagen deposition. PRP also increased the level of hydroxyproline, which is a positive value for wound healing. From this, we can infer that PRP shows its effect with a complex mechanism, especially in cutaneous wound healing.

In the study conducted by Van Eps *et al*.[6], based on the Modified Hopkins Adhesion Score, a significantly higher adhesion score was obtained after six months in the group which received facial repair with PRP compared to the group which did not (1.63± 0.92 *vs*. 2.75± 0.70). Also, in this study, it was determined that more recurrence developed after 6 months in the group that was not treated with PRP. In terms of incisional hernia recurrence rates in experimental work, since it has not been recommended much due to working disadvantages (the disproportion of the incision length to the size of the rat, difficult surgical technique), there are very few publications on this subject and these studies are found more among human studies[25,26].

When, by measuring TNF-α and TGF-β, we looked at in what way PRP affects wound healing with this mechanism of action, TNF-α levels were found to be significantly higher in all groups compared to the control group ($p<0.001$). It was found to be highest in the group wherein fecal peritonitis was created and PRP applied. TGF-β levels were detected higher in the PRP treated groups than the control group. These findings in consistence with literature that PRP increases growth factors and through this mechanism speeds up wound healing[27,28]. In many studies that have been carried out, concentrated growth factors have been found to speed up wound healing at a rate of 30-40%[29,30].

As a result, it might be said that the absence of harmful side effects, the fact that it doesn't shape a widespread scar tissue, doesn't cause malignant transformations, is easily available and can be obtained in a less costly way makes it an alternative - supportive treatment. Several studies in the literature are unanimous with respect to our study. However, further studies are needed to show the protective role of PRP in patients who carry risk factors for incisional hernia (such as obesity, immunosuppressive therapy, diabetes) is investigated.

Conclusion

When platelet rich plasma was given histologically and biochemicaly as wound healing parameters cellular infiltration, collagen accumulation, and tissue hydroxyiproline levels were not increased but neovascularization, fibroblast activation and TNF Alfa levels were increased and PRP accelerated wound healing.

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