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Use of platelet-rich plasma in deep second- and third-degree burns

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ABSTRACT

Unfortunately burns are a common occurrence, leading to scarring or death. Platelet-rich plasma (PRP) contains many growth factors that can accelerate wound healing. We analyzed the use of PRP in deep second-degree (dSD), deep second-degree associated with diabetes mellitus (dSDD), and third-degree (TD) burns in rats. Sixty syngeneic rats divided into three groups (dSD, dSDD, and TD) were burned, half receiving topical PRP and half being used as control; 10 additional rats per group were used for PRP preparation. On day 21, the animals were sacrificed and skin biopsies were collected. dSD and dSDD wounds treated with PRP showed faster wound closure, reduction in CD31-, CD68-, CD163-, MPO-, and in TGF-β-positive cells, and an increase in MMP2-positive cells. The neo-epidermis was thinner in the control of both the dSD and dSDD groups and granulation tissue was less reduced in the control of both the dSDD and TD groups. These results indicate that PRP can accelerate the healing process in dSD and dSDD, but not in TD burns.

1. Introduction

Burns have a high prevalence in modern life [1]. In the United States, 450,000 patients receive medical treatment related to burns every year, with up to 40,000 hospital admissions and 3500 deaths [2].

Initial studies concerning burns were focused on improving the overall survival rate. The understanding of the physiopathology of burns has led to enhanced resuscitation techniques with drastic reduction in the number of deaths. Recently, the focus of investigation shifted towards minimizing morbidity and improving the quality of life of the patient. Some secondary complications can be avoided by shortening the time needed for wound closure [3].

Platelet-rich plasma (PRP) has been used to accelerate healing [4]. PRP is a concentrate of autologous platelets in a small volume of plasma that is obtained by sequestering and concentrating freshly drawn blood. The platelet count should ideally be 4–5 times the baseline count of whole blood [5]. Degranulation of platelet granules, which contain growth factors, induces cellular proliferation, mitosis, chemotaxis, matrix formation, collagen synthesis, and angiogenesis are essential in the healing process [5].

Diabetes mellitus (DM) affects all phases of healing, leading to delayed closure and poor esthetic and functional scarring

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[6,7]. Although skin biopsies of rats with DM exhibit a drastic reduction in growth factors, with PRP applications the healing pattern of burns is similar to that of normal rats [8].

The purpose of this experimental study was to analyze the potential of PRP to accelerate tissue healing in deep second-degree (dSD) burns, deep second-degree burns associated with diabetes mellitus (dSDD), and third-degree (TD) burns in rats.

2. Materials and methods

2.1. Animals

The study was approved by the Ethics Committee on Animal Research of the Biology Institute Roberto Alcantara Gomes, Rio de Janeiro State University–Brazil (protocol number CEUA056/2012). All procedures rigorously followed current guidelines for animal experimentation [9].

Ninety young male Wistar rats (275–300 g) were randomly assigned to three groups of 30 animals each, respectively submitted to dSD, dSDD, and TD. All groups were subdivided into control ($n = 10$) and PRP-treated ($n = 10$) animals, with the 10 remaining animals being used for PRP preparation.

The animals had unrestricted access to food and water, were housed in appropriate cages under controlled temperature and humidity, and were kept on a 12-hour light/dark cycle.

2.2. PRP preparation

PRP was prepared as previously described [10]. Briefly, the rats were anesthetized with an intramuscular injection of 80 mg/kg ketamine (Agener União; Embu-Guaçu, Brazil) and 12 mg/kg xylazine (Agener União; Embu-Guaçu, Brazil). Blood (6.5 ml) was collected by cardiac puncture into a syringe containing 0.7 ml of 10% sodium citrate. Blood was then centrifuged at 160 *g* for 20 minutes. A red lower fraction (red cell component) and an upper straw-yellow turbid fraction (serum component) were obtained, with an intermediate layer between them, i.e. the buffy coat that contained the platelets and represented about 5% of the volume of the tube. A point was marked at 2 mm below the line dividing the two fractions. All the content above this point was pipetted and transferred to another tube, in which a line corresponding to 0.72 ml (10% of the total original volume of whole blood + sodium citrate) was drawn from the tube's bottom. The sample was then submitted to a new centrifugation at 400 *g* for 15 minutes, resulting in two components: one above the line drawn on the tube–platelet-poor plasma (PPP), and the other below the line, platelet-rich plasma (PRP). PPP was pipetted and discarded, leaving only PRP. Next, the PRP fraction was activated with 0.05 ml of 10% calcium chloride solution per 1 ml of PRP.

2.3. Platelet count

To confirm if PRP was adequately prepared, both whole blood and prepared PRP were subjected to platelet count using a hematology analyzer (CELL-DYN Ruby; Abbott Diagnostics, São Paulo, Brazil).

2.4. Induction of diabetes mellitus

Seven days before the burn, the animals in the dSDD group received a single intraperitoneal injection of streptozotocin (45 mg/kg body weight in 0.1 M citrate buffer, pH 4.5) (Sigma, St. Louis, MO) after a 12-h fast. Seven days later, blood glucose was measured (Accu-Check Advantage II; Roche Diagnostics, Mannheim, Germany) and DM status was defined as blood glucose levels higher than 300 mg/dl.

2.5. Burn procedure

Two days prior to creation of the burn, the animal's dorsum was shaved with electric clippers and commercial depilatory cream (VEET$^{(TM)}$–Rickitt Benckiser; Cali, Colombia). Animals were anesthetized as described previously and the entire procedure was performed under aseptic conditions. A burn was created as described previously [11]. Briefly, the animal was laid on its side with a 2-cm high Styrofoam board adjacent to it. The skin was stretched and fixed with four hypodermic needles (26 G) so that the head of the burn apparatus would not only fit completely but would also permit a complete and perpendicular contact of the weighted apparatus–1 kg of lead (Fig. 1).

The head of a high power commercial soldering iron (Weller 80 Watts–Cooper Hand Tools; Sorocaba, Brazil) was removed and replaced with a cylindrical aluminum head 23 mm in diameter. We have published a previous paper determining that temperatures of 70 °C and 80 °C should be used to obtain the expected deep second- and third-degree burns, respectively [11].

Using Meeh's formula ($A = 10 \times W^{2/3}$, where: A = area in cm^2, 10 is a constant and W = weight in grams) to calculate the surface area of the animal, the burn represented about 1% of the total surface area [12].

In the PRP-treated subgroup, the PRP preparation was activated after the burn was completed, and ten minutes later, the gel was formed and applied to the burn. To maintain the PRP in place, a round clear plastic cover 27 mm in diameter was sutured over the burn wound and secured in place with occlusive dressing in order to prevent removal by the animals. The same plastic disk and dressing were applied to the control group.

2.6. Macroscopic analyses

To evaluate wound closure, a transparent plastic sheet was placed over the wound and its margins were traced. After

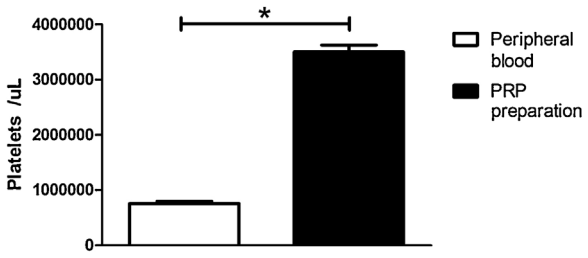

Fig. 1 – Platelet Count—increase of 465% in platelet count in PRP when compared to peripheral blood. *P < 0.005.