Effect of Platelet-rich Plasma on Acute Dermonecrotic Injuries Caused by The Venom of LoxoscelesIntermedia

Running Title: Platelet-rich plasma on dermatologic injuries.

Camila Maria Ribeiro PachecoI, Ana Isabel Bernuci Gouveia Horbux do AmaralI, Ivo IlvanKerppersII, Thais Barbosa de OliveiraII, Larissa GulogurskiRibeiroII, Marcos Paulo PoloweiRolãoII, Andressa PanegalliHosniII, Andressa Leticia MiriII

University Campo Real, Brazil
University Estadual do Centro-Oeste

Corresponding author
Ivo IlvanKerppers
Neuroanatomy and Neurophysiology Laboratory.
Simão Varela de Sá St. # 03, Guarapuava, Paraná; Brazil. zipcode: 85040-080;
Fax: + 55 042 36298155
Email: labneurosubmissoes@outlook.com

ABSTRACT: Loxoscelism is a term used to describe cases of accident with spiders of Loxosceles gender. The injury caused by the spider bite varies from 1 to 30 cm of diameter, with a red halo covering the circumference and a pale area, which is denominated marmorata plate. Platelets have properties related to adhesion, platelet aggregation, release of growth factors, which induce angiogenesis, and promote vascular growth and proliferation of fibroblasts. Therefore, platelet-rich plasma is a product able to potentialize the grafts integration, as well wounds healing. The present research has the intention of studying the PRP action in dermal-necrosis induced by Loxoscelesintermedia venom. In this research three New Zealander albino rabbits were used. Four areas were marked to a posterior intradermal injection. In one of the areas saline (PBS) was injected as a negative control, in the other three areas an aliquot of 10 mg of crude venom diluted in saline was injected. One of these areas was used as a control, in the other one 150 µL of PRP was applied and in the third one, 300 µL of the same substance was injected, being the respective doses applied again in each of the injuries. When analyzing the results, it was shown that the PRP is able to soften the superficial symptoms like bruising, redness and swelling in dermal-necrotic lesions caused by the poison in the acute phase. Obtaining an accelerated process of wound healing.

Keywords: platelet-rich plasma; Loxoscelesintermedia; dermo-necrosis.

INTRODUCTION

Cases of arachnid accidents are currently very common and can be considered a public health problem in Brazil. In 2008, 20,996 cases were recorded, 38% of which were caused by spiders of the Loxosceles genus. A further 14.1% were associated with the Phoneutriagenus and 0.5% were caused by Latrodectus[1]. Loxoscelism is the term used to describe accidents associated with spiders of the Loxosceles genus. This type of accident is more common in the south of Brazil, particularly in the state of Paraná,

which has reported significant increases in recent years[2]. Most cases occur between November and March, which is the hottest period of the year in Brazil[3].

Spiders of the species Loxoscelesintermediahave a small body size, ranging from 1 cm to 5 cm, a reddish-brown color and a diet composed of smaller insects[4,5]. They are generally docile and only sting when they feel threatened. Most cases of spider bites occur when a person presses the spider against their body while dressing or sleeping[6]. The severity of a spider-related accident depends on the species involved, the gender of the spider, the quantity of venom injected, the location of the bite and the person´s sensitivity to the venom, which is usually greater the younger the person is[1].

The venom of the Loxoscelesintermediaspider has a crystalline aspect and contains proteins, nucleic acids, free amino acids, monoamines, neurotoxic polyamines and inorganic salts[7]. Its harmful activities are believed to be related to the presence of proteolytic toxins, which characteristically degrade proteoglycans, fibronectin and fibrinogen[8].

Other related toxins are known as sphingomyelinases, which trigger lysis among human erythrocytes and platelets in a manner that depends on catalytic activity[9,10]. Loxolisine A is associated with the necrotic and hemorrhagic effects caused by poisoning. The venom acts on vascular endothelial cells, which trigger cascades of the complement system, platelet aggregation and intense inflammatory activity in the bite area. Consequently, small vessels are obstructed, leading to edema, hemorrhage and focal necrosis[11].

Loxoscelism can exhibit two clinical forms: cutaneous and cutaneous-visceral. The cutaneous-visceral form (minority of cases) involves cutaneous impairment, anemia, jaundice and hemoglobinuria, and may even lead to acute kidney failure and death[12]. The cutaneous form (majority of cases) involves a slow and progressive development, characterized by the appearance of a wound at the bite area between 24 and 72 hours after the accident. The bite is initially painless, but after 2 to 6 hours, pain and other associated symptoms such as edema, erythema, swelling, itchiness and sensitivity may occur in the area[2].

The wound can range from 1 cm to 30 cm in diameter, with a red circle around the circumference and a pale zone, which is known as the marble plate. The following phenomena have been recorded at this stage: the accumulation of polymorphonuclear leukocytes; the formation of an abscess in the area of the wound; thickening of the endothelium of blood vessels and the presence of inflammatory cells[10]. A study conducted by Ospedal et al.[13] injected the venom of L. intermediainto rabbits and recorded the appearance of tissue injuries 4 hours after the injection, with tissue damage and lysis of tissues deeper than the dermis, as well as damaged blood

vessels, blisters on the endothelium, fibrinogenolysis and thrombosis. Necrotic cutaneous injuries, which involve the formation of an eschar, can lead to deforming sequelae[14].

Platelets are involved in the processes of hemostasis, scar formation and reepithelialization and are recruited when a vessel is injured and the underlying collagen is exposed. These cells come from the bone marrow. They are anucleated and possess a complex cytoplasm. They are also rich in mucopolysaccharides, glycoprotein material and phospholipids, which are responsible for their adhesiveness and aggregability[15]. Platelets also liberate growth factors (GFs), which can stimulate angiogenesis, promoting vascular growth and the proliferation of fibroblasts. These, in turn, increase collagen synthesis. According to Lorenzi[16], the number of platelets in the human body ranges from 150,000 to 400,000 per µL.

These properties make platelet-rich plasma (PRP) a product that is capable of enhancing the integration of skin grafts and the healing of wounds. PRP contains plasma, leukocytes and platelets and is a rich source of essential GF´s and osteoconductive proteins, which also serve as a matrix for epithelial migration and the formation of bones and connective tissue[17]. The concentration of platelets in PRP used for therapy should be significantly greater than the plasma so that it can promote the adequate liberation of growth factors in the injured area[18].

PRP exhibits the following properties: healing; adhesion; an increased capacity for cellular division; collagen synthesis; accelerated cellular differentiation; efficient healing of compression ulcers, chronic and surgical wounds and skin grafts[19,20]. Therefore, the aim of the present study was to investigate the effect of PRP on dermonecrotic injuries induced by the venom of the Loxoscelesintermedia spider. This is a technically straightforward and relatively inexpensive technique that, theoretically, could be useful in the treatment of Loxoscelic accidents.

MATERIALS AND METHODS

Obtaining the venom

The venom was obtained from adult spiders that had spent a week resting and fasting, although they did have access to water ad libitum. The spiders (Loxoscelesintermedia) had to be immobilized prior to extracting the venom. This was done using tweezers and an electric shock of 15 volts to the ventral region of the cephalothorax, which induced the secretion of venom[8]. The venom was aspirated from the chelicerae using an automatic micropipette and deposited in a microtube containing saline solution. After collection, it was lyophilized and frozen at -80ºC until use[11]. The

venom was obtained with the assistance of the Laboratory of Extracellular Matrix and Venom Biotechnology in the Federal University of Paraná.

Blood collection to obtain platelets

The platelet-rich plasma (PRP) used in the present study came from healthy human donors through the performance of venipuncture. All hands were sterilized and gloves were put on before sample collection. The donor´s arm was inclined downwards at shoulder height. The patients arm was sterilized and bound. The protective cover of the hypodermic needle was removed and the puncture was performed at an oblique angle of 30°, with the bevel of the needle pointed upwards. The patients arm was released when the required amount of blood had entered the syringe[21]. The tube used to obtain the plasma contained EDTA anticoagulant.

Obtaining platelet-rich plasma (PRP)

In order to ensure that the PRP obtained was apt for use, the procedure was sterile and did not cause lysis of the platelets or damage them in any way. When damaged, platelets lose their capacity to secrete growth factors and consequently, their function[22]. The process used is known as the simplified version, since it uses mini centrifuges to obtain the PRP[18]. The collection tubes with EDTA contained close to 4 mL of human blood. They were centrifuged at a rotation of 900 rpm for 10 minutes to separate red cells, white cells and platelets based on their density[22]. A micropipette was used to aspirate the upper segment to the limit of the mist zone corresponding to the plasma and platelets[23].

The test tube containing the plasma and platelets was submitted to further centrifugation, this time at 1800 rpm for 10 minutes[22]. Afterwards, a platelet button formed at the bottom of the tube, separated from the supernatant plasma. Close to 50% of the plasma was withdrawn using a micropipette and stored in another tube, to be considered as platelet-poor plasma (PPP). The remaining material was re-suspended, yielding the PRP[23]. A concentration of 560,000/μL platelets was used for the PRP of 300 μL, whereas 280,000/μL was used for the PRP of 150 μL.

Study design and authorization

The present study received approval from the Animal Use Ethics Committee under protocol number 077-2013. Three New Zealand albino rabbits were obtained from the vivarium of the Catholic University of Paraná for use in this study. In order to ensure greater homogeneity among the rabbits, they each weighed approximately

three kilograms and were kept in individual cages in laboratory conditions, with access to water and rations ad libitum[20].

Trichotomywas performed on the dorsolateral of the animals, with mean measurements of 5 cm in width and 12 cm in length. Four areas were demarcated for intradermal injections. Saline solution (PBS) was injected into one of these areas to be used as the negative control. The other three areas received injections of one aliquot of 10 µg of crude venom, diluted in saline solution. One of these areas was used as a positive control. Another received aliquots of 150 µL of PRP at periodic intervals of 12 hours and the remaining area received aliquots of 300 µL of PRP, also at periodic intervals of 12 hours.

After the macroscopic analysis of the evolution of the injuries, the rabbits were euthanized. This procedure involved the intramuscular application of ketamine hydrochloride and xylazine hydrochloride (0.2 ml for both) in the gluteus region for analgesia. After the analgesic effect was confirmed, 1mL/Kg of intracardiac potassium chloride was injected[24]. The biological material was collected by a specialized disposal company.

Assessment of the injury and surrounding area

Assessment of the dermonecrotic injuries and surrounding areas was conducted using photographic images obtained 2, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hours after the poison was injected into the animal[25,26]. In the photographs, surface areas were visually examined for ecchymosis, erythema, edema and necrotic eschar[11].

Basic histology of the injured tissue was also used in the analysis (after 48h, 72h and 96h). Blocks were cut on microtome to a thickness of five micrometers and then stained using hematoxylin-eosin. The wounds were examined using an optical microscope at 20X magnification[27].

RESULTS

Macroscopic Analysis

During the macroscopic analysis, it was possible to monitor the evolution of the dermonecrotic injuries (Figure 1). In the early stages, the areas on which PRP were applied were swollen, with the most intense form of erythema and controlled ecchymosis. As time passed, the erythema and edema in the area of the PRP application decreased and the wound became more controlled. The gravitational spread of the venom was less than in areas that had only been inoculated with the venom. The venom of the Loxoscelesintermediaspider is characterized by this method of spreading. With PRP, it is possible to visualize the response related to the control of

the injury. When comparing the application of 150 µL and 300 µL of PRP, the latter produced a better response.

Histological Analysis

Superficial dermis, epidermis and preserved cutaneous attachments were observed for all times in the histology of the location where the PBS was applied (Figure 2).

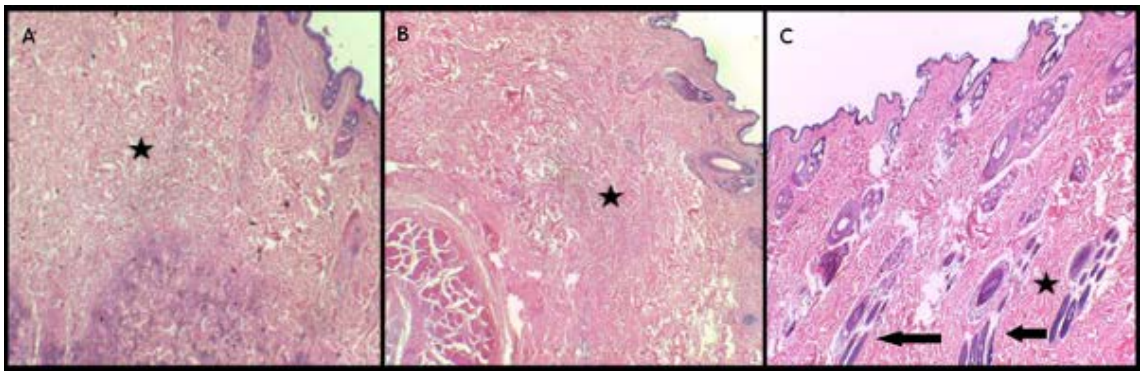

Figure 2: PBS Histology: A: 48 hours; B: 72 hours and C: 96 hours. Arrows how hair follicles. ★ : Dermis with preserved collagen fibers.

After 48 hours, analysis of the tissue that was only inoculated with venom (Figure 3) exhibited marked edema and dense suppurative interstitial inflammatory infiltrate, with foci of necrosis around the injection area. After 72 hours, edema and interstitial inflammatory infiltrate were noted. After 96 hours, foci of dermal-epidermal necrosis were observed, in association with dense suppurative superficial and deep inflammatory processes.

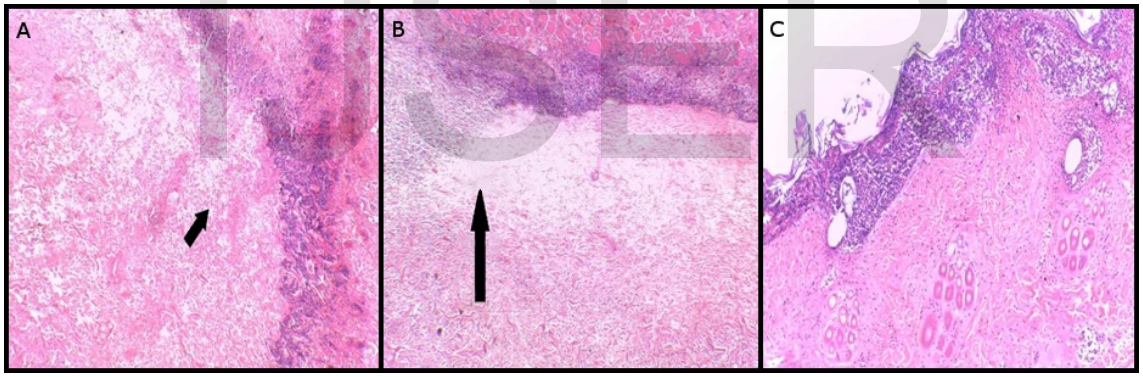

Figure 3: Histology of the tissue with venom: A: 48 hours; B: 72 hours and C: 96 hours.

Figure 4 displays the histology of the tissue that received the venom and 150 µL of PRP. After 48 hours, the following symptoms were noted: subcutaneous tissue and skin with areas of necrosis; thick collagen fibers; edema and acute inflammatory infiltrate. The edema decreased after 72 hours. After 96 hours, a palisade inflammatory process was noted in both deep and superficial dermis.

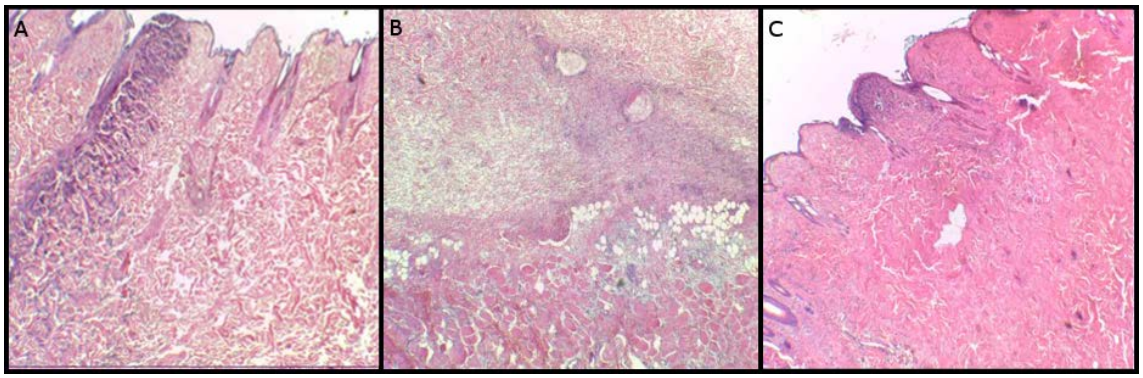

Figure 4: Histology of the tissue that received the venom and 150 µL of PRP: A: 48 hours; B: 72 hours and C: 96 hours.

When comparing the histology of the tissue that only received the venom with tissue that received the venom plus 300µL of PRP (Figure 5), it is notable that, after 48 hours, there was a reduction in edema and maintenance of the inflammatory process where PRP was used. After 72 hours, the tissue exhibited angiolymphatic vascular ectasia, with decreased edema and inflammation in deep and superficial dermis, as well as a discreet thickening of collagen fibers / fibrosis. After 96 hours, the tissue exhibited a reduction in the inflammatory process / vascular ectasia.

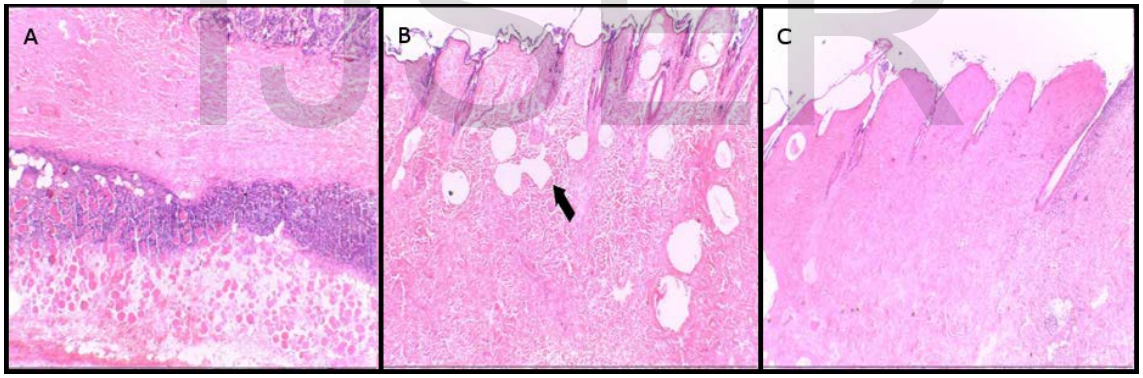

Figure 5: Histology of the tissue that received the venom and 300 µL of PRP: A: 48 hours; B: 72 hours and C: 96 hours.

When we compare the inflammatory infiltrate from the time of 96h for the tissue on which only venom was applied and the tissue on which venom and 300µL of PRP were applied (Figure 6), there is a clear decrease in the inflammatory process, thereby demonstrating that the application of PRP accelerated the healing process. This is contrary to other results, such as the thickening of collagen fibers.

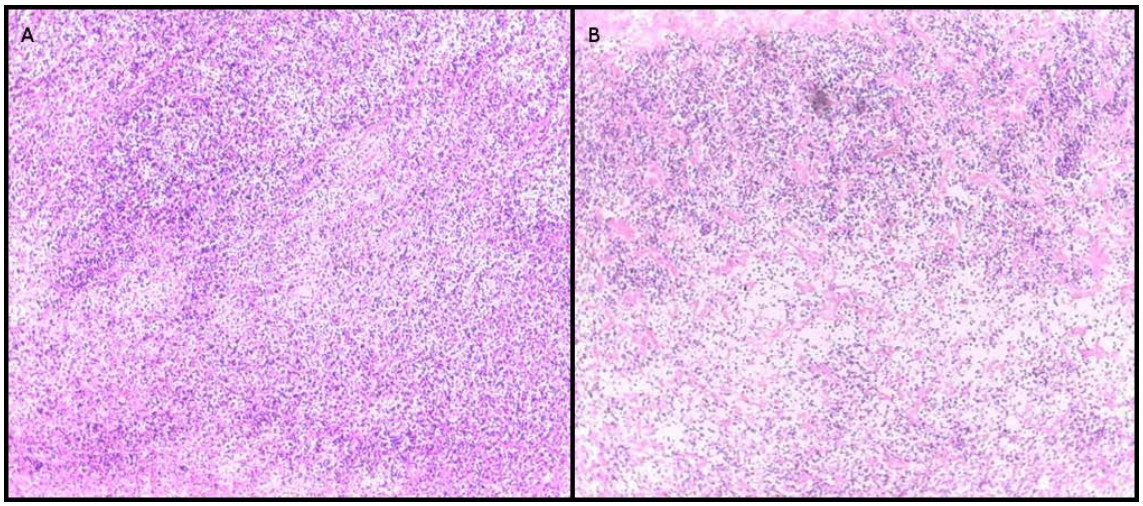

Figure 6: Comparison of the inflammatory infiltrate: A: Venom; B: Venom + 300µL of PRP.

DISCUSSION

Loxoscelic accidents cause dermonecrotic injuries to the area where the venom was inoculated, leading to the occurrence of edema and erythema[2]. The following histological symptoms have been recorded in these cases: thickening of the endothelium of blood vessels; vasodilation; intravascular coagulation; degeneration of blood vessels; dermal and subcutaneous bleeding. Marble plaque formation and gravitational scattering have been recorded after just a few hours[8].

Ospedal et al.[13] injected the venom of Loxoscelesintermedia into albino rabbits from New Zealand and assessed the injury 4, 12, 24, 48 and 120 hours after the injection. After 4 hours, the histology of the skin of the rabbits exhibited epithelial tissue and keratinized cells of preserved epidermis, although the collagen fibers of the conjunctive tissue were disorganized, indicating the occurrence of edema. After 12 hours, histological characteristics of inflammation were noted, including necrosis of hair follicles in the dermis, disorganized skeletal muscle fibers, edema and leukocyte infiltrate in the interstitium. After 24 hours, the injury exhibited a preserved epidermis (epithelium and keratinized cells), although the collagen fibers of the conjunctive tissue were loose and disorganized near the epidermis and leukocyte infiltrate was also noted, indicating edema and an evolving inflammatory action. After 48 hours, the histological findings revealed tissue destruction. After 120 hours, the tissue exhibited massive hemorrhage, collagen necrosis, destroyed hair follicles and myonecrosis[13].

Tavares[11] induced thrombocytopenia in rabbits by inoculating them with the venom of the Loxosceles gaucho spider and reported that the absence of platelets resulted in a more intense dermonecrotic injury, with greater development of

ecchymosis and necrotic eschar. These results suggest that the cells were intimately associated with the containment of phenomena that caused the injury.

The use of PRP in dermonecrotic injuries has become an auxiliary form of therapy for the healing process and the containment of injuries. Anitua[27] stressed that the principal reason for applying PRP is to accelerate the healing process through growth factors (PDGF, TGF-β and IGF), which stimulate the sequence of necessary cellular events. These in turn, optimize the cellular activity of mitogenesis, angiogenesis and chemotaxis, which is the fastest way of stimulating the regeneration process.

D'élia[28] obtained satisfactory results when using PRP to consolidate the osteotomies of autograft. Almeida et al.[29] studied a clinical case of rhytidectomy with flap reflection and noted reductions in edema and ecchymosis in the post-operative period.

Vendramimet al.[20] conducted histological studies of skin grafts performed on rabbits using liquid PRP, gel PRP and without PRP. The authors assessed the integration of the grafts, the intensity of collagenization and the inflammatory response, as well as the quantity of fibroblasts and macrophages. Their results showed increases of 31.6% for fibroblasts and 57% for macrophages, as well as greater collagen production and inflammatory responses when PRP were used. The best results were achieved when liquid PRP was sued with the skin grafts.

The limitations involved in using PRP in clinical cases include the diversity of existing protocols related to how to obtain them, as well as the correct aliquot to be used and the frequency of application. The variations inherent in patients could affect the attainment of PRP, given that healthy patients exhibit distinct cellular characteristics from those with pathologies[30]. In addition, there are other variables associated with the quality of the PRP, including the centrifugation force, the centrifugation time and the reduction in plasma volume. Therefore, it is of paramount importance that the following question is considered prior to defining the most adequate method: is this the method that results in the greatest concentrations of platelets in an adequate volume without unduly prolonging the preparation time of the product?[17]

Pereira[15] compared seven protocols for obtaining PRP and reported no statistically significant differences among them in terms of the capacity of platelet concentration and the levels of growth factors. However, it is notable that there are a number of protocols that are more effective in avoiding the residual contamination of PRP with erythrocytes and leukocytes.

The results of the present study differed from those found in the literature. Although the technique has not yet been standardized, PRP could be an alternative

option in the treatment of patients who have suffered loxoscelic accidents. The best response in the histology and macroscopic analysis was associated with the application of 300 µL of PRP.

Macroscopically, the areas where PRP were applied exhibited more edema and more accentuated erythema in the early stages due to the high cellular concentration in the area. However, after 72 hours, both the edema and the erythema had decreased as a result of the increased diameter of the lymphatic vessels and the decrease in cellular infiltrate, thereby demonstrating that the area was already in a more advanced healing phase. Further studies are necessary to assess the processing and mechanisms of PRP activity, with a view to developing standardized clinical trials.

CONCLUSION

Analysis of the results confirmed that PRP can ease superficial symptoms such as bruising, redness and swelling in acute dermonecrotic injuries caused by the venom of Loxoscelesintermediaand can accelerate the healing process. Therefore, this technique could be used to control dermonecrotic injuries, although further studies should be conducted to determine the aliquot and frequency of application prior to clinical use.

REFERENCES

1. BRASIL. MINISTÉRIO DA SAÚDE. *Guia de Vigilância Epidemiológica*. 7ª ed., caderno 14. Brasília. 2009.

2. BERTOLDI, I.; ERZINGER, G. S. Estudos de casos clínicos da ação do veneno das aranhas do gênero Loxosceles e suas correlações clínicas hematológicas no Estado do Paraná. *Revista Saúde e Ambiente.*v.6, n.1, 2005.

3. MARQUES-DA-SILVA, E.; SOUZA, S.R.; FISHER M.L. Loxoscelesspiderbites in thestateof Paraná, Brazil: 1993-2000.*J. Venom. Anim. Toxinsincl. Trop. Dis,* v. 12, n.1, p. 110-123, 2006.

4. FISCHER, M.L. *Biologia e Ecologia de Loxosceles (Araneae: Sicariidae) no município de Curitiba, Paraná.*Dissertação (Mestrado em Zoologia) – Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, 1996.

5. SWANSON, D.L.; VETER, R.S. Loxoscelism. *Clinics in Dermatology*, v.24, p.213- 221. 2006.

6. FUTRELL, J.M. Loxoscelism. *American Journal Medicine Science*, v.304, p.261- 267, 1992.

7. CHRISTOFF, A. O. *Efeitos do veneno e da toxina dermonecrótica recombinante LiR e cDT1 de Loxosceles intermedia em fígado de rato.*Dissertação (Pós-Graduação em Ciências Biológicas- Farmacologia) - Setor de Ciências Biológicas da Universidade Federal do Paraná, Curitiba, 2008.

8. RAMADA, J. S. *Desenvolvimento de ensaio in vitro para avaliar potência neutralizante de soro antiloxoscélico de uso terapêutico*. Dissertação (Mestrado em Processos Biotecnológicos)- Setor de Tecnologia, Universidade Federal do Paraná, Curitiba, 2012.

9. MOREIRA, D.C. *Estudo da atividade hemolítica do veneno de Loxosceles intermedia (Aranha Marrom) e seus mecanismos moleculares.*Dissertação (Mestrado em Biologia Celular e Molecular)- Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, 2008.

10. da SILVA, P. H. et al. Brown SpidersandLoxoscelim. *Toxicon.*v.44, n.7, p.693-709, 2004.

11. TAVARES, F. L. *Contribuição ao estudo da ação in vitro do veneno da aranha marrom, Loxoscelesgaucho, sobre plaquetas humanas e de coelho. Participação das plaquetas na dermonecrose induzida experimentalmente pelo veneno loxoscélico em coelhos.*Tese (Doutorado em Ciências)- Departamento de Patologia, Universidade de São Paulo, São Paulo, 2007.

12. WILLE, A. C. M. *Estudo dos efeitos citotóxicos do veneno de Loxosceles intermedia e de toxinas recombinantes sobre células subendoteliais.* Dissertação (Mestrado em Biologia Celular e Molecular)- Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, 2010.

13. OSPEDAL, K. Z. et al. Histopathological findings in rabbits after experimental acute exposure to the Loxoscelesintermedia(Brown spider) venom. *I. J. Exp. Path***.** v. 83, n.6, p.287-94. 2002.

14. CHAIM, O. M. *Estudo da atividade citotóxica da proteína dermonecrótica do veneno de aranha marrom (Loxosceles intermedia) com ênfase no efeito nefrotóxico.*Dissertação (Mestrado em Biologia Celular e Molecular)- Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, 2005.

15. PEREIRA, R. C. F.*Avaliação de sete protocolos de obtenção de plasma rico em plaquetas (PRP).*Dissertação (Mestrado em Medicina Veterinária)- Área de Concentração em Clínica Médica, da Universidade Federal de Santa Maria, Santa Maria, 2012.

16. LORENZI, T. F. *Manual de hematologia: propedêutica e clínica.*4. ed. Rio de Janeiro: Guanabara Koogan, 2011. 710 p.

17. SCARSO FILHO, J. *Avaliação do plasma rico em plaquetas na proliferação celular- estudo in vitro.*Tese (Doutorado em Implantodontia)- Departamento de Estomatologia, Universidade Federal de Santa Catarina, Florianópolis, 2002.

18. SCARANTO, M. K. *Plasma Rico em Plaquetas.*Monografia (Especialista em Periodontia)- Departamento de Estomatologia, Universidade Federal de Santa Catarina, Florianópolis, 2002.

19. UEBEL, C.O. *Ação do plasma rico em plaquetas e seus fatores de crescimento na cirurgia dos microimplantes*. Tese (Doutorado em Medicina) Porto Alegre: Pontifícia Universidade Católica do Rio Grande do Sul, 2006.

20. VENDRAMIN, F. S.; FRANCO, D; SCHAMALL, R. F.; FRANCO, T. R. Utilização do plasma rico em plaquetas (PRP) autólogo em enxertos cutâneos em coelhos. *Rev. Bras. Cir. Plást***.** v.25, n. 1, p. 4-10. 2010.

21. ARCHER, E. et al. *Procedimentos e protocolos*. Rio de Janeiro: Guanabara Koogan, 2005.

22. ALVES, S. B. C. R.; MONTEIRO, M. P. G.; PIRES, N. R.; SANTOS, A. V. P. Metodologia para obtenção do plasma rico em plaquetas: Estudo preliminar*. Rev. Eletrônica Novo Enfoque.* v.15, ed. especial, p. 83-89. 2012.

23. JUNIOR, R. R.; NEGREIROS, R. M.; ELIAS, F. M.; JORGE, W. A. Utilização de plasma rico em plaquetas em enxertos ósseos para reparação de defeitos ósseos. *Rev. de Odontologia da Universidade Cidade de São Paulo.*v.20, n.3, p. 295-300. Set-dez. 2008.

24. KALAS, E. et al. Modelo de anestesia em coelhos para procedimentos no tórax. *Acta Cir. Bras.* v.16, n.2 São Paulo, 2001.

25. PEREIRA, N.B et al. Histopathological characterization of experimentally induced cutaneous loxoscelism in rabbits inoculated with Loxoscelessimilisvenom. *J. Venom. Anim. Toxins incl. Trop. Dis*.v. 18, n.3, p. 277-286. Aug. 2012.

26. RESENDE, C. et al. Granuloma periférico de células gigantes – imunohistoquímicaanti-TNF-alfa. *Rev Periodontia.*v.17, n.4, p. 104-111. 2007.

27. ANITUA, E. et al. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. *Journal of Orthopaedic Research.* v.23, n.1 , p. 281–286.2005.

28. D'ÉLIA, C.O. *Comparação do plasma rico em plaqueta associado com aspirado de medula óssea ao enxerto autólogo de ilíaco na consolidação das osteotomias da tíbia proximal: estudo prospectivo randomizado*. Dissertação (Mestrado em Ciências)- Setor de Ciências da Saúde da Faculdade de Medicina da Universidade de São Paulo, São Paulo, 2009.

29. Almeida A.R.H. de, Menezes J.A.D.E., Araújo G.K.M. de & Mafra A.V.C. Utilização de plasma rico em plaquetas, plasma pobre em plaquetas e enxerto de gordura em ritidoplastias: análise de casos clínicos. *Rev. Bras. Cir. Plast.,* 23:82-88, 2008.

30. RUSSELL, R.P. et al. Variability of Platelet-rich Plasma Preparations. *Sports MedArthrosc.* v.21, n.4, p.186-1990. 2013.