Author’s response to reviews

Title: Growth factor-enriched autologous plasma improves wound healing after surgical debridement in odontogenic necrotizing fasciitis: a case report

Authors:

Rubi Lopez-Fernandez MD (dra_rubylepez@yahoo.com.mx)
Jorge Ramirez-Melgoza MD (sapic04@hotmail.com)
Nora E Martinez-Aguilar MD (noramar_dr@yahoo.com.mx)
Alicia Leon-Chavez PhD (alileon04@yahoo.com.mx)
Daniel Martinez-Fong PhD (dmartine@fisio.cinvestav.mx)
Juan A Gonzalez-Barrios PhD (jantgonzalez@issste.gob.mx)

Version: 5 Date: 6 May 2010

Author’s response to reviews: see over
Reviewer: Joseph Alsousou

Comments to authors:

1) "it seems that the authors have not distinguish between GF enriched plasma and Platelet Rich plasma (PRP). The methods used to prepare the plasma seems to be a method for PRP. the authors did not use any GF filtering technique to enrich the plasma. It seems that they simply produced Platelet rich plasma in low concentration. It is well known that GF present in the platelet dense granules and are only released when the platelets are activated. It will be very misleading to readers if the authors did not clarify the product they have used."

Response

- We did use the correct method to obtain the Growth factor-enriched plasma (PRGF), which is different from that for PRP preparation. There are three major differences in the methods to obtain Platelet rich plasma (PRP) and Growth factor-enriched plasma (PRGF) (Anitua et al., Pract Proced Aesthet Dent. 2001;13:487-93). 1) PRP is prepared from large volumes of blood (50 - 500 mL), whereas small blood volumes (5 - 40 mL) are used to prepare PRGF. 2) Two centrifugation cycles at 3,000 rpm for 30 min are required for PRP, whereas GFEP requires only one centrifugation cycle at 1,800 rpm for 8-10 min. 3) The bovine thrombin is the activator for PRP, whereas PRGF is activated by calcium chloride (10%) (Lannnddesberg, 2000). As can be seen in the first version of our paper (pp 6, P1, L1), we used the three essential conditions to obtain GFEP; i.e., small blood volume (15 mL), one centrifugation cycle (1800 rpm for 8 min), and 10% CaCl₂ solution as activator. Those references were added in our revised version to support the clarity of the method used to obtain GFEP and activate GF release.
- We agree with reviewer’s comment about “GFs present in the platelet dense granules are only released when the platelets are activated”. To induce platelet degranulation and the consequent release of GFs, we added fifty µL of 10% CaCl₂ solution, as reported by Lannnddesberg, 2000 (See pp 6, P 1, L1 of the first version of our paper).
- Taking into account the reviewer’s suggestion, we clarified the product we used by adding all the responses to the reviewer’s comments (See pp 6, P1, L1-15).

2) "Also it is not clear how they have applied the PRP on the wound, did they use spray, swap, or applied it directly, in which case they will need large amount of PRP to cover the size of the wound."

Response

- Taking into account the reviewer’s suggestion, we detailed the procedure for gel application (See pp 6, P 1, L9-11). The text addition was as follows, Approximately 1.5 mL of this gel was directly applied in the center of the wound and then manually spread to cover the total area of the surgical wound. This procedure was repeated every third day until the completion of the wound healing (Figure 2).
3) “PRP was applied 8 times every three days: there is no reference to justify this method. Did they bleed the patient 8 times every three days?”

Response

• Since 1.5 mL of gel fraction containing GFs were obtained from each 15 mL of peripheral blood, the patient was bleed every three days. This was clarified in the text (See pp 6, P 1, L 11-12).

4) “I also note that the patient had low platelet count (78) which could be a contraindication for platelet rich plasma use. did the author consider this?”

Response

• The reviewer’s appreciation about the low platelet count (thrombocytopenia) is correct. However, we believe that thrombocytopenia does not contradict the use of platelet rich plasma, because GF’s are able to activate their receptors at low concentrations, in the range of picomolar (pM) to nanomolar (nM) (See Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003; 83(3):835-870). Despite thrombocytopenia, the procedure to obtain PRGF is and effective to provide the concentrations of GFs required to activate their receptors thus exert their trophic effects. Considering the reviewer’s suggestion, this issue was discussed emphasizing the effectiveness of GF-enriched autologous plasma despite thrombocytopenia (See pp 8, P 2, L 4-8).
• Werner et al. (2003) reference was added in both the text and the list of references.

5) “using PRP to accelerate wound healing is extensively reported in the medical literature. I am not sure about the originality of this method.”

Response

The originality of this work consists in the local application of PRGF is able to avoid the pedicled graft rotation for the covering of the muscle exposed zone by surgical wound debridement. Before to this work the final result of conventional treatment in the patient look like the next picture.
Reviewer: Alaa Abd-Elsayed

1) “Introduction: provide the reported incidence of necrotizing fasciitis.”

Response

- Taking into account the reviewer’s request, we added the information focusing into the cervical necrotizing fasciitis, whose incidence is well known and reported. We changed the lines 1 to 5 of the first paragraph as follows:

  Cervical necrotizing fasciitis (CNF) is an uncommon, rapidly progressive, and potentially lethal infection comprising skin, subcutaneous tissue, superficial fascia, and occasionally the deep fascia. Its rapid progression results in necrosis and severe systemic toxicity. The incidence of CNF is 2.6% out of the infections of head and neck [1].

- As support for CNF incidence, we added the reference [1] as in-text citation and in the list of references (see Tung-Yiu, 2000).

2) “Case report: try to find an alternative to the symbol located at the second line after "Department of the"

Response

- Taking into account the reviewer’s suggestion, we substituted the symbol 1o with the original name of the Hospital (See pp 4, P 3, L 2).

3) “Page 5: add differential to the WBCs.”

Response

- According to the reviewer’s request, we added the differential counts of WBCs (See pp 5, P 1, L 2-5).