## Project Review

### "Experimental model for the study of the effects of platelet derived growth factors on muscles"

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Platelets are an intriguing autologous source of growth factors (GFs) which have been demonstrated to be able to modulate the recruitment, duplication, activation and differentiation of cells involved in bone- and soft-tissue healing. Doping related issues are still matter of debate when considering this therapeutic approach for the treatment of sport-related injuries in particular because of the IGF-1 content in the platelets alpha granules. Certainly, the use of PRP for the treatment of bone, tendon, cartilage and ligament injuries cannot be considered as a technique able of enhancing the physical performances but its muscle injection is still a matter of debate. Moreover, some authors suggested the hypothesis that the use of PRP for ameliorating muscle healing may lead to late muscle fibrosis.

The aim of the present study is to analyse, in a murine model, the effect of the different techniques used for the preparation of platelets derived GFs [i.e. Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF)] on muscle repair processes. Muscle injuries, induced on animals, will be treated with the 2 different techniques. Morphological and morphometrical analysis as well as the evaluation of myonucleous with electron mycroscopy will be carried out on repaired muscles. The evaluation, both quantitative and qualitative, of the satellite cells will be performed with the immunohistochemistry technique combined with confocal microscopy. Functional evaluation will be performed with the grafting test. All of the analysis will be carried out with both internal and external controls represented by untreated muscles and muscles treated with recombinant growth factors as well as treated with adeno-associated-virus(AAV) mediated –VEGF gene transfer which has been demonstrated to be able to stimulate angiogenesis and repair processes when trasnfected into skeletal muscles. Long term follow up will be able to highlight eventual muscle fibrosis.

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## Results and conclusions

**Background** Ample evidences suggest that growth factors (GFs) may play a key role in the healing process, especially in the early stages of the inflammatory phase. Despite the reported clinical successes, there is still a lack of knowledge when considering the biological mechanism at the basis of platelet-rich plasma (PRP) activity during the muscle healing process. The aim of the present study was to analyze the early effects of PRP in an easily reproducible animal model.
**Materials and methods** 102 Wistar male adult rats were used in the present study. The muscle lesion was performed by scalpel on the flexor sublimis muscles. PRP was administered immediately after surgery. Treated, untreated and contralateral muscles were tested by morphological analysis, immunohistochemistry, RT-PCR analysis and Western Blot assay.

**Results** In the PRP treated muscles**,** the leukocyte infiltration was significantly higher when compared to both untreated and contralateral samples. The latter showed a higher leukocyte infiltration when compared to the untreated muscles. PRP treatment also modified the cellular composition of the leukocyte infiltration leading to an increased expression of the CD3, CD8, CD19 and CD68 and to a decreased CD4 antigen expression in both PRP treated and contralateral muscles. The analysis of the blood vessel density and the blood vessel diameters showed no statistically significant differences when comparing the three groups analyzed. At day 2 and 5 after PRP administration there was a significant expression of Pax7 and MyoD1. The analysis of pro- and anti-inflammatory cytokines expression showed that PRP induced a more pronounced and/or early inflammatory response by expression of IL-1β and TGF-1β.

**Discussion** The results of the present study showed that PRP treatment increased the physiological early inflammatory response further to a muscle injury with a parallel modification of the pattern of cellular recruitment. Moreover, the local PRP treatment may exert, directly or, more plausibly, indirectly, a systemic effect when healing processes were concerned, at least limited to the very first inflammatory phase. The results of the present study might suggest the hypothesis that PRP promoted an early inflammatory response together with a more efficient production of pre-myogenic progenitor population, satellite cell and myoblasts activation/proliferation during muscle regeneration. Conversely, the terminal differentiation markers (i.e., myogenin, Mrf4) as well as others pro-inflammatory cytokines (i.e., IL-6, IL-10, TNFα) and VEGF-A were not additionally modulated by PRP treatment. Further experimental studies are needed to fully understand the local and systemic mechanism of action before apply PRP in routine clinical practice as well as in order to deeply understand possible systemic effects on muscle performance.