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This is the author's final version of the contribution published as:

Borrione, P.; Grasso, L.; Racca, S.; Abbadessa, G.; Carriero, V.; Fagnani, F.; Quaranta, F.; Pigozzi, F.. Systemic effects of locally injected platelet rich plasma in a rat model: an analysis on muscle and bloodstream. JOURNAL OF BIOLOGICAL REGULATORS & HOMEOSTATIC AGENTS. 29 (1) pp: 251-258.

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Systemic effects of locally injected Platelet Rich Plasma in a rat model: an analysis on muscle and bloodstream

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Conflict of interest: in this work there are no financial or other relationships that might lead to a conflict of interest.

Keywords: Platelet Rich Plasma, muscle injury, systemic effect, inflammatory phase

SUMMARY

Abundant evidences suggest that growth factors, contained in platelets alpha granules, may play a key role in the early stages of the muscle healing process with particular regard to the inflammatory phase. Although the contents of the platelet-rich plasma preparations have been extensively studied, the biological mechanisms involved as well as the systemic effects and the related potential doping implications of this approach are still largely unknown. The aim of the present study was to investigate whether local platelet-rich plasma administration may modify the levels of specific cytokines and growth factors both in treated muscle and bloodstream in rats. Additional aim was to investigate more deeply whether the local platelet-rich plasma administration may exert systemic effects analyzing contralateral lesioned but untreated muscles. The results of the present study showed that platelet-rich plasma treatment induced a modification of certain cytokines and growth factor levels in muscle but not in the bloodstream, suggesting that local platelet-rich plasma treatment influenced directly or, more plausibly, indirectly the synthesis or recruitment of cytokines and growth factors in the site of injury. Moreover, the observed modifications of cytokine and growth factor levels in contralateral injured but not treated muscles, strongly suggested a systemic effect of locally injected platelet-rich plasma.

49 **INTRODUCTION**

50 The repair response of the musculoskeletal tissue generally starts with the
51 formation of a blood clot and the following degranulation of platelets, which
52 release locally growth factors (GFs) and cytokines (1). This
53 microenvironment results in chemotaxis of inflammatory cells as well as
54 activation and proliferation of local progenitor cells. Alpha granules are
55 storage units within platelets, which contain pre-packaged GFs in an inactive
56 form. Abundant evidences suggest that GFs, contained in platelet alpha
57 granules, may play a key role in the early stages of the healing process with
58 particular regard to the inflammatory phase being able of modulating the
59 recruitment, duplication, activation and differentiation of the cells involved in
60 the healing process (2-6). The efficacy of those GFs should be, in theory,
61 directly proportional to their local concentration. This hypothesis is at the
62 base of the use of platelet-rich plasma (PRP) in several circumstances, all of
63 them characterized by the need of activating, modulating, speeding up or
64 ameliorating the process of tissue repair.

65 With regard to sport medicine, doping related issues are still matter of debate
66 when considering this therapeutic approach for the treatment of sport-related
67 injuries, in particular because of the Insulin-like growth factor-1 (IGF-1)
68 content in the platelets alpha granules as well as the blood manipulation
69 procedures. With particular regard to the muscle injection of platelets derived
70 GFs, several issues still need to be clarified. Assuming that this procedure, as
71 it has been demonstrated by several studies, is able to ameliorate the muscle
72 tissue repair processes, it is still unclear whether the locally injected

73 concentrated amount of GFs may exert systemic effects. Indeed, although the
74 contents of the PRP preparations themselves have been extensively studied
75 (7-12), the biological mechanisms involved in treatment with PRP as well as
76 its systemic effect with the related potential doping implications are largely
77 unknown. With this regard, it has to be underlined that the World Anti-
78 Doping Agency (WADA) prohibited the use of intramuscular injection of
79 PRP in the 2010 list (13), then allowing its use in the 2012 list (14). This
80 modification has been introduced despite the suggested systemic effect of
81 locally injected GFs described by some authors (7, 15). Banfi et al. measured
82 the levels of some cytokines and GFs in the serum obtained from five male
83 subjects 30 minutes, three hours and 24 hours after the treatment with PRP in
84 order to evaluate the eventual systemic effect of this local injection. Authors
85 reported significant modifications of Vascular Endothelial Growth Factor
86 (VEGF), Epidermal Growth Factor (EGF) and Chemokine (C-C motif)
87 Ligand 2 (CCL2) levels 30 minutes after the treatment followed by a gradual
88 return near to basal values 24 hours after the injection (15). Wasterlain et al.
89 measured the levels of some GFs in the serum obtained from 25 subjects
90 treated with leukocyte-rich PRP (LR-PRP). Authors reported significant
91 increases of Insulin-like Growth Factor 1 (IGF-1), basic Fibroblast Growth
92 Factor (bFGF) and VEGF levels following the local PRP injection (7).
93 With the exception of these two studies, the possible systemic effect of
94 locally injected PRP is largely unknown. Conceivably, the systemic effect
95 should be studied observing whether the treatment with PRP is able to modify

96 the healing process in contralateral, but untreated with PRP, injured muscle,
97 as suggested by Borrione et al in a recent study (2).
98 The aim of the present study was to investigate whether the local PRP
99 administration may modify the levels of specific cytokines and GFs both in
100 treated muscle and bloodstream. The hypothesis at the base of this
101 speculation was that the injection of cytokines and GFs naturally present in
102 platelets and included in the WADA prohibited list could generate an increase
103 of normal function, strength and capacity in non injured muscles if a systemic
104 effect is really present.
105

106 **MATERIALS AND METHODS**

107 **Animals and Surgery**

108 Wistar male adult rats (n=60), 8-9 weeks old, weighing approximately 250g,
109 were used. Twenty-seven animals were sacrificed 2 days after surgery: seven
110 rats were subjected to muscle injury on the right flexor muscles and
111 immediately treated with PRP (treated group: TR), eight animals, used as
112 controls, were subjected to the same muscle injury and left untreated
113 (untreated group: UT), seven rats were subjected to muscle injury in both
114 anterior limbs: the right limb was treated with PRP while the injury on the left
115 limb remained untreated (contralateral group: CL). Five rats, left untreated
116 and uninjured, were used as controls (C). Twenty-five animals were
117 sacrificed 5 days after surgery (seven treated, seven untreated, six
118 contralaterals and five controls). Eight animals were analyzed 30 days after
119 surgery (two treated, two untreated, two contralaterals and two controls).

120 Animals were kept in cages in a room with controlled temperature and
121 humidity, with light/dark cycle of 12/12h, and fed with food and water *ad*
122 *libitum*. Animals underwent surgery under general anesthesia by
123 intramuscular injection of tiletamine + zolazepam (Zoletil) 3mg/kg. The
124 decision of treating all animals with Finadyne administered at a dose of 2.5
125 mg/kg/12h, independently of the presence of signs of suffering, was
126 determined by the intention of obtaining the same condition in the different
127 experimental settings since the use of anti-inflammatory drugs may affect
128 both the healing process and the first inflammatory response (16). The
129 surgical procedures were performed with the aid of a surgical microscope
130 (Zeiss OPMI7, Jena, Germany). A longitudinal incision was performed on the
131 right arm (or both arms) from the elbow region to the wrist in order to access
132 the flexor sublimis muscles of the upper joint of the fingers. The muscle was
133 then injured transversely and medially using a scalpel. The wedge-shaped
134 lesion had a length of approximately 3 mm, a width of 2 mm and a depth of 3
135 mm. After the incision, the injury sites of the treated animals were
136 immediately filled with PRP. The flexor muscles were withdrawn after 2, 5 or
137 30 days and analyzed. Animals were daily monitored to assess their state of
138 wellness, to prevent self-mutilation, skin ulcers, muscle contractures and
139 suffering. All procedures were carried out in accordance with the Local
140 Ethical Committee and the European Communities Council Directive of 24
141 November 1986 (86/609/EEC). All procedures were approved by the local
142 Animal Care Committee and supervised by a veterinary.

143 **Blood Collection and PRP preparation**

144 Blood was collected by intracardiac drawing. Briefly the needle (21G) was
145 inserted at the base of the sternum at 20° angle just lateral of the midline. 3-
146 3.5 ml of blood were slowly aspirated in a syringe containing 1 ml of 3.8%
147 sodium citrate as anticoagulant in order to avoid platelet activation and
148 subsequent degranulation. Blood was then transferred in sterile tubes
149 containing sodium citrate and underwent a first centrifugation at 220 g for 15
150 minutes. The top layer plasma was transferred, in another sterile tube without
151 anticoagulant. To objectively determine the number of platelets and
152 investigate the presence of other cells, before proceeding with the second
153 centrifugation, a complete blood count was performed using a cell counter
154 ADVIA 2021 (Bayer, Leverkusen, Germany) on a small amount of the
155 plasma layer obtained after the first spin centrifugation (platelets content:
156 361.43±32.48 x10^3/µl; white blood cells content: 0.01±0.082 x10^3/µL). A
157 second centrifugation at 1270 g for 5 minutes allowed the platelets to fall to
158 the bottom of the tube. The most of acellular plasma was removed and
159 discarded. The pellet was re-suspended in 100 µL of plasma to obtain a
160 concentration of 4 times greater than the initial condition. This platelets
161 enriched preparation was activated with 20 µL of 10% calcium chloride
162 (Braun, Melsungen, Germany, 1000 IE / ml CaCl2-2SG) room temperature
163 and after jellification, immediately inserted through tweezers into the injured
164 muscle of the same animals from which blood has been drawn. The wound
165 was then sutured and washed with saline solution (17-18).

166 **Cytokines and growth factors**

167 Blood samples were collected before surgery and animal sacrifice. Muscle
168 samples (200-300 mg) were lysed in RIPA buffer (25 mmol/L Tris-HCl pH
169 7.6 , 150 mmol/L NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS;
170 Sigma Aldrich) supplemented with Halt™Protease Inhibitor Cocktail (Sigma
171 Aldrich). Plasma and muscle tissue samples were analyzed by Milliplex plus
172 kit (Millipore, Billerica, Massachusetts) to quantify the levels of the
173 following 10 molecules: Granulocyte-colony stimulating factor (G-CSF),
174 Granulocyte-macrophage colony-stimulating factor (GM-CSF), Epidermal
175 growth factor (EGF), Tumor necrosis factor alpha (TNF-α), Interleukin 1
176 alpha (IL1-α), Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 6 (IL-6),
177 Interleukin 10 (IL-10), Interleukin 13 (IL-13).

178 **Statistical analysis**

179 Cytokines and Growth factors data were analyzed by two-way ANOVA
180 analysis. Multiple comparisons were further performed with Bonferroni‘s
181 post hoc test. The effects of the time for each cytokines and growth factors in
182 each experimental condition were analyzed by one-way ANOVA. Differences
183 were considered statistically significant when p-value < 0.05. All data are
184 presented as mean ± SD. The little variation of number of rats in 2 day group
185 and 5 day group depended by accidental deaths, probably caused by
186 intracardiac drawing or anesthesia.

187

188 **RESULTS**

189 **Cytokines and growth factors analysis**

190 In blood samples, the levels of all the investigated molecules remained
191 unmodified in all the experimental conditions as well as in all the time points
192 considered (data not showed).
193 IL-4, IL-6, IL-10, IL-1α, TNF-α and EGF showed significant modifications in
194 treated and contralateral muscle samples. On the contrary, G-CSF, GM-CSF,
195 IL-5 and IL-13 showed no modifications when compared to basal values.
196 **IL-4.** At day 2, TR, CL and UT showed lower values in comparison to C. The
197 reduction was statistically significant only in TR (Table I). At days 5 and 30,
198 TR showed a significant increase of IL-4 levels when compared to C and UT
199 (Table I) while cytokine values in CL and UT remained lower in comparison
200 to C. The increase in time of IL-4 levels in TR was statistically significant
201 ($p<0.05$, one way-ANOVA).
202 The evaluation of data was supported by two-way ANOVA analysis that
203 revealed significant effects for interaction ($F=5.975$; $p<0.001$), treatment
204 ($F=7.346$; $p<0.001$) and time ($F=8.064$; $p<0.001$).
205 **IL-10**. 2 days after surgery, IL-10 showed a significant peak in TR (Table I).
206 The cytokine in CL showed a behavior similar to what observad in TR with
207 higher values when compared to C, but the difference was not statistically
208 significant. At 2 days, TR and CL values were higher in comparison to UT
209 whereas UT showed lower values when compared to C (Table I).
210 At day 5, IL-10 levels in TR and CL decreased showing values lower than C
211 (significant difference in CL). UT continued to show values lower than C
212 (Table I).

213 Interestingly, IL-10 showed a trend, even if not statistically significant,
214 toward a progressive increase in TR, CL and UT from day 5 to day 30 after
215 surgery. Two-way ANOVA analysis showed significant effects for interaction
216 ($F=5.618$; $p<0.001$), treatment ($F=20.39$; $p<0.0001$) and time ($F=13.14$;
217 $p<0.0001$).

218 **IL-1α** showed a significant peak level at day 2 in TR in comparison to all the
219 other groups. UT showed values lower than C while CL revealed values
220 similar to C (Table I).

221 At 5 days after surgery, IL-1α levels decreased in TR maintaining otherwise
222 higher values when compared to all the other groups (Table I).

223 At day 30 IL-1α values in TR reached values found in C. The decrease of IL-
224 1α values from day 2 to day 30 was statistically significant ($p<0.05$, one way-
225 ANOVA). Two-way ANOVA analysis confirmed significant effects for
226 interaction ($F=49.17$; $p<0.001$), treatment ($F=146.0$; $p<0.0001$) and time
227 ($F=30.40$; $p<0.0001$).

228 **IL-6** showed a significant peak in TR and CL at day 2 when compared to C
229 and UT respectively (Table I). IL-6 values in UT did not differ from those
230 observed in C.

231 At day 5, the values in TR remained higher than in C and UT (Table I).

232 At 30 days after surgery, the cytokine values in TR decreased showing values
233 similar to all the other groups. The IL-6 decrease in TR and CL from day 2 to
234 day 30 was statistically significant ($p<0.05$, one way-ANOVA). The
235 evaluation of data was supported by two-way ANOVA analysis that showed

236 significant effects for interaction ($F=95.24$; $p<0.001$), treatment ($F=179.2$;
237 $p<0.0001$) and time ($F=277.0$; $p<0.0001$).
238 At day 2, **TNF-α** showed UT levels lower than in C ($p<0.05$). At day 5 TNF-
239 α in TR was characterized by a significant peak level when compared to the
240 other experimental groups. At day 30, UT, TR and CL presented values lower
241 than in C (Table I). The variations in time of the values of TNF-α in TR and
242 UT resulted significant ($p<0.05$, one way-ANOVA). Two-way ANOVA
243 analysis showed significant effects for interaction ($F=11.16$; $p<0.001$),
244 treatment ($F=14.72$; $p<0.0001$) and time ($F=29.07$; $p<0.0001$).
245 **EGF** levels in all injured muscles were significantly lower when compared to
246 C values in all considered time points. In particular, at day 2 TR, CL and UT
247 presented values lower than in C. UT levels were higher than those observed
248 in TR and CL (Table 1).
249 At day 5, the values detected in all injured muscles continued to remain lower
250 than those observed in C without significant differences among them. These
251 results were still present 30 days after surgery (Table I).
252 The comparison between EGF values in time in TR and UT groups showed
253 two different trends. The UT values at day 30 were lower than those observed
254 at days 2 and 5 ($p<0.01$, one way-ANOVA). On the contrary, EGF values in
255 TR presented a progressive increase in the same time interval ($p<0.001$, one
256 way-ANOVA). Two-way ANOVA analysis confirmed significant effect for
257 interaction ($F=3.422$; $p<0.01$), treatment ($F=51.74$; $p<0.0001$) but not for the
258 time.
259

260 **DISCUSSION**

261 The results of the present study showed that PRP treatment induced a
262 modification of certain cytokines and GFs in muscle but not in the
263 bloodstream, suggesting that local PRP treatment influenced directly or, more
264 plausibly, indirectly the synthesis or recruitment of cytokines and GFs in the
265 site of injury.

266 Since cytokines and GFs have a short half-life (15), we can hypothesize that
267 cytokines and GFs present in the site of injury after 2, 5 and 30 days from
268 PRP application, were not directly derived from PRP application (data
269 confirmed by the absence of relevant data in bloodstream). Conceivably, the
270 variation of cytokines and GFs concentration observed in muscles resulted
271 from biosynthetic activity of other cells (e.g.macrophages/monocyte)
272 recruited in the site of injury.

273 Moreover, the observed modifications of cytokines and GFs levels in
274 contralateral injured but not treated muscles, strongly suggested a systemic
275 effect of locally injected PRP. Indeed, several of the analyzed molecules,
276 namely IL-1α, IL-4, IL-6, IL-10, TNF-α and EGF, showed a different
277 behavior in the three different experimental conditions: treated, untreated and
278 contralateral. In particular IL-1α, IL-4, IL-6, IL-10 and TNF-α showed a
279 significant modification 2 and 5 days after PRP treatment.

280 The local increase of cytokines can be determined by different factors. It is
281 known that muscle healing process progresses through a constant series of
282 overlapping phases (degeneration and inflammation, regeneration,
283 remodelling) resulting in the restoration of the anatomic continuity and

284 function (19). The first stage usually starts with the formation of a blood clot
285 followed by the degranulation of platelets that release locally GFs and
286 cytokines. The following phases are controlled by complex and dynamic
287 molecular mechanisms involving local and systemic factors interacting with
288 many different cell types recruited to the site of injury from the surrounding
289 tissues and/or circulation (1). Generally, during the acute phase, following a
290 muscle injury, polymorphonucleated leukocyte are the most abundant cells
291 presents in the lesion site (19-22) and they are replaced by monocytes within
292 the first days. Monocytes are then activated into macrophages and involved in
293 the proteolysis and phagocytosis of the necrotic material (19, 23, 24).
294 Previous studies demonstrated that PRP treatment increases the leukocyte
295 infiltration in the injured muscle (2). Conceivably, the higher concentration of
296 macrophages in the muscles treated with PRP may easily explain the
297 observed increased concentration of IL-1α, in the treated group (25, 26).
298 These data were confirmed by a recent study carried out by our group in
299 which it has been observed a significant increase of NF-κB-p65 at 2-day post-
300 injury. On the contrary, at 5-day post-injury, while in the PRP group the level
301 of NF-κB-p65 was still significantly higher than in the C group, in the UT
302 group the NF-κB-p65 protein returned to approximately the same level as in
303 the C group. The trend of NF-κB-p65 was directly correlated to the IL-1α
304 trend (18).
305 The amplification and modulation of the first inflammatory phase induced by
306 PRP treatment may also be explained by the observed reduction of IL-10
307 levels following PRP injection. Indeed, the reduction of IL-10 levels results in

308 an increased macrophage recruitment, enhancing consequently the
309 inflammatory condition during the first 2 days following the treatment (27).
310 The observed increase of IL-4 and TNF-α levels at day 5 is a direct
311 consequence of the amplification of the early inflammatory response. Indeed,
312 macrophages recruited in the site of injury are subsequently activated,
313 producing additional chemoattractors, thus resulting in an increased leukocyte
314 recruitment in the site of injury. Conceivably, when considering the short half
315 life of these cytokines, it could be assumed that the observed increased levels
316 of IL-4 and TNF-α at day 5 after PRP treatment is the result of the synthesis
317 of these cytokines by the leukocytes recalled in the site of injury. This
318 hypothesis may explain the persistence of high levels of cytokines until 30
319 days after the treatment. Indeed, IL-4 is able to protect lymphoid cells from
320 apoptosis (28, 29), thus favoring the persistence of the amplified
321 inflammatory response. Furthermore, IL-6 levels were found increased only
322 in treated and contralateral muscles but not in untreated samples.
323 Conceivably, this finding supports the hypothesis of a stimulated IL-6
324 production by infiltrating lymphocytes and excludes its lesioned muscle
325 origin. This observation strongly supports the conclusions of previous studies
326 indicating that PRP injection induces an amplification and modulation of the
327 early inflammatory response resulting in an increase of the inflammatory
328 infiltration in the site of injury, data further confirmed by present study (2,
329 18). Indeed, changes in cytokine values were predominantly recorded at day 2
330 and day 5 with gradual reduction at day 30 after surgery with the exception of
331 IL-4 in treated group. This evidence suggests that the inflammatory response

332 in the treated group continued beyond 30 days after surgery, though to a
333 lesser extent.
334 The hypothesis of a possible systemic effect of locally injected PRP
335 preparation was effectively also confirmed by the results of the present study
336 since several analyzed molecules such as IL-10, IL-6, TNF-α and EGF in
337 contralateral muscles showed an intermediate behavior between treated and
338 untreated samples. The observation that none of the analyzed molecules
339 showed any statistically significant modification in the bloodstream following
340 PRP local administration reinforces the hypothesis that certain, not yet
341 identified, locally produced molecule may exert systemic effects being able of
342 modifying the inflammatory response of contralateral injured but not treated
343 muscles. The peculiarity of this investigation was to analyze "contralateral"
344 muscles (the same animal was injured on both limbs and only one was treated
345 with PRP while the other, the contralateral one, was left untreated) and
346 observe whether the treatment with locally injected PRP, may influence the
347 healing process even far from the site of injury. The results obtained using
348 this experimental model strongly suggested that local PRP treatment may
349 influence inflammatory responses even far from the site of injection. When
350 considering its application on athletes, no evidence supports the hypothesis of
351 possible action on non injured muscles, thus excluding possible muscle
352 performance enhancing properties. Certainly, the issue of the systemic effect
353 of locally injected PRP preparation needs further investigations.
354 In conclusion, the results of the present study confirmed that PRP treatment
355 influences the early inflammatory phase of the healing process. This

356 observation may have an immediate clinical translation. Indeed, the
357 demonstrated modulation of the inflammatory response may explain the pain
358 reduction usually observed after PRP administration and accounting for the
359 early mobilization of the patients (30). Moreover, suggests that an early
360 treatment after the injury may results in better clinical responses.
361 In literature, PRP has been studied *"in vitro "* and *"in vivo"* in the field of
362 maxillofacial surgery and general surgery, and more recently in muscle and
363 tendon healing but little is known about a possible systemic effect deriving by
364 the local use of PRP. Further experimental studies are needed in order to
365 understand the biological mechanisms at the base of the inflammatory process
366 following the local treatment with PRP preparations, focusing on which
367 mediators exert systemic effect. For example a microarray analysis could be
368 useful to investigate how IL-1α, IL-4, IL-6, IL-10, TNF-α, EGF, IL-5 and IL-
369 13 expression could be modulated in muscles. The easy reproducibility
370 achieved in this study has allowed us to create a solid foundation on which
371 future studies will be carried out in order to deeply understand the molecular
372 dynamics of the inflammatory process modulated by PRP administration. A
373 potential limitation of the present study was represented by the low number of
374 animals analyzed 30 days after surgery (2 animals in each experimental
375 condition). These data should be considered the result of a pilot study carried
376 out in order to obtain useful indications for future analysis aimed to analyze
377 the long-term effects of PRP preparations.
378 The little variation of number of rats in 2 day group and 5 day group depends
379 by accidental deaths probably caused by intracardiac drawing or anesthesia.

380 **ACKNOWLEDGEMENTS**

381 This study was supported by a grant from World Anti-Doping Agency
382 (WADA) and from the Italian ministry of health, Commission for the
383 surveillance of Doping (CVD).

384

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Attachment 1. Cytokines and growth factors in bloodstream at 2, 5 and 30 days after surgery.

IL-4	**2 days**	**5 days**	**30 days**	**IL-10**	**2 days**	**5 days**	**30 days**
C	<3.1	<3.1	<3.1	**C**	11.85±7.99	18.73±11.36	14.93±5.77
UT	<3.1	<3.1	<3.1	**UT**	14±8.34	16.74±10.93	17.75±10.22
TR	<3.1	<3.1	<3.1	**TR**	11.01±4.92	18.48±10.48	13.72±6.3
CL	<3.1	<3.1	<3.1	**CL**	12.68±5.70	16.75±11.31	13.38±9.94
IL-1α	**2 days**	**5 days**	**30 days**	**IL-6**	**2 days**	**5 days**	**30 days**
C	< 6.1	<6.1	<6.1	**C**	72.33±34.5	75.32±12.01	109.24±50.5
UT	<6.1	<6.1	<6.1	**UT**	50.02±10.93	82.88±35.53	90.17±40.20
TR	<6.1	<6.1	<6.1	**TR**	113.14±100.2	117±12.73	108±45.12
CL	<6.1	<6.1	<6.1	**CL**	97.59±80.90	58.30±21.48	112.41±19.2
TNF-α	**2 days**	**5 days**	**30 days**	**EGF**	**2 days**	**5 days**	**30 days**
C	7.82±4.09	6.26±1.01	6.55±2.82	**C**	<0.17	<0.17	<0.17
UT	4.47±2.89	5.80±1.62	4.65±1.22	**UT**	<0.17	<0.17	<0.17
TR	7.39±5.34	6.51±0.01	7.36±3.76	**TR**	<0.17	<0.17	<0.17
CL	7.34±1.18	4.61±1.41	6.06±0.64	**CL**	<0.17	<0.17	<0.17
G-CSF	**2 days**	**5 days**	**30 days**	**GM-CSF**	**2 days**	**5 days**	**30 days**
C	< 2.61	< 2.61	< 2.61	**C**	< 6.73	< 6.73	< 6.73
UT	< 2.61	< 2.61	< 2.61	**UT**	< 6.73	< 6.73	< 6.73
TR	< 2.61	< 2.61	< 2.61	**TR**	< 6.73	< 6.73	< 6.73
CL	< 2.61	< 2.61	< 2.61	**CL**	< 6.73	< 6.73	< 6.73
IL-5	**2 days**	**5 days**	**30 days**	**IL-13**	**2 days**	**5 days**	**30 days**
C	< 8.67	< 8.67	< 8.67	**C**	<4.77	<4.77	<4.77
UT	< 8.67	< 8.67	< 8.67	**UT**	<4.77	<4.77	<4.77
TR	< 8.67	< 8.67	< 8.67	**TR**	<4.77	<4.77	<4.77
CL	< 8.67	< 8.67	< 8.67	**CL**	<4.77	<4.77	<4.77

Table I. Cytokines and growth factors in muscles at 2, 5 and 30 days after surgery.

IL-4	**2 days**	**5 days**	**30 days**	**IL-10**	**2 days**	**5 days**	**30 days**
C	4.05±0.95	4.13±0.33	3.96±1.7	**C**	0.63±0.34	0.64±0.34	0.60±0.23
UT	2.30±0.61*	3.41±0.72	3.29±1.33	**UT**	0.06±0.05**	0.02±0.01	0.30±0.03
TR	1.77±0.45*	6.24±1.92*##	7.06±2.78*##	**TR**	1.09±0.41**##	0.46±0.21	0.61±0.04
CL	3.45±0.61*	3.63±0.91	2.78±0.82	**CL**	0.93±0.26*##	0.16±0.08*	0.29±0.17
IL-1α	**2 days**	**5 days**	**30 days**	**IL-6**	**2 days**	**5 days**	**30 days**
C	0.96±0.21	0.74±0.24	1,2±0,20	**C**	6.08±2.17	6.07±3.1	5.99±1.9
UT	0.14±0.08	0.23±0.08	1,32±0,06	**UT**	17.53±4.61	1.59±0.53	0.85±0.5
TR	6.66±1.33**##	3.41±0.51**##	1,27±0,07	**TR**	327.02±40.9**##	165.22±65.11**##	0.69±0.43
CL	1.32±0.54	0.94±0.38	1,05±0,18	**CL**	298.51±6.4**##	1.55±0.93**	0.47±0.39
TNF-α	**2 days**	**5 days**	**30 days**	**EGF**	**2 days**	**5 days**	**30 days**
C	0.11±0.03	0.10±0.03	0.12±0.02	**C**	1.66±0.05	1.66±0.07	1.65±0.06
UT	0.03±0.01*	0.09±0.03	0.02±0.02*	**UT**	1.19±0.12**	1.14±0.18**	0.88±0.07**
TR	0.13±0.03#	0.31±0.10**##	0.02±0.02*	**TR**	0.73±0.08**##	0.86±0.11**	1.16±0.11**
CL	0.09±0.05	0.14±0.08	0.06±0.02	**CL**	0.79±0.13**##	0.94±0.12**	0.75±0.06**

C: control group, UT: untreated group, TR: treated group, CL: contralateral group.

Multiple comparison Bonferroni's test: *p<0,05 vc C; **p<0,01 vs C; # p<0,05 vs UT; ## p<0,01 vs UT