Treatment of Muscle Injuries by Local Administration of Autologous Conditioned Serum: A Pilot Study on Sportsmen with Muscle Strains

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Abstract

Muscle injuries represent a major part of sports injuries and are a challenging problem in traumatology. Strain injuries are the most common muscle injuries after contusions. These injuries can lead to significant pain and disability causing time to be lost to training and competition. Despite the frequency of strain injuries the treatment available is limited and is generally not sufficient to enhance muscle regeneration efficiently when fast resumption of sport activity is a primary target. A number of growth factors play a specific role in regeneration and it has been proven that a previously described method of physically and chemically stimulating whole blood (to produce autologous conditioned serum) induces concentration increases in FGF-2, HGF, and TGF-β1. A preliminary study was conducted on muscle strain injuries in professional sportsmen receiving either: 1. autologous conditioned serum (ACS) or 2. Actovegin/Traumeel® treatment as control. Assessment of recovery from injury was done by: 1. sport professional's ability to participate to 100% under competition conditions in their respective sport and 2. MRI analysis. A significant difference in the recovery time from injury was demonstrated: 16.6 ± 0.9 in the ACS treated instead of 22.3 ± 1.2 (mean ± SEM) days in the Actovegin/Traumeel® control group ($p = 0.001$). MRI analysis supported the observed acceleration of the lesion recovery time. We conclude that ACS injection is a promising approach to reduce the time to recovery from muscle injury.

Key words
Growth factors · regeneration · FGF-2 · skeletal muscle

Abbreviations

ACS	autologous conditioned serum
bFGF	basic fibroblast growth factor (also FGF-2)
HGF	hepatocyte growth factor
IGF-1	insulin-like growth factor-1
IL-1β	interleukin 1 beta
IL-1Ra	interleukin 1 receptor antagonist
IL-7	interleukin 7
NGF	nerve growth factor
PDGF-AB	platelet derived growth factor
TGF-β1	transforming growth factor beta 1

Introduction

Muscle injuries account for up to 30% of the injuries sustained in sports events [7]. More than 90% of muscle injuries are caused either by excessive contusion or strain of the muscle [6,8]. In professional sport, some of these injuries can lead to significant pain and disability causing loss of training and competition time. Muscle strain may be a consequence of eccentric exercise, when the muscle develops tension during this type of lengthening contraction [25]. These injuries are especially common in sports that require sprinting or jumping [7].

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Despite the frequency of muscle injuries, the best treatment for strains is not clearly defined. The immediate treatment is known as the RICE principle as it includes rest, ice, compression, and elevation [12]. The target of this first aid principle is to minimize the formation of a large haematoma, which has a potential of affecting the size of scar tissue at the end of the repair process. Further therapy depends on the severity of the injury but the suggested treatments currently include NSAIDs (non-steroidal anti-inflammatory drugs), therapeutic ultrasound, hyperbaric oxygen, and operative treatment in the case of severe muscle ruptures with complete loss of function [12]. It has been suggested that the strength of the injured muscle should be 90% of the uninjured control side before the return to performance is safe [4]. Magnetic resonance imaging (MRI) has also been demonstrated as a good method for estimating recovery following muscle strain injury [4,19].

In the regeneration process several growth factors including IGF-1, PDGF, FGF-2, HGF, NGF, and TGF-β1 have been shown to play key roles [1,11,14]. Of these trophic substances, FGF-2, HGF, and TGF-β1 are thought to be key regulators in the chemotaxis and activation of muscle satellite cells [5,16,21,24]. For FGF-2 in particular, one source are the infiltrating macrophages during the inflammatory reaction [22] and another source are the muscle satellite cells themselves [13]. FGF-2 has also been shown to accelerate regeneration in animal experiments [14–16]. Thus, in conclusion, FGF-2 appears to play a major role in muscle regeneration.

A previously described method of physically and chemically stimulating autologous whole blood under specific conditions [17] has been tested here for a number of growth factors and cytokines. Because the resulting autologous conditioned serum (ACS) was found to have elevations in growth factors of interest for regeneration, we decided to conduct a preliminary trial on professional sportsmen with strain injuries. Prior promising results on the beneficial effects of ACS treatment on the regeneration of injured muscle in animal experiments [23] encouraged us to test this new method in patients.

Materials and Methods

Autologous conditioned serum (ACS)

Whole blood (50 ml) from each patient was withdrawn intravenously (without anti-coagulants) into 60-ml syringes (Perfusor Syringes, Becton Dickinson, USA). The syringes contained about 200 glass beads of medical grade each with 2.5 mm in diameter and a surface area of 21 mm^2 prepared as described in detail by Meijer et al [17]. The whole blood incubation was carried out aseptically at 37 °C, 5% CO_2 (Kendro, Germany). After 24 hours incubation, serum was retrieved and centrifuged (3500 rpm, 10 min, Megafuge, Kendro, Germany). The ACS was stored at – 20 °C until used. The control serum was not incubated but was centrifuged immediately as described above and similarly stored at – 20 °C until it was used in the ELISA test.

ACS ELISA tests

Growth factor and cytokine levels in the ACS were measured by ELISA in a pre-pilot study using the blood from 22 different subjects to see the effectiveness of the conditioning process. ELISA kits were purchased from R&D Systems (USA) and Biosource (USA) and employed according to the manufacturer's instructions. The growth factors tested were PDGF-AB, IGF-1, FGF, TGF-β, HGF, TNF-alpha, IL-7, IL-1Ra, and IL-1β.

ACS safety tests on serum

The presence of microbial contaminants (bacteria, fungi, and mycoplasm) and serological parameters (human immunodeficiency virus [HIV] 1 & 2, hepatitis B virus [HBV], hepatitis C virus [HCV] and Syphilis) in serum produced in syringes was assessed by external, accredited clinical laboratories.

Treated patient groups

The study conducted is a non-randomised and non-blinded pilot study. For years, the standard in our practice for the treatment of muscle strains has been a local injection of Actovegin®, a deproteinised dialysate from bovine blood, and Traumeel®, a homeopathic antiinflammatory drug with extracts of arnica, calendula, camomile amongst others. Thus our control group in this pilot study is a retrospective analysis of 11 patients treated with this Actovegin®/Traumeel® therapy [9,18].

Based on successful preclinical results in animal experiments [23], we started using the ACS in the treatment of muscle strains. The number of patients in this group was 18. All the patients treated with ACS signed an informed consent form. Table **1** shows the strained muscle groups as well as the practiced sport for all the patients in the study.

According to Jarvinen's classification of muscle strains [12], the patients included in both the control and the treatment group had muscle fiber tears defined as "moderate strains"(second degree). These moderate strains are characterized by a stretch injury with detection of a bleeding in the MRI scan and a moderate but not complete loss of strength.

Therapy regime and evaluation of recovery

The muscle strains were diagnosed by an MRI scan (Magnetom Symphony Quantum 1,5 Tesla, Siemens) latest one day after injury. Initial treatment was performed according to the RICE principle. For the ACS group, blood was taken from the patient imme-

Table **1** Types of muscle strains treated

Strained muscle	*Autologous conditioned serum patients*	*Actovegin®/ Traumeel® control patients*
Hamstring	6 (3 FB, 2 BB, 1 IH)	5 (4 FB, 1 IH)
Adductor	6 (2 FB, 1 BB, 3 IH)	4 (2 FB, 2 IH)
Iliopsoas	2 (FB)	1 (FB)
Gluteus	1 (IH)	0
Abdominal oblique	1 (IH)	0
Gastrocnemius	1 (BB)	1 (FB)
Rectus femoris	1 (FB)	0

FB: football (soccer); BB: basketball; IH: ice hockey

Table **2** Recovery time after moderate muscle strains in professional sportsmen

Strained muscle	*Recovery time (days) in autologous conditioned serum group*	*Recovery time (days) in control Actovegin®/ Traumeel® group*
Hamstring	12, 14, 16, 17, 18, 21	16, 18, 23, 24, 28
Adductor	10, 15, 17, 18, 21, 23	19, 24, 25, 26
Iliopsoas	17, 21	24
Gluteus	20	
Abdominal oblique	8	
Gastrocnemius	14	18
Rectus femoris	16	
Mean	16.6	22.3
SE	0.9	1.2

diately after MRI diagnosis and prepared as described above. Injection treatment started two days after diagnosis. 2.5 ml of the ACS was extended by 2.5 ml of saline in a 5-ml syringe to have an appropriate volume for distribution into the injured area. A bacterial filter (Millex-MP, 0.22 µm Filter Unit, Millipore S.A., Molsheim, France) was placed between syringe and needle. Another syringe was filled with 5 ml of local anaesthetic (Meaverin 0.5%). After the muscle injury was palpated and its extension marked, the local anaesthetic was injected in portions of 1 ml by placing 5 needles (27 G × 1 1/2") covering the injured area. The local anaesthetic was used to minimize the tonus of the muscle close to the injury and thus to prevent muscle fiber shortening. Using these same needles, the serum was then injected in portions of 1 ml. The treatment started two days after the diagnosis and was then administered every second day. The mean number of treatments per patient was 5.4.

In the control group, 3 ml of Actovegin® were mixed with 2 ml of Traumeel® in a 5-ml syringe. The principles of application were the same as those in the ACS group, starting two days after diagnosis and being repeated every second day. The mean number of treatments with Actovegin®/Traumeel® was 8.3.

In addition to the injections, the patients of both groups underwent the same rehabilitation program with lymph drainage, mild stretching and massage. Exercise was allowed in a pain free range. Furthermore, the patients took oral antiphlogistics on a natural base ("Phlogenzym" tablets from MUCOS Pharma; components: Bromelain 90 mg, Trypsin 48 mg; Rutosid $3H_2O$ 100 mg).

The success of the treatment was evaluated by the sport professional's ability to participate to 100% under competition conditions in their respective sport. The decision here was a combination of the physiotherapist's standard examination and the subjective judgement of the individual sport professional. The examination by the physiotherapist included isokinetic tests to confirm that muscle-strength imbalances had been corrected and the strength of the injured limb had been restored to at least

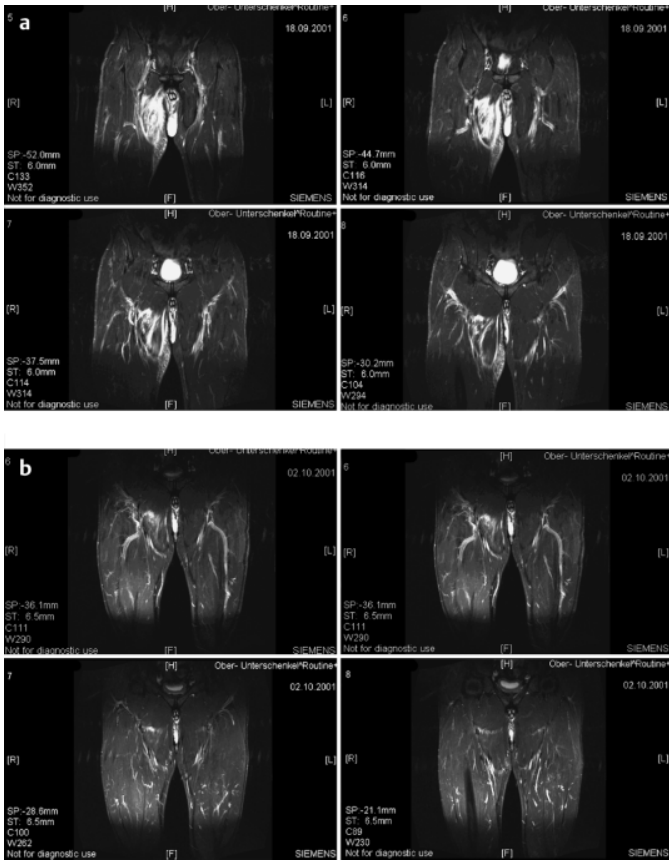

Fig. **1 a** and **b** MRI scans of a patient treated with the autologous conditioned serum (ACS). **a** At diagnosis, the patient presented with an adductor strain with important oedema and bleeding into the muscle. **b** MRI at 14 days after treatment showed a nearly complete regression of the initial diagnostis findings and a restitution of the muscle tissue. Furthermore this sportsman had recovered 100% of the muscle function.

90% of that of the unaffected limb muscle. To visualize the success of the treatment, a control MRI scan was implemented between the 14th and the 16th day after injury for the patients of both groups.

Statistical analysis

All results shown here are the means ± SEM. The statistical significance of differences was determined by Student's *t*-tests.

Results

Autologous conditioned serum group

In this group we have treated 18 professional sportsmen with muscle strains as defined above. Table **2** shows the number of days taken till recovery and return to 100% performance level. The average time to recovery was 16.6 days (± 0.9). The control MRI scans taken at 14 – 16 days after injury showed a nearly complete regression of the findings in the first scan concerning oedema/bleeding into the muscle and restitution of the muscle structure (Fig. **1**). There were neither local (allergic reactions, inflammation) nor systemic side effects seen by treating the patients with the ACS.

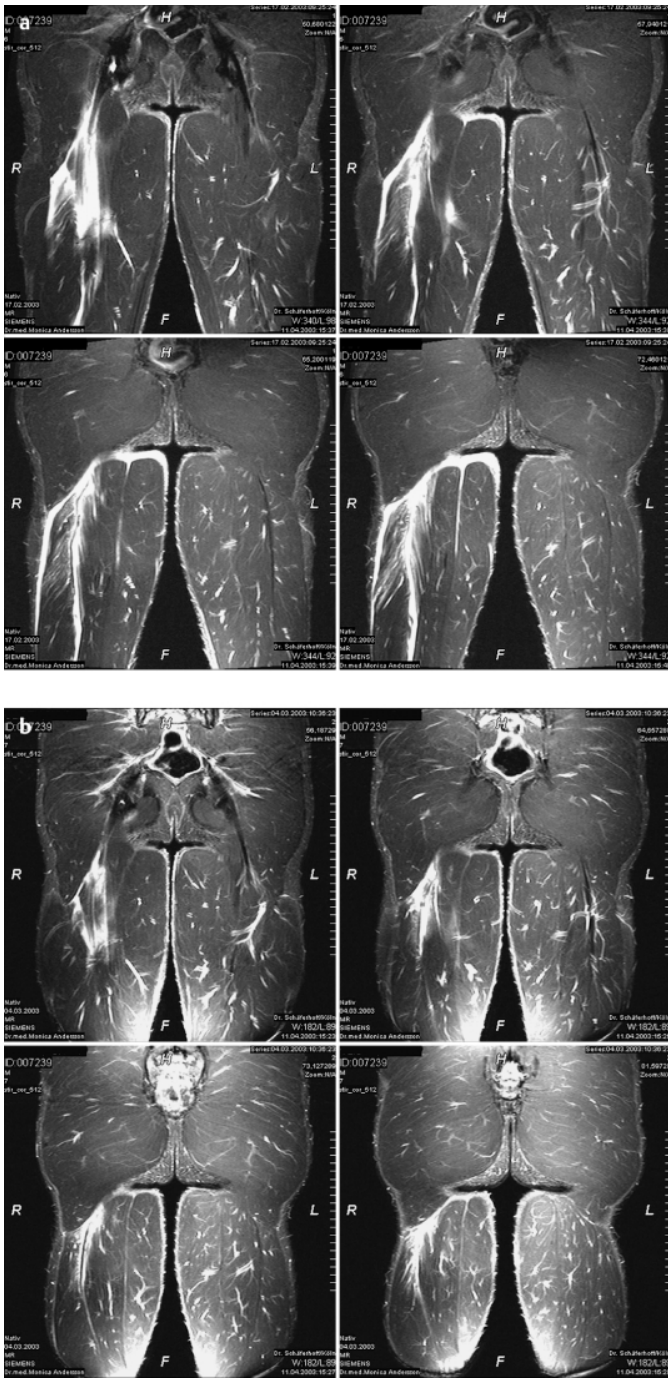

Fig. **2 a** and **b** MRI scans of a patient treated in the control group with Actovegin ®/Traumeel®. **a** At diagnosis, the patient presented with a hamstring strain with important oedema and bleeding into the muscle. **b** MRI at 15 days after treatment with Actovegin ®/Traumeel® showed only a mild regression of the initial diagnostis findings. The sportsman had only recovered 70% of the muscle function.

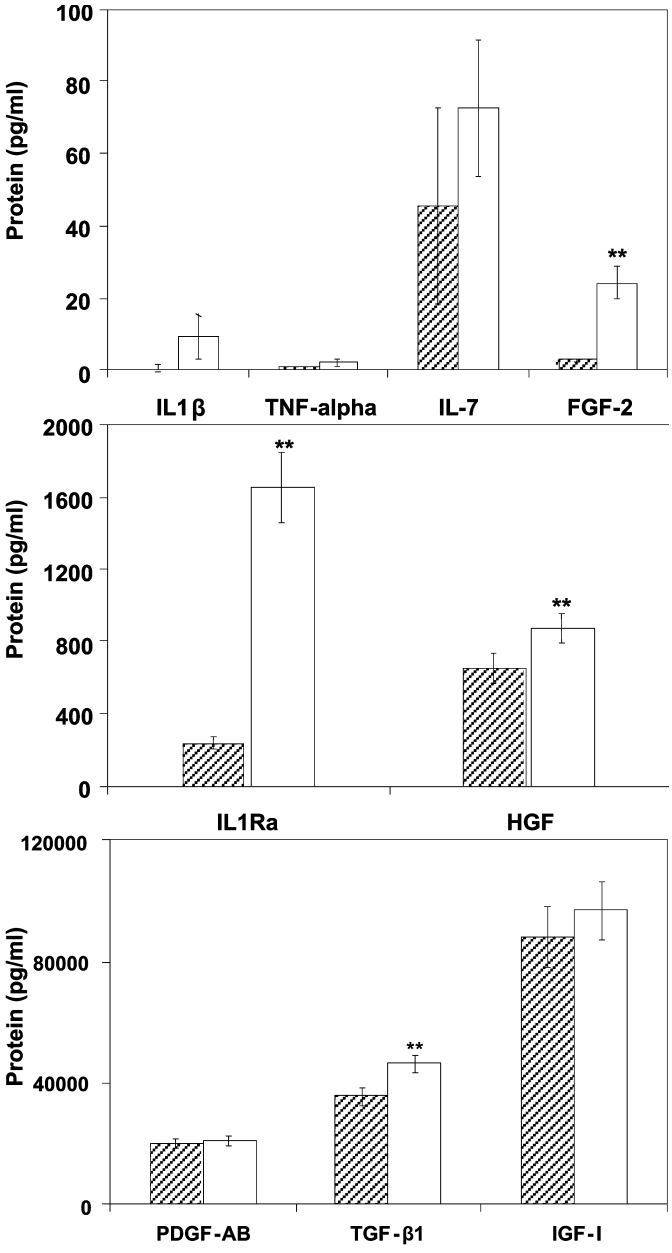

Fig. **3** Levels of growth factors in human conditioned serum. The levels (pg/ml) of IL1-β, TNF-alpha, IL-7, FGF-2, IL-1Ra, HGF, PDGF-AB, TGF-β1, and IGF-1 in human serum samples (n = 22) measured before (filled bars) and after (open bars) the 24-hour incubation in the bead containing syringes are reported as means ± SEM. ** $p < 0.001$, * $p < 0.05$.

Control group

In this group we have treated 11 professional sportsmen with muscle strains as defined above. The average time to recovery was 22.3 days (± 1.2) (Table **2**). The control MRI scans taken at 14 – 16 days after injury showed only a mild regression of the findings in the first scan concerning oedema/bleeding into the muscle (Fig. **2**). There were neither local (allergic reactions, inflammation) nor systemic side effects seen by treating the patients with the Actovegin®/Traumeel®.

ELISA tests

The results of the ELISA tests (Fig. **3**) performed on the human ACS showed an increase in the concentration of FGF-2 (750%), IL-1Ra (600%), HGF (35%), and TGF-β (31%). IGF-1, PDGF, IL1β, and IL7 concentrations were also slightly increased but not significantly. No change in TNF-alpha concentration was found.

Discussion

This preliminary study shows that the functional recovery time after moderate muscle fibre strains (second degree strains) is accelerated with the use of the ACS in comparison to the use of Actovegin®/Traumeel® control therapy. The target in the treatment

of these injuries was to reduce the recovery time and by this allow a fast return to 100% performance level. Apparently, it would have been desirable to have collected data on muscle strength recovery, but strength recordings during the recovery period would have included the risk of reinjury. We therefore depended on the athletes' subjective impression of their readiness to resume exercise at competitive level.

The MRIs showed a nearly complete regression of the oedema and bleeding and a restitution of the muscle tissue in the patients of the ACS-treated group 2 weeks after the injury. On the other hand, in the control group only a mild regression of the oedema/bleeding was observed in the MRI at the same time point. Regarding the return to 100% performance level, the treated group showed an acceleration of about 6 days or a 30% benefit over the control group (16.6 ± 0.9 vs. 22.3 ± 1.2 days). Still, it must be noted here that although the control study MRIs at day 14 to 16 showed some oedema/bleeding, these patients could perform up to 70 – 80% of their preinjury sports performance level. However, returning to full competition under such conditions should impose the risk of a reinjury.

The clinical performance results are also supported by the ELISA data on the ACS and by the histological findings we have reported in mice [23]. Of particular interest are the significant increases in the FGF-2 (750%, $p > 0.05$), TGF-β1 (31%, $p > 0.05$), and HGF (35%, $p > 0.05$) concentrations that were found in the conditioned serum. IL-1Ra was also increased significantly (600%, $p > 0.05$) and although this cytokine is not mentioned in the literature as being of interest for muscle regeneration, we could speculate over a possible role in the competitive inhibition of the inflammatory cytokine IL-1α, thus favouring myogenic differentiation [10].

Similar important increases for FGF-2 and similar moderate increases for TGF-β1 were also seen in a mouse injury model treated with ACS [23]. We assume that these elevations are the result of the manipulation of the blood with the glass beads [17]. IGF and PDGF-AB which also play a role in muscle regeneration [11] showed no significant increase of concentration. The same was true for the cytokines, IL-β, TNF-alpha, and IL-7, for which, however, no particular influence on the regeneration process has been suspected until now.

Thus because of the well described properties of FGF-2 in muscle regeneration, in particular as a chemoattractive agent and a mitogen to satellite cells [2,20,24], the important increase in the FGF-2 concentration (750%) observed in the ACS can explain the efficacy of the local injection of this treatment.

In conclusion, we report that the autologous conditioned serum shortened the time to recovery after strain injury in a preliminary study group of professional sportsmen. These results were confirmed by MRI scans. The conditioned serum contains increased levels of growth factors which are involved in regeneration, mostly FGF-2, with mild elevations in HGF and TGF-β1. These results are sustained by animal experiments in a mouse model of muscle injury [23] where it was demonstrated histologically that at 1 week after injury, the ACS increases the diameter of the centronucleated regenerating cells, which is a sign of faster regeneration.

Therefore the treatment of strain injuries with ACS – along with the utilisation of an adequate post-injury muscle strengthening program – may be a promising way of returning athletes to full activity and full muscular function in a shorter period of time than with conventional treatment. Furthermore, this method is feasible, simple and, because of the autologous character, has no side effects.

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