

Review Article

Effect of Actovegin® Versus Cortisone on PMA-Induced Inflammation on Human Cells

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Abstract

Purpose: The effect of Actovegin® versus Cortisone was investigated on PMA induced human Peripheral Blood Mononuclear Cells (PBMCs).

Methods: PBMCs (1 × 10 cells/ml) from ten blood donors (5 f, 5 m; 45–55 years) were grown in medium and exposed to Actovegin® or Cortisone in the presence or absence of PMA. Supernatants were collected to assess the concentration of cytokines/substances: IL-6, TNF- α , IL-8, IL-1 beta, and IL-10. The Reactive Oxygen Species (ROS) were assessed by a ROS-GloTM H₂O₂ assay.

Results: Stimulation of cells with PMA (1 μ g/ml) (without Actovegin® or Cortisone) significantly ($p < 0.05$) increased the secretions of IL-6, TNF- α , IL-8, IL-1 beta, and IL-10 from PBMCs, compared to controls (without PMA). Addition of Actovegin® (20 μ g/ml) or Cortisone (0.7 and 7 μ g/ml) plus PMA significantly decreased the secretion of TNF- α , compared to controls (without Actovegin® or Cortisone). Addition of Actovegin® (1 and 20 μ g/ml) plus PMA significantly decreased the secretion of IL-8, compared to controls (without Actovegin®). However, addition of Cortisone (0.7 and 7 μ g/ml) plus PMA did not influence the secretion of IL-8, compared to controls (without Cortisone). Addition of Actovegin® (20 μ g/ml) plus PMA significantly decreased the secretion of IL-1Beta, compared to controls (without Actovegin®). Addition of Cortisone (0.7 μ g/ml) plus PMA increased the secretion of IL-1Beta, compared to controls (without Cortisone). Addition of Actovegin® (20 μ g/ml) plus PMA significantly increased the secretion of IL-10, compared to controls (without Actovegin). However, addition of Cortisone (0.7 and 7 μ g/ml) plus PMA did not influence the secretion of IL-10, compared to controls (without Cortisone). Addition of Actovegin® (20 μ g/ml) plus PMA significantly decreased the ROS formation, compared to controls (without Actovegin®). However, addition of Cortisone (0.7 and 7 μ g/ml) plus PMA did not influence the ROS formation, compared to controls (without Cortisone).

Conclusion: Both, Actovegin® and Cortisone, have anti-inflammatory effects on human cells. These findings may help to explain the clinically known positive effects of Actovegin® on athletic injuries with inflammatory responses (e.g., muscle injuries, tendinopathies). These results further indicate that Actovegin® can supply valuable components for the formation and function of an efficient antioxidative and/or anti-inflammatory system, which may contribute to the reduction of an inflammation and in some inflammation factors even superior to Cortisone.

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Abbreviations: MRI– Magnetic Resonance Imaging; NADPH– Nicotinamide Adenine Dinucleotide Phosphate; NLRP3– Nucleotide-Binding Oligomerization Domain-Like Receptor Containing-Pyrin Domain 3; PAMP– Pathogen-Associated Molecular Pattern; PBMCs– Human Peripheral Blood Mononuclear Cells; PKC– Protein Kinase C; PMA Phorbol 12-Myristate 13-Acetate; ROS– Reactive Oxygen Species; TLR4– Toll-like Receptor 4

WADA– World Anti-Doping Agency

Introduction

Cortisone is a corticosteroid and it has been used as a medical drug for the past 70 years in the treatment of various musculoskeletal conditions. This includes its use for joint pain such as inflammation reactions, rheumatoid arthritis, osteoarthritis, and many others. There are numerous review articles to corticosteroids (cortisone) including the history, the effectiveness, and adverse effects in the treatment of joint pain [1,2]. The current evidence would suggest that the use of corticosteroids provides moderate evidence for short-term pain reduction and improvement in function. There are multiple potential adverse effects, such as toxic damage to articular cartilage, as well as numerous systemic side effects, including increase in blood glucose levels, a reduction in immune function and an increased risk of infections [1,2].

Actovegin® is also a medical drug obtained from natural calf blood. Over 60 years, many medical indications are treated by Actovegin®, e.g., acute stroke [3,4] or postpartum hemorrhage (intravenous infusion) [5], skin ulcers (topical medication) [6], and long bone fractures (intraarterial infusion) [7], malfunction of the blood circulation in the brain and trophic disturbances (e.g., ischemic insult, craniocerebral injury) [8], impairment of peripheral blood circulation (e.g., angiopathy and ulcus cruris) [7,9,10], wound healing issues (e.g., torpid wounds, decubitus) [7,11-13] and mucosal lesions after radiation [7,14-17].

Muscle injury incidence varies from 30 to 55%; therefore, it is one of the most common sports-related injuries [18-20]. Twelve percent of all muscle injuries are hamstring injuries, which are 2.5 times more frequent than, for example, quadriceps injuries [21,22]. It has been shown that muscle healing can be promoted by administration of anti-inflammatory drugs [23]. However, anti-inflammatory drugs also can have an adverse effect on the entire healing process [24]. Moreover, a recent systematic review illustrates the potential myotoxicity of local anesthetics and non-steroidal anti-inflammatory drug injection, while there is no evidence that Actovegin® has such a side effect [25]. A variety of treatments such as growth factor injection therapy is still

very experimental and has shown initial results in some pilot studies; however, due to their performance enhancing and anabolic properties, they are prohibited by the World Anti-Doping Agency (WADA) [26].

In 1990 Pfister and Koller [27] first described intramuscular injection of Actovegin® as treatment of muscle injuries in a partially blinded case control study with 102 patients [27]. Their study showed a reduction in recovery time in a treatment group of 5.5 weeks, compared to 8.3 weeks for the control group [27]. However, in this study, the diagnosis of specific muscle injuries was only based on clinical findings and was not graded according to imaging, e.g., Magnetic Resonance Imaging (MRI). Furthermore, Actovegin® was mixed with anesthetics before injection resulting in pharmacodynamic and pharmacokinetic alterations [27].

In vivo and in vitro studies suggest that Actovegin® contains some active components, although they were not identified [6,28-30]. In a previous in vitro study, an enhancement of the mitochondrial oxidative phosphorylation was registered in permeabilized human muscle fibers (obtained from overweight and untrained subjects) acutely exposed to Actovegin® [31]. Hitherto, the effect of stand-alone Actovegin® therapy in muscle precursor cells highly relevant in skeletal muscle regeneration was not investigated in vivo and/or in vitro studies. To investigate effects of various substances/solutions on muscle precursor cell proliferation, optimal experimental conditions are represented by C2C12 muscle cells [30]. In our recent study, the effect of a stand-alone Actovegin® addition on the proliferation of C2C12 muscle cells was described, and Actovegin® increased the proliferation of muscle cells [32]. Furthermore, in this study the ingredients of Actovegin® were identified and the active substances on muscle proliferation were discussed in detail [32].

There is much media attention and there are many anecdotal beliefs regarding Actovegin® injection therapy. In the lay press, controversial discussions between proponents and opponents have been published in recent years regarding the use of Actovegin® in high performance athletes. In our recent study a risk assessment was given and it could be demonstrated that Actovegin® may not be classified as a doping agent [32]. Furthermore, some clinical studies for Actovegin® confirm its safety [28,33,34]. The effect of anti-inflammatory drugs on muscle regeneration is controversial discussed. It was described that anti-inflammatory drugs can improve muscle regeneration by reducing degeneration and inflammation [23]. However, in other studies, it was described that anti-inflammatory drugs are not conducive to the healing process [24,35].

Human Mononuclear Cells of the Peripheral Blood (PBMCs) are a useful tool to investigate anti-inflammatory effects of substances or antigens, as these immune cells of the

peripheral blood actively participate in the healing processes after inflammation [36,37].

In the present study the effects of Actovegin® versus Cortisone were investigated on inflammation reactions on human PBMCs. Our hypothesis was that Actovegin® and Cortisone have anti-inflammatory effects on human cells.

Materials and Methods

Cell culture and cell exposure

Stimulation assays were performed according to Summer et al. [36], with the optimizations reported by Ständer et al. [38]. Heparinized blood was taken from anonymized healthy blood donors (five females, five males, 45 – 55 years, non-smokers, no drug administration, no medication). After isolation of Peripheral Blood Mononuclear Cells (PBMCs) by density centrifugation, PBMCs of each blood donor were separately cultivated with Phorbol 12-Myristate 13-Acetate (PMA) (1 µg/ml, Sigma-Aldrich, Munich, Germany) with or without Actovegin® or Cortisone in different concentrations in quadruplicate. Cells (1 × 10⁶ cells/ml) were grown in RPMI 1640 medium in 96-well plates at 37°C for 24 h. Actovegin® (200 mg/5 ml; Lot-No. 10946788; Takeda Austria GmbH, Linz, Austria) was directly diluted in cell culture medium (1 and 20 µg/ml) or to Cortisone (0.7 and 7 µg/ml), exactly as described in a recent investigation on muscle cell proliferation [32]. Cell cultures were exposed to these Actovegin® or Cortisone concentrations in the presence or absence of PMA (1 µg/ml) for 24 h. After the exposure, culture supernatants were collected for cytokine/substance analysis.

The ten blood donors were healthy individuals with normal blood cell counts with 1500–3000 lymphocytes/µl blood and 280–500 monocytes/µl blood. As for the healing process after inflammation all blood cells support the healing process we wanted to simulate a quite physiological situation with all mononuclear blood cells as already described in our previous study [39].

Cytokine assays

The amount of IL-6, TNF-α, IL-8, IL-1 beta, and IL-10 was assessed by a multiplex cytometric bead assay according to the manufacturer's protocol (BD, Biosciences, Heidelberg, Germany) in a FACS Canto flow cytometer.

ROS assessment

The Reactive Oxygen Species (ROS) were assessed in an identical experimental assay by ROS-Glo™ H2O2

Assay (Promega, Mannheim, Germany) according to the manufacturer protocol.

Statistical analyses

Individual data from independent experiments were now summarized as medians (25–75% quartiles). Statistically significant differences between mean values were calculated using now the one-way ANOVA-Test followed by Games Howell post hoc test (SPSS Statistics 23, IBM, Armonk, NY, USA). The level of statistical significance was set to $p < 0.05$.

Results

Stimulation of cells with PMA (1 µg/ml)(without Actovegin® or Cortisone) significantly ($p < 0.05$) increased the secretions of IL-6, TNF-α, IL-8, IL-1 beta, and IL-10 from PBMCs, compared to controls (without PMA) (Table 1).

Addition of Actovegin® (1 and 20 µg/ml) or Cortisone (0.7 and 7 µg/ml) plus PMA did not influence the secretion of IL-6, compared to controls (without Actovegin® or Cortisone) (Figure 1).

Addition of Actovegin® (20 µg/ml) or Cortisone (0.7 and 7 µg/ml) plus PMA significantly ($p < 0.05$) decreased the secretion of TNF-α, compared to controls (without Actovegin® or Cortisone) (Figure 2).

Addition of Actovegin® (1 and 20 µg/ml) plus PMA significantly ($p < 0.05$) decreased the secretion of IL-8, compared to controls (without Actovegin®). However, addition of Cortisone (0.7 and 7 µg/ml) plus PMA did not influence the secretion of IL-8, compared to controls (without Cortisone) (Figure 3).

Addition of Actovegin® (20 µg/ml) plus PMA significantly ($p < 0.05$) decreased the ROS formation, compared to controls (without Actovegin®). However addition of Cortisone (0.7 and 7 µg/ml) plus PMA did not influence the ROS formation, compared to controls (without Cortisone) (Figure 6).

Discussion

Until today, the effect of anti-inflammatory drugs on muscle healing after injury is controversially discussed: Anti-inflammatory drugs can improve muscle regeneration by reducing degeneration and inflammation [23], in contrast, it is described that anti-inflammatory drugs are not conducive to the healing process [24,35].

Table 1: Cytokine and ROS formation of PBMC after stimulation with PMA for 24h (without Actovegin® or Cortisone) (mean ± sem, n=10). rlu = relative light unit (luminescence).

	IL-6	TNF alpha	IL-8	IL-1 beta	IL-10	ROS
Medium	0.0 pg/ml	0.0 pg/ml	1319 pg/ml ± 1000	0.0 pg/ml	0.0 pg/ml	212160 rlu ± 2345
PMA	783 pg/ml ± 124	678 pg/ml ± 39	11968 pg/ml ± 2322	755 pg/ml ± 358	1.2 pg/ml ± 1.0	1096560 rlu ± 4521

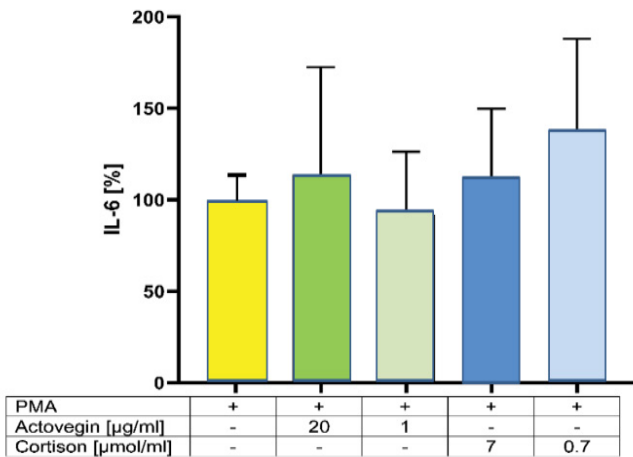


Figure 1: IL-6 formation of PBMC after stimulation with PMA (set as 100%) and addition of Actovegin vs. Cortisone in two different concentrations (mean ± sem, n=10).

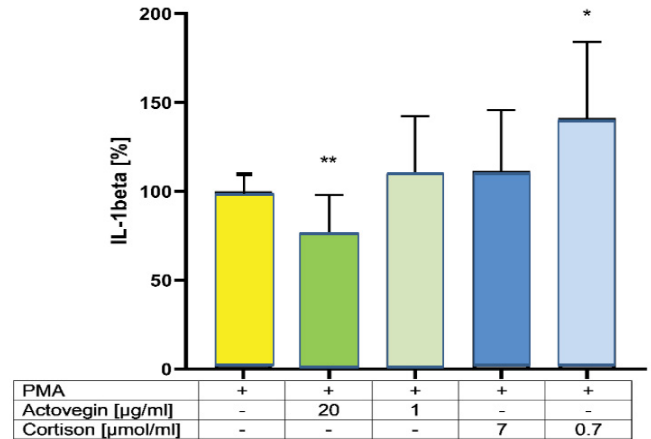


Figure 4: IL-1beta formation of PBMC after stimulation with PMA (set as 100%) and addition of Actovegin vs. Cortisone in two different concentrations (mean ± sem, n=10; * p<0.05, **p<0.01).

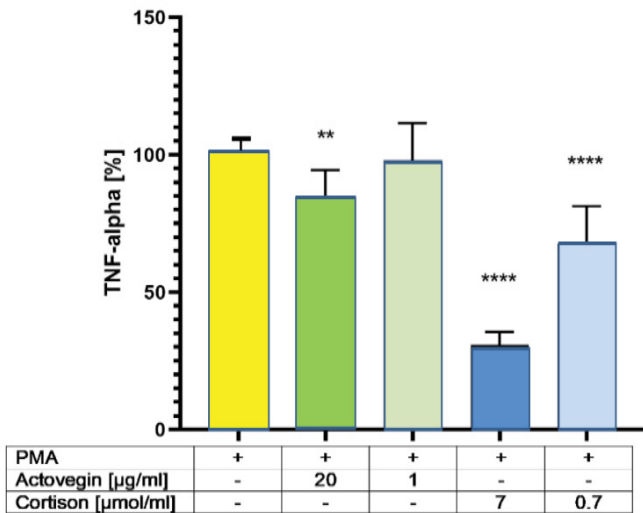


Figure 2: TNF-Alpha formation of PBMC after stimulation with Phorbol 12-Myristate 13-Acetate (PMA) (set as 100%) and addition of Actovegin vs. Cortisone in two different concentrations (mean ± sem, n=10; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

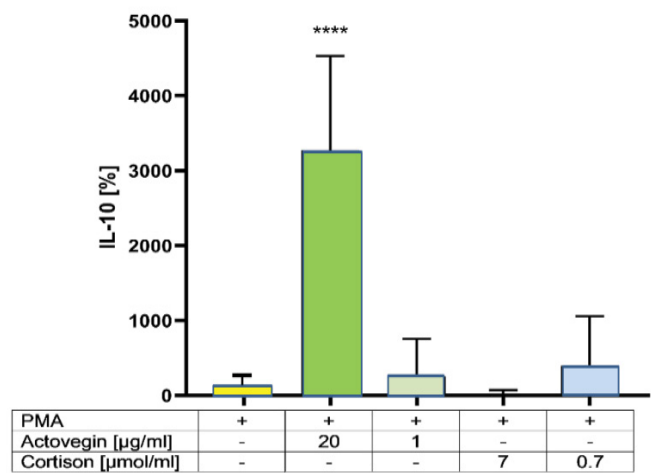


Figure 5: IL-10 formation of PBMC after stimulation with PMA (set as 100%) and addition of Actovegin vs. Cortisone in two different concentrations (mean ± sem, n=10; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

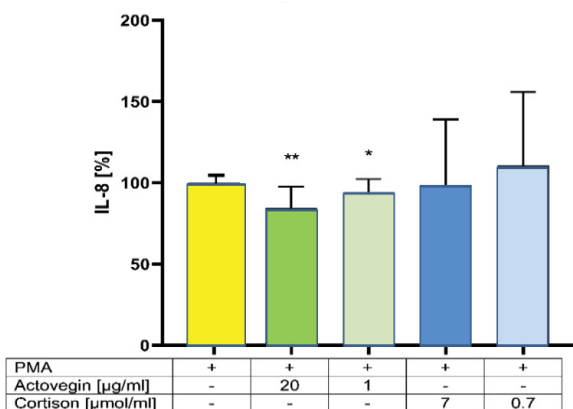


Figure 3: IL-8 formation of PBMC after stimulation with PMA (set as 100%) and addition of Actovegin vs. Cortisone in two different concentrations (mean ± sem, n=10; *p<0.05, **p<0.01).

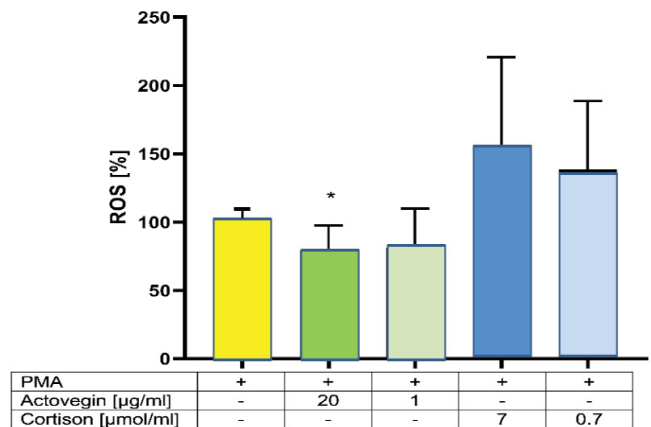


Figure 6: ROS formation of PBMC after stimulation with PMA (set as 100%) and addition of Actovegin vs. Cortisone in two different concentrations (mean ± sem, n=10; *p<0.05).

PBMCs are a useful tool to investigate inflammation reactions. PBMCs (i.e., lymphocytes and monocytes) play a central role in muscle repair and regeneration during the inflammation that follows muscle injury. Therefore, we used in our model the PBMCs as described in previous study [40]. Here, we study the effect of Actovegin® versus Cortisone in Phorbol 12-Myristate 13-Acetate (PMA)-induced inflammation on human PBMCs.

PMA is a potent tumor promoter via activation of the signal transduction enzyme Protein Kinase C (PKC) [41-43]. The PKC pathway is critically involved in function and development of B cells, as well [44]. PMA is routinely used as an inducer for endogenous superoxide production, as well, since it has been demonstrated to induce superoxide as the major ROS [45,46]. ROS are generated by NADPH oxidase in a process called the respiratory burst. This key enzyme catalyzes the generation of superoxide and hydrogen peroxide using electron provided by the hexose monophosphate shunt [47-49]. NADPH oxidase of phagocytic cells can also be activated by protein kinase C agonists such as PMA [50].

PMA initiates acute inflammatory responses in mammals that are characteristic for the host reaction to tissue injury and/or infection [51].

Tissue response following injury or surgical trauma involves participation of blood-derived components, cytokines and growth factors. PMA induces high cytokine responses in PBMCs after 24 h stimulation including proinflammatory cytokines as IL-1beta, IL-6, IL-8 and TNF- α but also anti-inflammatory cytokines as for example IL-10 and is, therefore, widely used as a suitable positive control in *in vitro* PBMCs stimulation experiments, where high cytokine production is expected and where freshly isolated PBMCs are used [52,53].

Wu et al. [54] described IL-1 beta, IL-6, IL-8, IL-10 or TNF- α as important inflammatory cytokines/substances. In the present study the effects of Actovegin® versus Cortisone on the release of these cytokines/substances were investigated in PMA-stimulated human PBMCs. The PMA-induced increase in the secretion of the pro-inflammatory cytokine IL-1 beta was dose dependently inhibited by addition of Actovegin® but dose dependently increased by Cortisone. Whereas the secretion of the pro-inflammatory TNF- α was dose-dependently decreased with both, Actovegin® and Cortisone. However, the secretion of the anti-inflammatory cytokine IL-10 was significantly increased by addition only with Actovegin® but not with Cortisone. Oxidative damage plays a key role in inflammatory reactions and can induce several injuries (e.g., septic shock, atherosclerosis) induced by PMA which is known to enhance the formation of Reactive Oxygen Species (ROS) [46,55]. PMA can induce the activation of TLR4 in the cell wall, which can induce ROS, e.g., superoxide [46]. In the present study a dose

dependent decrease of ROS was observed after addition with Actovegin®, but not with Cortisone.

Two questions are arising from our results:

- 1) what is the mechanism of Actovegin's® effect of ROS formation?
- 2) why is there a significant effect of Actovegin® on IL-1beta, IL-8, and IL-10 production but not with cortisone?

Ad 1 (ROS formation)

Possible explanations are that Actovegin® may support the antioxidative systems in the cells consisting of enzymatic and non-enzymatic antioxidants. Enzymes like catalase and substances such as glutathione as the major non-enzymatic antioxidant reduce oxidative stress by decreasing the levels of Reactive Oxygen Species (ROS). Concentrations of ROS are crucial in the formation of inflammatory cytokines such as IL-1beta and IL-6 as well [56]. In our recent study high levels of cystathionine in Actovegin® were detected, compared to the human adult serum/plasma [32]. Cystathionine is a precursor of cysteine synthesis which in turn is a component of the tripeptide glutathione. Both cysteine and glutathione contain sulfhydryl-groups and can, therefore, effectively act as antioxidants [57]. Thus, an antioxidative effect of Actovegin® may be at least in part explained by the high availability of cystathionine.

Another antioxidative system represents the enzyme catalase (oxidoreductase). Human catalase is a peroxisomal enzyme. It is implicated in inflammation, ethanol metabolism, apoptosis, aging and cancer [58]. It is a common enzyme found in nearly all living organisms predominantly in the liver, kidney and erythrocytes [58]. It is a very important enzyme in protecting the cell from oxidative damage by Reactive Oxygen Species (ROS). Catalase has one of the highest turnover numbers, each second one molecule can convert millions of hydrogen peroxide molecules to water and oxygen [58]. Superoxide is also biologically toxic and is employed by the immune system to kill invading microorganisms. Superoxide can be converted in cells into hydrogen peroxide which can further be catalyzed by catalase. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long, with four iron-containing heme groups that allow the enzyme to react with the ROS. Most intersubunit contacts are confined to the amino-terminal arms and the wrapping domains. The amino-terminal domain becomes almost completely buried between neighboring subunits in the tetramer. There are numerous salt bridges at the interfaces between monomers, mostly involving glutamic acid, asparagine acid, and arginine [59,60]. In our recent study high levels of glutamic acid, asparagine acid, and arginine in Actovegin® were detected, compared to the human adult serum/plasma [32]. The antioxidative effect of Actovegin® may be explained by the high availability of these Actovegin® ingredients. Therefore,

the observed antioxidative effect of Actovegin® may be better understandable.

Ad 2 (differential effects after Actovegin® or Cortisone addition)

PMA has been shown to exert its well-known effect on monocyte differentiation via caspase-1 activation [61]. Ribeiro and colleagues [62] could further demonstrate that activated Protein Kinase C (PKC) further activates mTORC1/S6K pathway [62]. This observation is important for the interpretation of our results, since PMA is a potent activator of PKC [63]. PMA is known to promote tumor outgrowth through activation of serine/threonine-specific protein kinases. These serine/threonine-specific protein kinases appear to be responsible for the abnormal phosphorylations of CD23 protein in healthy B cells, as well [64]. Antagonism of this mechanism might be relevant for the effect of Actovegin® as well.

In this study a significant decrease was found only for the pro-inflammatory cytokines IL1-beta and IL-8 after addition of Actovegin® with PMA, compared to the release without Actovegin®, but not with addition of cortisone. In contrast the addition of cortisone even increased the IL-1beta formation, compared to Actovegin® addition.

IL-1beta is a key inflammatory mediator driving the host response to infection, injury, and disease. IL-1beta driven inflammation has often disastrous consequences, and thus represents a therapeutic target [65]. Caspase 1 is activated by recruitment to a molecular platform called an inflammasome [66] and caspase 1 is considered to belong to the inflammatory group [67]. Inhibition of caspase 1 would be anti-inflammatory by preserving cell viability and, therefore, limiting the release of Pathogen-Associated Molecular Pattern (PAMPs), consequently resulting in less inflammation [68]. Inhibition or deletion of caspase 1 improves, e.g., outcome after myocardial infarction [69-71]. Studies describing clinical use of anti-IL-1 therapies focus almost on the use of biologicals such as IL-1Ra (anakinra) or anti-IL-1b antibodies such as canakinumab and other substances [72] but were not successful [65].

ICEberg is a protein that inhibits generation of IL-1beta by interacting with caspase-1 [73]. ICEberg is induced in human cells by pro-inflammatory stimuli, suggesting that it may be part of a negative feedback loop. Consistent with this, enforced retroviral expression of ICEberg inhibits IL-1beta generation [54]. The distribution of surface charge is complementary to the homologous prodomain of caspase-1, suggesting that charge-charge interactions mediate binding of ICEberg to the prodomain of caspase-1 [74]. Humke et al. [74] detected in ICEberg in main domains (helix 1-6) the following aminoacids: Arginine (Arg), Lysine (Lys), Glutamic acid (Glu) and Asparagine acid (Asp). The surface of ICEberg contains three highly charged patches [74]. In

our recent study it could be demonstrated that Actovegin® contains many physiological substances in significantly higher concentrations, compared to human adult serum [32]. The ICEberg aminoacids Arg, Lys, Glu, and Asp were found with 2-, 4-, 14-, and 14-fold higher concentrations, compared to human adult serum [32]. For the intact ICEberg synthesis and ICEberg function, these aminoacids are necessary and must be also available in the cells. The significantly decreased IL-1beta release, after Actovegin® application may be explained by the successful synthesis of the enzyme ICEberg, which is only possible by availability of these relevant aminoacids. Then ICEberg may powerfully inhibit caspase 1 and may, therefore, result in an anti-inflammatory effect.

For an intact antioxidative and/or anti-inflammatory system with many proteins, not only amino acids are necessary, for the anabolic and catabolic pathways just like energy (e.g., ATP) and important inorganic substances (e.g., potassium, chloride, sodium, phosphate) are also necessary. ATP may be formed from increased availability and uptake of glucose. In the recent Actovegin® analysis for glucose a fourfold higher level, and for potassium, chloride, sodium, and phosphate up to tenfold higher levels were detected, compared to the corresponding substance levels in the adult human physiological serum/plasma [32]. Therefore, the observed anti-inflammatory effect of Actovegin® may be explained by the high availability of these Actovegin® ingredients in cells.

It is to note that TNF- α and IL-6 are also pro-inflammatory cytokines. However, the addition of Actovegin® or cortisone with PMA did not lead to a decrease of the release of IL-6. Therefore, IL-6 does not contribute to the reduction of inflammatory reactions with Actovegin® or with Cortisone.

The addition of Cortisone decreased the secretion of TNF- α , compared to the Actovegin® addition. Only in this case cortisone was superior to Actovegin®. Why cortisone is superior to Actovegin® only in TNF- α inhibition has to be clarified in further studies. Cortisone is a powerful drug but it also has many side effects as described above, especially with prolonged use [1,2].

IL-10 is an anti-inflammatory cytokine. The addition of Actovegin® (20 μ g/ml) with PMA did lead to an increase of the IL-10 release; therefore, IL-10 significantly contributes to the reduction of inflammatory reactions with Actovegin®, but not with cortisone. Why? High contents of following amino acids were found in the IL-10 protein structure: glutamic acid, asparagin acid, leucine, glycine, isoleucine, alanine, lysine, valine, tyrosine, histidine, methionine, threonine, arginin, and histidine. In our recent study it could be demonstrated that Actovegin® contains these physiological amino acids in up to 14-fold higher concentrations, compared to human adult serum [32]. For an optimal IL-10 synthesis and function, these aminoacids are necessary and must be also available

in the cells. The significantly increased IL-10 release, after Actovegin® application (and not by cortisone) may be explained by the successful synthesis of the IL-10, which is only possible by availability of these relevant aminoacids. Then IL-10 may be powerful synthesized and may therefore, result in a higher anti-inflammatory effect, compared to cortisone.

Our hypothesis is confirmed. Both, Actovegin® and Cortisone exert an anti-inflammatory effect, by dose-dependently diminishing the PMA-induced release of the pro-inflammatory TNF- α . However, a dose-dependent diminish of the PMA-induced release of the pro-inflammatory IL-8, IL-1 β and ROS was observed only for Actovegin® but not for Cortisone - likewise an increase of the release of the anti-inflammatory IL-10 was found only for Actovegin® but not for Cortisone.

It is mentioned that the transferability of in vitro results to the human physiological situation is limited but it is to note that this study has also a new direct relation to inflammations in sports medicine. Actovegin® is not only used in the above mentioned scopes of application and in skeletal muscle, but also as anti-inflammatory medication in skeletal muscle and in tendinopathies, e.g., on the patellar and achilles tendon [3-17,32]. Actovegin® is used as peritendinous injection (not intra-tendinous). Clinical experience indicates that inflammatory response and adhesions in the peritendinous tissue can be reduced with several injections of Actovegin® [75]. The present study was conducted to analyse if Actovegin® has an anti-inflammatory effect, compared to Cortisone. The data support the clinical therapeutic findings and can help to explain how Actovegin® may work as a therapeutic agent when it is injected into inflamed tissue, e.g., around the patellar tendon, the achilles tendon or other locations that are mechanically inflamed and therefore could be an alternative biocompatible drug for these treatments, compared to cortisone.

Conclusion

Both, Actovegin® and Cortisone exert an anti-inflammatory effect, by dose-dependently diminishing the PMA-induced release of the pro-inflammatory TNF- α . However, a dose-dependent decrease of the pro-inflammatory IL-8, IL-1 β and ROS and an increase of the anti-inflammatory IL-10 were observed only for Actovegin® but not for Cortisone. These findings may help to explain the positive effects of Actovegin® on inflammation injuries, compared to Cortisone. Therefore, Actovegin® may also contribute to the reduction of inflammation reactions.

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Author contributions

Conceptualization, FXR, CH, BS, HS; Methodology, FL, BS, MS, HS; Data analysis and interpretation, BS, FXR, CH, MS; Manuscript preparation, FXR, CH, MS, BS; Review/editing, DT, RH, WB, PT, CH; All authors read and approved the final manuscript for submission.

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Data availability

All data generated or analysed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional and/or national research committee (LMU Munich, Project Nr: 19-331 KB) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Research involving human and animal participants

This article does not contain any studies with animals performed by any of the authors.

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