



Actovegin® reduces PMA-induced inflammation on human cells

Franz-Xaver Reichl¹ · Christof Högg¹ · Fangfang Liu¹ · Markus Schwarz² · Daniel Teupser² · Reinhard Hickel¹ · Wilhelm Bloch³ · Helmut Schweikl⁴ · Peter Thomas⁵ · Burkhard Summer⁵

Received: 3 December 2019 / Accepted: 16 May 2020 / Published online: 23 May 2020
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Abstract

Purpose The effect of Actovegin® was investigated on PMA- and LPS-induced human peripheral blood mononuclear cells (PBMCs).

Methods PBMCs (1×10^6 cells/ml) from five blood donors (2 f, 3 m; 45–55 years) were grown in medium and exposed to Actovegin® in the presence or absence of PMA or LPS. Supernatants were collected to assess the concentration of cytokines (TNF- α , IL-1beta, IL-6 and IL-10). The reactive oxygen species (ROS) were assessed by a ROS-Glo™ H₂O₂ assay.

Results Stimulation of cells by PMA or LPS (without Actovegin®) significantly increased the secretion of IL-1beta, IL-6, IL-10 and TNF- α from PBMCs, compared to controls. Pre-treatment of cells with Actovegin® (1, 5, 25, 125 μ g/ml) plus PMA significantly decreased the secretion of IL-1beta from PBMCs, compared to controls (PMA without Actovegin®). In contrast, addition of Actovegin® (1, 5, 25, 125 and 250 μ g/ml) plus LPS did not alter the IL-1beta production, compared to controls (LPS without Actovegin®). TNF- α , IL-6 and IL-10 do not contribute to the reduction of inflammatory reactions with Actovegin®.

Conclusions Actovegin® can reduce the PMA-induced IL-1beta release and the ROS production from PBMCs. These findings may help to explain the clinically known positive effects of Actovegin® on athletic injuries with inflammatory responses (e.g., muscle injuries, tendinopathies).

Keywords Sports · PMA · LPS · ROS · IL1-beta · Human PBMCs

Abbreviations

LPS	Lipopolysaccharide	PAMP	Pathogen-associated molecular pattern
MRI	Magnetic Resonance Imaging	PBMCs	Human peripheral blood mononuclear cells
NADPH	Nicotinamide adenine dinucleotide phosphate	PKC	Protein kinase C
NLRP3	Nucleotide-binding oligomerization domain-like receptor containing-pyridin domain 3	PMA	Phorbol 12-myristate 13-acetate
		ROS	Reactive oxygen species
		TLR4	Toll-like receptor 4
		WADA	World Anti-Doping Agency

Communicated by Fabio Fischetti.

✉ Franz-Xaver Reichl
reichl@lmu.de

- ¹ Department of Conservative Dentistry and Periodontology, University Hospital, LMU Munich, Goethestr. 70, 80336 Munich, Germany
- ² Institute for Laboratory Medicine, University Hospital, LMU Munich, Munich, Germany
- ³ Molecular and Cellular Sport Medicine, German Sport University, Cologne, Germany
- ⁴ Department of Conservative Dentistry and Periodontology, University Hospital, Regensburg, Germany
- ⁵ Department of Dermatology and Allergy, University Hospital, LMU Munich, Munich, Germany

Introduction

Actovegin® is a medical drug obtained from natural calf blood. Over 60 years, many medical indications are treated by Actovegin®, e.g., acute stroke (Boiarinov et al. 1998; Derev'yannykh et al. 2008) or postpartum hemorrhage (intravenous infusion) (Appiah 2002), skin ulcers (topical medication) (Biland et al. 1985), and long bone fractures (intra-arterial infusion) (Buchmayer et al. 2011), malfunction of the blood circulation in the brain and trophic disturbances (e.g., ischemic insult, craniocerebral injury) (Somogyi et al. 1979), impairment of peripheral blood circulation

(e.g., angiopathy and ulcus cruris) (Buchmayer et al. 2011; Chanh et al. 1980; Lanner and Argyropoulos 1975), wound healing issues (e.g., torpid wounds, decubitus) (Buchmayer et al. 2011; Mochida et al. 1989; Neinhart 1967; Schonwald et al. 1991) and mucosal lesions after radiation (Basu et al. 1985; Bauer and Locker 1974; Beetz et al. 1996; Buchmayer et al. 2011; Spessotto et al. 1993).

Muscle injury incidence varies from 30 to 55%; therefore, it is one of the most common sports-related injuries (Ekstrand et al. 2011; Jarvinen et al. 2000; Verrall et al. 2001). Twelve percent of all muscle injuries are hamstring injuries, which are 2.5 times more frequent than, for example, quadriceps injuries (Askling et al. 2003; Woods et al. 2004). It has been shown that muscle healing can be promoted by administration of anti-inflammatory drugs (Abramson and Weissmann 1989). However, anti-inflammatory drugs also can have an adverse effect on the entire healing process (Obremsky et al. 1994; Shen et al. 2005). 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 (Reurink et al. 2014). 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) (2019).

1990 Pfister and Koller first described intramuscular injection of Actovegin® as treatment of muscle injuries in a partially blinded case control study with 102 patients (Pfister and Koller 1990). 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 (Pfister and Koller 1990). 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 (Pfister and Koller 1990).

In vivo and in vitro studies suggest that Actovegin® contains some active components, although they were not identified (Biland et al. 1985; Pforringer et al. 1994; Wright-Carpenter et al. 2004; Yaffe and Saxel 1977). 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® (Sondergard et al. 2016). 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 condition are represented by C2C12 muscle

cells (Yaffe and Saxel 1977). In our recent study, for the first time 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 (Reichl et al. 2017). Furthermore, in this study the ingredients of Actovegin® were identified and the active substances on muscle proliferation were discussed in detail (Reichl et al. 2017).

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 (Reichl et al. 2017). Furthermore, some clinical studies for Actovegin® confirm its safety (Maillo 2008; Pforringer et al. 1994; Ziegler et al. 2009).

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 (Abramson and Weissmann 1989); however, in other studies, it was described that anti-inflammatory drugs are not conducive to the healing process (Obremsky et al. 1994; Shen et al. 2005).

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 (Summer et al. 2010; Thomas et al. 2013).

In the present study the effect of Actovegin® was investigated on inflammation reactions on human PBMCs.

Our hypothesis was that Actovegin® has an anti-inflammatory effect on human cells.

Materials and methods

Cell culture and cell exposure

Stimulation assays were performed according to Summer et al. (2010), with the optimizations reported by Ständer et al. (2017). Heparinized blood was taken from anonymized healthy blood donors (2 females, 3 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 either with Phorbol 12-myristate 13-acetate (PMA) (1 µg/ml, Sigma-Aldrich, Munich, Germany) or with lipopolysaccharide (LPS, 10 ng/ml) with or without Actovegin® in different concentrations in quadruplicate. Cells (1×10^6 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 to 0–1–5–25–125–250 µg/ml exactly as described in a recent investigation on muscle cell proliferation (Reichl et al. 2017). Cell cultures were exposed to these Actovegin® concentrations in the presence or absence of PMA (1 µg/ml) for 24 h. After the exposure, culture supernatants were collected for cytokine analysis.

The five 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 (Summer et al. 2018).

Cytokine assays

The amount of IL-1beta, IL-6, IL10 and TNF-α 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 with PMA (1 µg/ml) (without Actovegin®) significantly ($p < 0.05$) increased the secretion of IL-1beta, IL-6, IL-10 and TNF-α from PBMCs, compared to controls without PMA (Table 1).

Similarly, stimulation with LPS (10 µg/ml) (without Actovegin®) significantly ($p < 0.05$) increased the secretion of IL-1beta, IL-6, IL-10 and TNF-α from PBMCs, compared to controls without LPS (Table 1).

Addition of Actovegin® (1, 5, 25, 125 µg/ml) plus PMA (1 µg/ml) significantly ($p < 0.05$) decreased the secretion of IL-1beta from PBMCs, compared to the control condition with PMA only (without Actovegin®) (Fig. 1).

In contrast, addition of Actovegin® (1, 5, 25, 125 and 250 µg/ml) plus LPS (10 ng/ml) did not result in a change of IL-1beta production, compared to the control condition with LPS only (without Actovegin®) (Fig. 2).

IL-6, IL-10 and TNFα production were not significantly changed by the addition of Actovegin® (1, 5, 25, 125 and

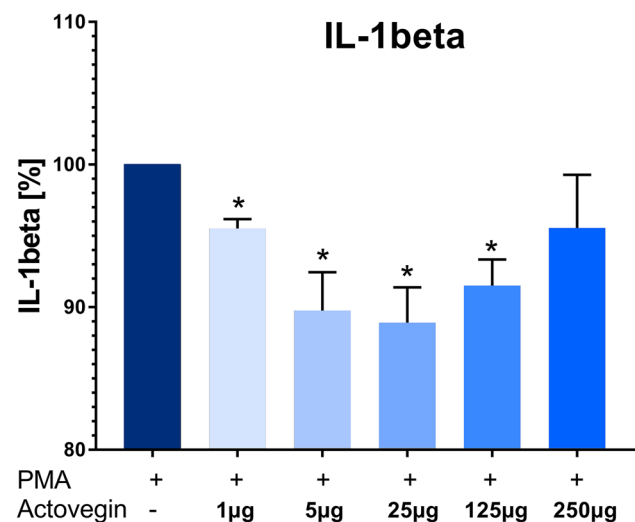


Fig. 1 IL-1beta production by human peripheral blood mononuclear cells (PBMCs) after stimulation with Phorbol 12-myristate 13-acetate (PMA) (set as 100%) and addition of Actovegin® in 5 different concentrations (mean ± sem, $n = 5$; * $p < 0.05$)

Table 1 Cytokine production by human peripheral blood mononuclear cells (PBMCs) after stimulation with Phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide (LPS) for 24 h (without Actovegin®) (mean ± sem, $n = 5$)

	IL-1beta	IL-6	IL-10	TNFα
Medium	0.0 pg/ml	0.0 pg/ml	0.0 pg/ml	0.0 pg/ml
PMA	2920.98 pg/ml ± 1010.66	2320.45 pg/ml ± 1753.71	2.98 pg/ml ± 1.02	3005.15 pg/ml ± 596.21
LPS	3623.13 pg/ml ± 381.2	11316.4 pg/ml ± 3367.12	314.5 pg/ml ± 70.8	2520.25 pg/ml ± 937.1

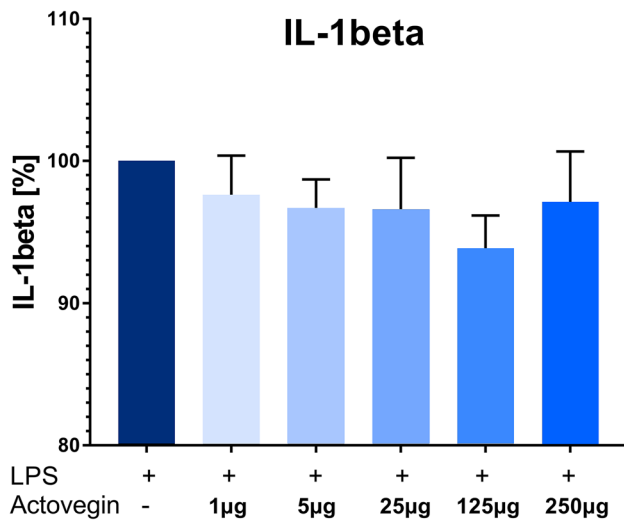


Fig. 2 IL-1beta production by human peripheral blood mononuclear cells (PBMCs) after stimulation with lipopolysaccharide (LPS) (set as 100%) and addition of Actovegin® in five different concentrations (mean \pm sem, $n=5$)

250 µg/ml) after stimulation with PMA or LPS (data not shown).

Addition of Actovegin® without PMA or LPS stimulation had no effect on ROS production, compared to controls (without Actovegin® and with medium only) (Fig. 3a).

Addition of Actovegin® (1, 125 and 250 µg/ml) significantly reduced the PMA-induced ROS production, compared to the control condition with PMA only (without Actovegin®) (Fig. 3b).

Addition of Actovegin® (125 and 250 µg/ml) significantly reduced the LPS-induced ROS production, compared to control condition with LPS only (without Actovegin®) (Fig. 3c).

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 (Abramson and Weissmann 1989), in contrast, it is described that anti-inflammatory drugs are not conducive to the healing process (Obremsky et al. 1994; Shen et al. 2005).

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 (Tidball 1995).

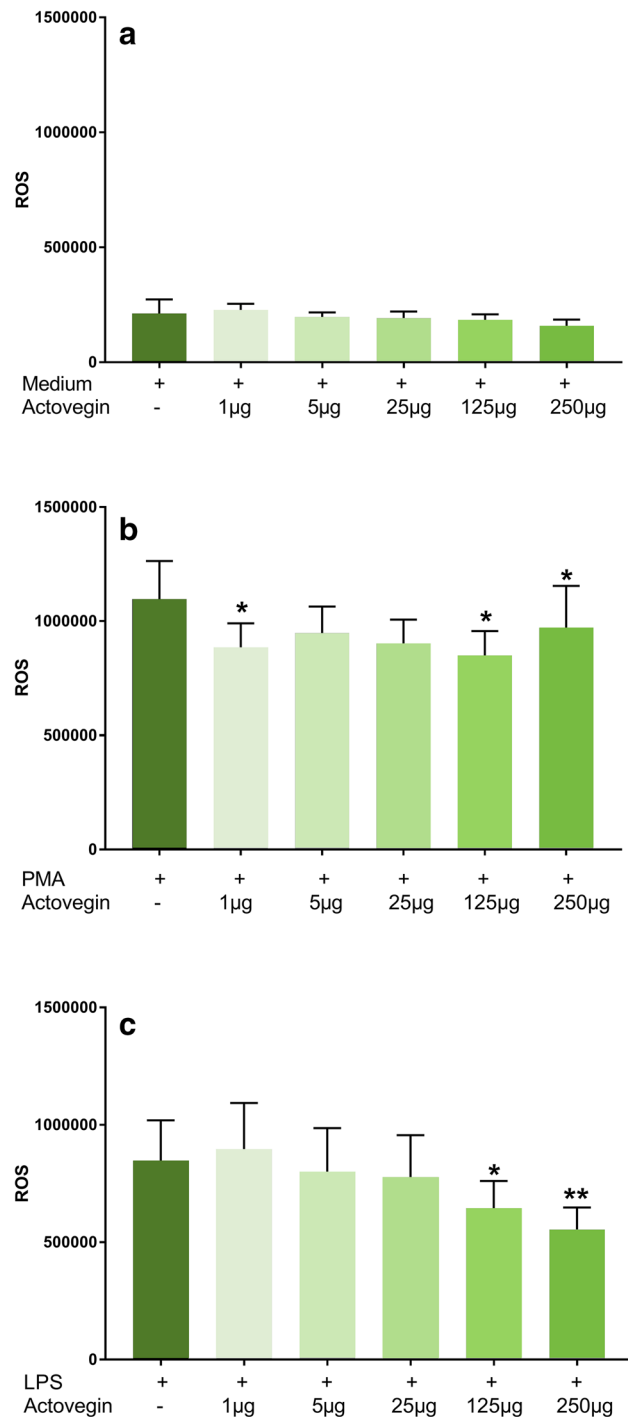


Fig. 3 ROS production by human peripheral blood mononuclear cells (PBMCs) after stimulation with Actovegin® alone (Fig. 3a), Phorbol 12-myristate 13-acetate (PMA) and Actovegin® (Fig. 3b) and lipopolysaccharide (LPS) and Actovegin® (Fig. 3c) (mean \pm sem, $n=5$; * $p < 0.05$, ** $p < 0.01$)

Here, we study the effect of Actovegin® in Phorbol 12-myristate 13-acetate (PMA)- and lipopolysaccharide (LPS)-induced inflammation on human PBMCs. LPS acts as the prototypical endotoxin, because it binds receptor

complexes in many cell types, which promotes the secretion of pro-inflammatory cytokines (Abbas and Lichtman 2007). In our study the addition of Actovegin® did not influence the LPS-induced release of interleukines.

PMA is also a potent tumor promoter via activation of the signal transduction enzyme protein kinase C (PKC) (Castagna et al. 1982; Blumberg 1988; Niedel et al. 1983). The PKC pathway is critically involved in function and development of B cells, as well (Ghamlouch et al. 2014). 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 (Huang et al. 2014; Swindle et al. 2002). 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 (Bastian and Hibbs 1994; Segal 1995; Jones et al. 2000). NADPH oxidase of phagocytic cells can also be activated by protein kinase C agonists such as PMA (Segal and Abo 1993).

PMA initiates acute inflammatory responses in mammals that are characteristic for the host reaction to tissue injury and/or infection (Basyreva et al. 2016).

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 pro-inflammatory cytokines as IL-1beta, IL-6 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 (David et al. 2003; Jeurink et al. 2008).

In the present study the effect of Actovegin® on the release of the important inflammatory cytokines IL-1 beta, IL-6, IL-10 and TNF- α (Wu et al. 2003) was investigated in PMA- and LPS-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®.

Oxidative damage plays a key role in inflammatory reactions and can induce several injuries (e.g., septic shock, atherosclerosis) induced by PMA and/or LPS which are known to enhance the formation of reactive oxygen species (ROS) (Glebov and Zinchuk 2006; Swindle et al. 2002). PMA/LPS can induce the activation of TLR4 in the cell wall, which can induce ROS, e.g., superoxide (Swindle et al. 2002).

In the present study a dose dependent decrease of IL-1beta was observed after addition of Actovegin® only with PMA. Therefore, it could be shown that Actovegin® has an anti-inflammatory effect. Additionally, it could be shown that the addition of Actovegin® decreased the ROS production.

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 production in PMA-stimulated, but not in LPS-stimulated cells, i.e., what is the difference between LPS and PMA stimulation of PBMCs?

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 (Lavieri et al. 2016). In our recent study high levels of cystathionine in Actovegin® were detected, compared to the human adult serum/plasma (Reichl et al. 2017). 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 (Paul et al. 2018). 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 (Goodsell 2004). It is a common enzyme found in nearly all living organisms predominantly in the liver, kidney and erythrocytes (Goodsell 2004). 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 (Goodsell 2004). 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 (Boon et al.). In our recent study high levels of glutamic acid, asparagine acid, and arginine in Actovegin® were detected, compared to the human adult serum/plasma (Reichl et al. 2017). 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 effect after LPS vs. PMA stimulation)

LPS is a characteristic cell membrane component of Gram-negative bacteria, recognized by different immune cells—mainly monocytic cells—via Toll-like receptor 4 (TLR4) (Wassenaar and Zimmermann 2018). After LPS binding to TLR4, intracellular signaling pathways result in secretion of IL-1 β after utilizing the NOD-like receptor containing-pyrin domain 3 (NLRP3) inflammasome and the subsequent cleavage of pro-caspase-1 into caspase-1 (Wynick et al. 2016).

However, this effect seems not be discriminative between LPS and PMA, since PMA has been shown to exert its well-known effect on monocyte differentiation via caspase-1 activation (Niu et al. 2017). Moreover, there is another mechanistic similarity between LPS and PMA on monocytic cells: Ribeiro and colleagues could demonstrate that activated protein kinase C (PKC) further activates mTORC1/S6K pathway in a similar effect observed to LPS (Ribeiro et al. 2018). This observation is important for the interpretation of our results, since PMA is a potent activator of PKC (Leonard et al. 2015).

Altogether, it appears that the effect of LPS and PMA on monocytic cells seems to be similar. Therefore, the distinct effect of PMA on IL-1beta secretion in our PBMC model may not be attributable to the effect on monocytes. In contrast, there is quite distinct effect of PMA compared to LPS on lymphocytes: PMA is a well-established inducer of the differentiation of CLL B cells into plasmacytoid cells (Ghamlouch et al. 2014). In lymphocytes derived from healthy individuals, PMA stimulation results in a strong increase of CD23 phosphorylations, equal to the CD23 phosphorylation observed in B cells of patients with an active form of CLL (Madarova et al. 2018). To the best of our knowledge, there is no similar effect of LPS on lymphocytes.

The selective effect of Actovegin® on IL-1beta secretion in PMA-stimulated, but not in LPS-stimulated PBMCs may, therefore, be due to its effect on lymphocytes (most probably B cells) and not on monocytes.

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 (Madarova et al. 2018). 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 cytokine IL1-beta after addition of Actovegin® with PMA, compared to the release without Actovegin®, but not for the release of the pro-inflammatory

cytokines IL-6 and TNF- α and for the anti-inflammatory IL-10. Therefore, the next question arising from our results concerns the difference between the investigated cytokines. IL1-beta is a key inflammatory mediator driving the host response to infection, injury, and disease. IL1-beta driven inflammation has often disastrous consequences, and thus represents a therapeutic target (Dinarello 2011). Caspase 1 is activated by recruitment to a molecular platform called an inflammasome (Schroder and Tschopp 2010) and caspase 1 is considered to belong to the inflammatory group (Siegel 2006).

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 (Denes et al. 2012). Inhibition or deletion of caspase 1 improves, e.g., outcome after myocardial infarction (Pomerantz et al. 2001; Frantz et al. 2003; Holly et al. 1999).

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 (Lopez-Castejon and Brough 2011) but were not successful (Dinarello 2011).

ICEberg is a protein that inhibits generation of IL-1beta by interacting with caspase-1 (Druilhe et al. 2001). 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 (Wu et al. 2003). 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 (Humke et al. 2000).

Humke et al. (2000) 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 (Humke et al. 2000).

In our recent study it could be demonstrated that Actovegin® contains many physiological substances in significantly higher concentrations, compared to human adult serum (Reichl et al. 2017). The ICEberg aminoacids Arg, Lys, Glu, and Asp were found with 2-, 4-, 14-, and 14-fold higher concentrations, compared to human adult serum (Reichl et al. 2017). 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 powerful 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 aminoacids 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 (Reichl et al. 2017). 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® with PMA or LPS did not lead to a decrease of the release of TNF- α or IL-6; therefore, TNF- α and IL-6 do not contribute to the reduction of inflammatory reactions with Actovegin®.

IL-10 is an anti-inflammatory cytokine. The addition of Actovegin® with PMA or LPS did not lead to an increase of the release of IL-10; therefore, IL-10 does also not contribute to the reduction of inflammatory reactions with Actovegin®.

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 (Reichl et al. 2017; Boiarinov et al. 1998; Derev'yannykh et al. 2008; Appiah 2002; Biland et al. 1985; Buchmayer et al. 2011; Somogyi et al. 1979; Chanh et al. 1980; Lanner and Argyropoulos 1975; Mochida et al. 1989; Neinhart 1967; Schonwald et al. 1991; Basu et al. 1985; Bauer and Locker 1974; Beetz et al. 1996; Spessotto et al. 1993). Actovegin® is used as peritendinous injection (not intra-tendinous). Clinical experience indicate that inflammatory response and adhesions in the peritendinous tissue can be reduced with several injections of Actovegin® (Hotfiel et al. 2018). The present study was conducted to analyse if Actovegin® has an anti-inflammatory effect at all. 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.

Conclusion

Our hypothesis is confirmed. Actovegin® exerts an anti-inflammatory effect, by dose- dependently diminishing the PMA-induced release of the pro-inflammatory interleukin

IL-1beta in human PBMCs. This effect may be due to a specific effect on B cells. Moreover, we could demonstrate an anti-inflammatory effect by reduction of LPS- and PMA-induced ROS species. These results further indicate that Actovegin® can supply valuable components for the formation and function of an efficient antioxidative and/or anti-inflammatory system in cells, which may contribute to the reduction of an inflammation. These findings may also help to understand the positive effects of Actovegin® on inflammation injuries (right up to high performance athletes) and how Actovegin® may work as a therapeutic agent when it is injected into inflamed tissue.

Acknowledgements The authors would like to thank Mr. Stefan Schulz for his technical support.

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.

Funding Open Access funding enabled and organized by Projekt DEAL.

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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