EXTENDED ABSTRACT

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Combination of wine polyphenols, vitamin E and zinc improves cellular phenotypes of muscle fatigue and damage

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Abstract

Period of intense or prolonged muscle activity can reduce performance and cause muscle weakness. If the weakness is quickly reversible, it is named fatigue. If the weakness is slowly or poorly reversible and associated with structural changes, it is described as muscle damage and can lead to soreness.
We investigated the effect of wine polyphenols, vitamin E and zinc on human skeletal muscle cells (HSMC) incubated with A23187, a calcium ionophore that mimicks cellular effects of exercise-induced muscle fatigue and damage. Fatigue- and damage-like phenotypes were monitored by quantifying the secretion of lactate and IL-6, and the intracellular activity of creatine kinase.
As expected, administration of A23187 increased levels of lactate and IL-6 in the medium and decreased the intracellular activity of creatine kinase in HSMC. Administration of either wine polyphenols, vitamin E or zinc alone, caused a very limited protection of HSMC from A23187-induced effects. However, the simultaneous administration of all three compounds caused a marked and significant protection.
These results demonstrate that a combination of wine polyphenols, vitamin E and zinc protect muscle cells from injury normally associated with fatigue and damage, and suggest the possibility that this combination could be used to optimize exercise performance and recovery.

Introduction

Any period of intense or prolonged muscle activity can cause a decline in performance which can be measured as muscle weakness. By definition, if the weakness is largely reversible in minutes or hours, it is described as muscle fatigue. If the weakness is slowly or poorly reversible and associated with structural changes within the muscle, it is described as Exercise-Induced Muscle Damage (EIMD).

Muscle fatigue

Muscle fatigue is generally viewed as a result of insufficient energy and availability of key metabolites that enable contracting muscles to meet increased energy demand (Baird *et al*., 2012). Multiple mechanisms exist to explain fatigue, as for example acidosis and depletion of ATP, due to increased consumption or decreased provision of substrates allowing ATP production.

Muscle acidosis comes from lactic acid production, happening during glycolysis. This mechanism reflects that the aerobic ATP generation is insufficient and needs to be supplemented with anaerobic ATP generation. Lactic acid dissociates rapidly into lactate and hydronium ion. The latter induces lowering of pH and apparition of muscle acidosis, which is thought to interfere with mechanisms of muscle contractions. Part of the lactate diffuses out muscle cells and thus can be considered as a marker of muscle fatigue during which muscle are not able to sustain energy production to maintain and perform physical exercise (Finsterer, 2012).
Contracting skeletal muscles also release myokines such as interleukin-6 (IL-6). Usually absent from the plasma or present at very low quantities in healthy subjects at rest, IL-6 production exponentially increases in response to muscle contractions. Furthermore, high plasmatic levels of IL-6 are associated with an increased feeling of fatigue during physical

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exercise, inducing a significant reduction of performance in trained runners (Robson-Ansley *et al*., 2004). IL-6 is thought to act like an energy sensor and to be involved in mediating hepatic glycogenolysis and glucose uptake during exercise to maintain glycemic homeostasis (Finsterer, 2002).

Muscle damage and soreness

Unaccustomed or strenuous exercise can initiate mechanical muscle damage of varying degrees. Even if it is usual to make a distinction between muscle fatigue and muscle damage, the two phenomena undoubtedly overlap. Muscle damage is characterized by structural abnormalities including sarcomeric disorder and membrane damages, resulting in the release of cellular components, increase in muscle protein degradation and cell permeability, and also inflammatory processes (Baird *et al*., 2012). Damage is particularly pronounced in muscles which are stretched during contractions.

Muscle damage induces a cascade of events leading to immediate and/or delayed soreness (Allen *et al*., 2005). Muscle soreness refers to the immediate soreness perceived by the athlete while or immediately after participating in exercises. Muscle soreness is associated with muscle stiffness, aching pain, and/or muscular tenderness. These symptoms are experienced for only hours and are relatively transient. The symptoms associated to Delayed-Onset Muscle Soreness (DOMS) are the same as for immediate soreness but symptoms onset is at about 24 hours after the athletes have completed their exercise and are maintained during 2-3 days (Lewis *et al*., 2012).

Loss of cell myofiber proteins into the blood may occur at several stages along the continuum of muscle injury to muscle soreness. Creatine kinase (CK) is one of the proteins which are leaked into the circulation when muscle damages occur. Furthermore, it is almost exclusively present in the muscular tissue. That is why it is currently dosed in serum to evaluate muscle damages and injury (Baird *et al*., 2012).

Aim of the study

The aim of the study was to investigate the protective effect of the combination of three anti-oxidant ingredients which were wine polyphenols (W), zinc (Z) and vitamin E (V) on muscular fatigue and damage. To this end, we measured the *in vitro* release of lactate and IL-6 and the intracellular CK activity in normal human skeletal muscle cells (HSMC) subjected to a calcium influx, mimicking a physical exercise (Hurst *et al*., 2009).

Materials And Methods

Materials

HSMC, skeletal muscle cell growth medium and differentiation medium were purchased from Promocell (Germany). A23187 calcium ionophore and Hoechst probe were purchased from Sigma-Aldrich (USA). Lactate content was evaluated thanks to colorimetric enzymatic method (BioVision, USA). IL-6 content and CK activity were evaluated thanks to colorimetric ELISA method (Raybiotech, USA and BioChain, USA respectively).

Wine polyphenols, vitamin E and zinc were supplied by SEPPIC SA (France). Wine polyphenols came from a spray dried alcohol-free red wine extract polyphenols concentrate which contained at least 70% of polyphenols (equivalent catechins).

Physical exercise model in HSMC

HSMC were cultivated in cell growth medium at 37°C, 5% CO_2, 95% air atmosphere. When they reached 70-80% of confluence, culture medium was replaced by differentiation medium, inducing the differentiation of muscular cells in myotubes.

Ten days after, myotubes were treated or not with W 0.00005%, V 0.001% or Z 0.00015%, alone or in combination. Doses of treatment were chosen based on cytotoxicity trials. Tested doses were also consistent with the fact that serum level of vitamin E is five times higher than zinc level in humans. Polyphenols consumption and bioavailability differ greatly. But considering that polyphenols consumption can range from 20 mg to more than 1 g/day, consumption of 500 mg of polyphenols could bring the equivalent of the dose selected for polyphenols (equivalent catechins).

24 hours later, treatments were withdrawn and cells were exposed or not to A23187 calcium ionophore for 48 hours. At the end of this incubation, lactate and IL-6 contents were evaluated in cell supernatants. CK activity and DNA contents were evaluated in cell lysates (patent application filed).

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Results analysis

For each parameter, mean and standard deviation were calculated and normalized with DNA quantity. The percentage of stimulation was calculated for A23187-treated HSMC *versus* (*vs*) untreated HSMC. The percentage of protection was calculated for each tested product vs A23187-treated and untreated HSMC. Statistical significance was assessed using a two-tailed, paired Student's test, with $p<0.05$ being considered significant.

Results And Discussion

Release of lactate by HSMC (Figure 1)

A23187 induced a significant increase in the release of lactate by 70% in HSMC. W, Z and V products tested alone had no protective effect on release of lactate. Pre-treatment with WZV association reduced it by 85% in HSMC, significantly *vs* A23187-stimulated cells and *vs* each product alone-treated cells.

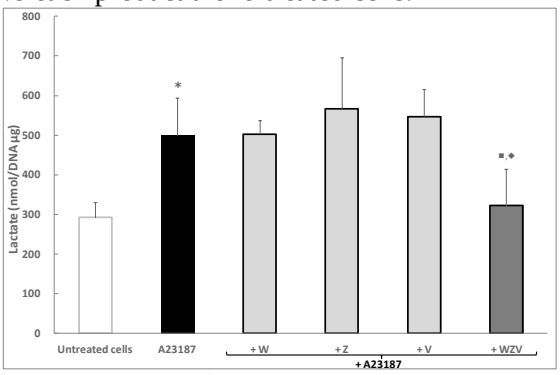

Figure 1: Release of lactate by HSMC stimulated with A23187 and pre-treated with each product alone or in combination. (✱): $p<0.05$ *vs* no treatment; (■): $p<0.05$ *vs* A23187; (◆): $p<0.05$ *vs* W, Z, V.

Release of IL-6 by HSMC (Figure 2)

A23187 induced a significant increase in the release of IL-6 by 211% in HSMC. W, Z and V tested alone had no protective effect on release of IL-6. Pre-treatment with WZV reduced it by 63% in HSMC, significantly *vs* A23187-stimulated cells and *vs* each product alone-treated cells.

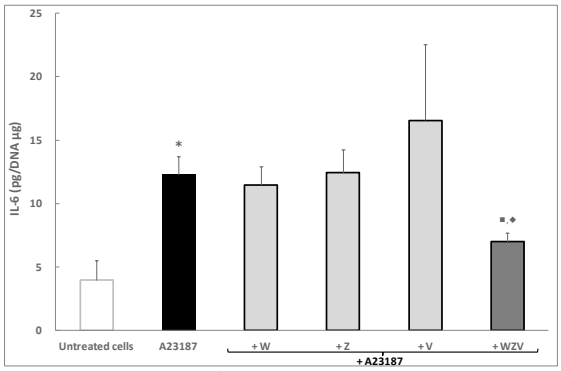

Figure 2: Release of IL-6 by HSMC stimulated with A23187 and pre-treated with each product alone or in combination. (✱): $p<0.05$ *vs* no treatment; (■): $p<0.05$ *vs* A23187; (◆): $p<0.05$ *vs* W, Z, V.

Intracellular CK activity in HSMC (Figure 3)

A23187 induced a significant decrease in the intracellular CK activity in HSMC by 56%. W and Z tested alone had no protective effect on intracellular CK activity, V had a protective effect of 47% ($p<0.1$). WZV association reduced the decrease of intracellular CK activity by 77%, significantly *vs* A23187-stimulated cells and *vs* W- and Z-treated cells.

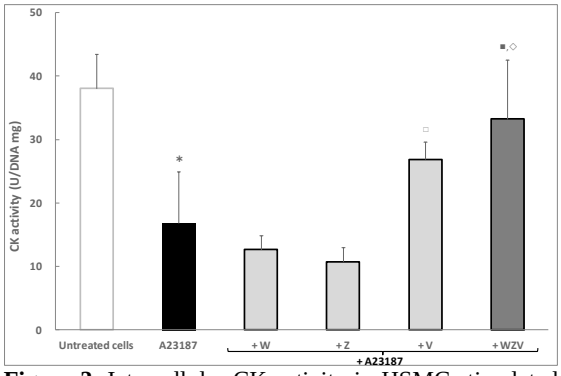

Figure 3: Intracellular CK activity in HSMC stimulated with A23187 and pre-treated with each product alone or in combination. (✱): $p<0.05$ *vs* no treatment; (■),(□): $p<0.05$ and <0.1 respectively *vs* A23187; (◇): $p<0.05$ *vs* W, Z.

The WZV association was able to protect HSMC from the release of lactate and IL-6 and the decrease in intracellular CK activity induced by A23187.

These results demonstrate that a combination of wine polyphenols, vitamin E and zinc protect human skeletal muscle cells from A23187-induced alterations in biomarkers normally associated with fatigue and damage that appear after physical exercise.

These findings raise the possibility that this combination could be used to optimize performance and recovery during and after physical exercise.

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