Effect of the Immunomodulator (AM3®) on Biochemical Muscular Damage Markers in Basketball Players

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ABSTRACT

The aim of this work was to explore the effects of AM3 (immunoferón®) on markers of muscle damage in professional basketball players. Twelve professional basketball players from Tau Cerámica Vitoria Sport Team (First Spanish Professional Basketball League) participated in the study. Muscle damage and haematological related biochemical parameters and handgrip strength were performed before beginning the intervention (April), after 6 weeks of AM3 treatment (May) and after 6 weeks post-treatment (July; coinciding with Euroleague Play off Final). During all study (April-July), significant increases were observed in creatinine (1.25±0.02 vs. 1.30±0.03 mg·dl⁻¹; p=0.012) and leucocyte counts (5.2±0.2 vs. 6.1±0.3 x10⁹·l⁻¹; P=0.02). However, no changes in the behaviour of the others biochemical indicators of muscular damage (CK, MB, AST, ALT, LDH), stress (cortisol) and haematological parameters (haemoglobin, haematocrit, RBC, neutrophils and lymphocytes)

Key Words: Immunomodulator, AM3, Exercise, Biochemical markers, Muscle damage
were presented. Regarding the handgrip strength, we detected significant higher levels in May and July with respect to April \((P=0.009)\). The study concludes that the use of AM3 maintains or not increases the serum levels of DOMs biochemical markers, especially in players with high physical demands, and can be considered in the recovery period.

INTRODUCTION

Strenuous physical exercise commonly results in exercise-induced muscle damage, especially when the exercise is intense and continuous, and involves eccentric muscle activity \((2)\). Eccentric contractions induce severe structural damage in muscles, contributing to delayed-onset muscle soreness (DOMS) and affecting their contractile properties \((1, 4)\). The magnitude of strength loss after DOMS may vary between 5–10% and 60%, depending on the characteristics of the protocol and the type of muscle actions used during the posttest \((18)\). The inflammatory response generates a transfer of fluid and cells to remove damaged contractile proteins and cellular debris from the damaged muscles \((20)\). Within days after exercise, these structural alterations are classically accompanied by physiological and subjective perceptions of muscle damage that delay recovery \((5)\). The efflux of intracellular enzymes [creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate deshydrogenase (LDH)], proteins and ions from the muscle tissue into the plasma are classically used to study the extent of muscle damage \((2, 8, 11, 25)\). The clearance time depends on the athlete’s training status, the type, intensity, and duration of exercise and on the biochemical characteristics of the molecule.

Regular exercise exerts a protective effect against diseases associated with chronic inflammation partially attributed to anti-inflammatory and antioxidant effects which are mediated by a reduction in visceral fat mass and by an induction of an anti-inflammatory environment after the exercise bouts \((13, 19, 22)\). However, a severe and excessive physical activity provokes a situation of tissue damage resulting from an excessive inflammatory reaction. In athletes, who require to train and compete frequently, the negative effects of DOMS can pose an obstacle to optimal performance. However, to reverse this situation, it has been suggested that the magnitude of muscle damage and the loss of muscle function might be attenuated after one bout of eccentric exercise with anti-inflammatory agents and/or immunomodulators, such as AM3 (Inmunoferón®) \((7, 10, 15)\). In general, this protective effect is confirmed by the reduced decrements and faster recovery of muscle strength, less swelling and DOMS, and attenuated changes in CK and myoglobin (MB) in the blood \((7, 10)\).
AM3 (Inmunoferón®, I.F. Cantabria, Madrid, Spain) is a commercially available immunomodulator. The glycoconjugated compound is a non-covalent association of a phosphorylated glycomanane polysaccharide (molecular weight 150 Kda) from the cell wall of Candida utilis, plus a reserve protein from non-germinated seeds of Ricinus communis Ricc3 (molecular weight 12 Kda) in proportions 5:1 (polysaccharide/protein). Both absorbed onto a calcium sulphate phosphate inorganic matrix (10).

We hypothesize that the intake of an immunomodulator, such as AM3 (Inmunoferón®) improves skeletal muscle recovery and therefore the performance by reducing inflammatory processes and the muscular damage. The aim of the present study was to examine the long-term effectiveness of AM3 intake on recovery of DOMS and biochemical markers in professional basketball players during a 6 weeks treatment in a period of regular competition. We have analyzed the enzymes and proteins indicative of muscle metabolism as serum Mb, CK, LDH GOT/AST, GPT/ALT, aldolase, urea and creatinine levels, and also, we have controlled the fatigue level by handgrip dynamometric test.

METHODS

Twelve professional basketball players (27.3±4.4 years old, 96.8±13 kg and 198±9.9 cm) from Tau Cerámica Vitoria Sport Team (First Spanish Professional Basketball League), volunteered to participate in the study. The experimental procedures, associated risks, and benefits were explained both verbally and written with information sheets to each player, and each player signed a written consent form before commencing participation. The protocol was designed in compliance with the recommendations for clinical research of the Declaration of Helsinki and was approved by the local ethics committee of the University of León (Spain).

A prior physical examination, including electrocardiographic and cardiopulmonary evaluation, to ensure that participants were in good health was performed by medical doctor team. None of the subjects smoked, drank alcohol, or were taking supplements and medications known to alter the hormonal response during the study. All subjects, under the supervision of the medical team, followed a similar diet throughout the competition season, and particularly, during the 10 weeks of the study. All players trained 2 times/day: a morning session of a 2-hours gym workout, and an afternoon session of a 3-hours basketball practice. It was repeated every day except on official matches (2 matches/week, Wednesday and Sunday).

The participants were required to attend the laboratory on 3 specific points
along the League and Euroleague final play offs: a) April (end of third mesocycle) coincident with the final of the Spanish League and Euroleague regular season. At this moment, all players started to intake AM3; b) May, after 6 weeks of treatment. At this moment, players stopped to intake AM3; and c) July, after 6 weeks post-treatment. At this moment the players were playing the Euroleague play offs final. AM3 was administered orally at 5 g/day for 6 consecutive weeks, between April and May, and then the next 6 weeks were used to analyse the effect of remained level of AM3 accumulated in the body (follow-up). Daily treatment compliance was supervised by the doctor’s team.

1. **Blood collection and biochemical analysis**

None of the basketball players were injured during the experimental period. DOMs serum biochemical markers were measured in the three selected points 24 hours after finishing the physical activity. Participants reported to the laboratory at 8:30 a.m. after overnight fast. After 30 min resting in a comfortable seat, blood samples were taken. Peripheral blood samples were obtained from the antecubital vein in suitable vacutainers with EDTA as anticoagulant to obtain plasma and without anticoagulant to obtain serum. Serum was separated from blood cells and stored at -20°C until analysis. Serum myoglobin (Mb) was determined by a chemiluminescent luminol reaction after adsorption to anti-myoglobin IgG onto a solid phase. %CK-MB fraction, calculated as (CK-MB/CK)x100, LDH, aldolase, ALT/GPT and GOT/AST were measured using classic enzymatic methods, in a Hitachi autoanalizer (Hitachi 917, Japan). Urea was measured using a colorimetric enzymatic method and creatinine was measured using the Jaffe’s reaction adapted kinetic method. All biochemical tests were carried out in an official hospital with the corresponding technique controls. Blood cortisol levels (COR) were determined using a radioimmunoassay kit (Diagnostic Products Corporation, USA). Hematological parameters were assessed using Sysmex XT-2000-i (Kobe, Japan).

2. **Dynamometric measurements**

During training, previous to the blood sampling day, the maximal handgrip strength of the dominant hand was measured with a Jamar® hand dynamometer (Sammons Preston, Bolingbrook, Illinois) (ICC: 0.85-0.98) (23). The test was performed with subjects comfortably standing with the shoulder adducted 90° forward at elbow joint. Dynamometer power was freely made without support; without touching the subject’s trunk. The hand position remained constant, with a downward direction. The palm did not flex on the wrist joint. Players were required to exert maximal strength on the dynamometer (maximum voluntary
contraction). All subjects performed 3 trials and the best performance was used. The dynamometer scale indicated handgrip strength in kilograms. Maximal grip strength was calculated in Newtons by multiplying the dynamometer index by 9.81.

3. Statistical Analysis

Data are shown as means±standard deviations. Statistical analysis were performed using the software SPSS, v.19.0 (SPSS Inc, Chicago) and graphics using GraphPad Prism® v.5.0 (GraphPad Software, Inc). ANCOVA for repeated measures was used to compare all determined parameters in the different study phases with a post-hoc Bonferroni test, and with Greenhouse-Geisser correction to test the existence of an interaction effect (time x group) (TxG) between time phases and changes in DOMS and hematological variables. Percentage of changes of all biochemical variables between different phases (AM3 Intake phase: April vs. May; Follow-up period: May vs. July and total study: April vs. July) were calculated as: Δ(%):[(Tfinal–Tinitial)/Tinitial]x100. Baseline and the percentage of changes in each study phase on DOMS markers between SBP and NSBP by parametric or non-parametric two independent-samples test were compared, after determining the normality (Shapiro-Wilk test). Differences were considered statistically significant when p<0.05.

RESULTS

All analysed biochemical parameters, showed maintenance of levels and are shown in Table 1. Non-significant differences were observed in the biochemical parameters between April and July. Only significant increases were observed in creatinine between April and July (1.25±0.02 vs. 1.30±0.03 mg·dL⁻¹). With respect of parameters no changes were evidenced neither biochemical indicators of muscular damage (CK, MB, AST, ALT, LDH), nor in stress parameter as cortisol concentration.
Table 1

Muscle damage parameters at baseline (April), at the end of AM3 treatment (May) and follow-up (July) in elite basketball players (n=12).

<table>
<thead>
<tr>
<th></th>
<th>April</th>
<th>May</th>
<th>July</th>
<th>p</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cK (U·L⁻¹)</strong></td>
<td>545.9±99.5</td>
<td>488.9±88.0</td>
<td>547.5±93.1</td>
<td>0.636</td>
<td>0.038</td>
</tr>
<tr>
<td><strong>cK-MB (U·L⁻¹)</strong></td>
<td>18.5±4.7</td>
<td>19.5±4.3</td>
<td>21.2±5.0</td>
<td>0.127</td>
<td>0.179</td>
</tr>
<tr>
<td><strong>MB (ng·mL⁻¹)</strong></td>
<td>34.1±3.7</td>
<td>32.3±3.4</td>
<td>36.9±2.6</td>
<td>0.537</td>
<td>0.050</td>
</tr>
<tr>
<td><strong>AST (U·L⁻¹)</strong></td>
<td>34.6±3.4</td>
<td>31.3±2.2</td>
<td>33.9±2.2b</td>
<td>0.257</td>
<td>0.116</td>
</tr>
<tr>
<td><strong>Cortisol (µg·dL⁻¹)</strong></td>
<td>17.7±1.2</td>
<td>17.2±1.3</td>
<td>18.9±1.6</td>
<td>0.276</td>
<td>0.109</td>
</tr>
<tr>
<td><strong>LDH (U·L⁻¹)</strong></td>
<td>383.5±18.7</td>
<td>395.0±22.2</td>
<td>406.0±19.5</td>
<td>0.421</td>
<td>0.071</td>
</tr>
<tr>
<td><strong>Aldolase (U·L⁻¹)</strong></td>
<td>7.0±0.5</td>
<td>7.2±0.5</td>
<td>7.5±0.6</td>
<td>0.687</td>
<td>0.032</td>
</tr>
<tr>
<td><strong>Creatinine (mg·dL⁻¹)</strong></td>
<td>1.25±0.02</td>
<td>1.23±0.02</td>
<td>1.30±0.03b</td>
<td>0.012</td>
<td>0.340</td>
</tr>
<tr>
<td><strong>Urea (mg·mL⁻¹)</strong></td>
<td>43.8±2.7</td>
<td>41.9±2.6</td>
<td>41.8±2.6</td>
<td>0.479</td>
<td>0.041</td>
</tr>
<tr>
<td><strong>ALT (U·L⁻¹)</strong></td>
<td>24.8±1.6</td>
<td>24.6±1.3</td>
<td>26.5±1.9</td>
<td>0.424</td>
<td>0.073</td>
</tr>
</tbody>
</table>

Data are mean ± error standard of the mean. CK: Creatine phosphokinase; CK-MB: CK-MB fraction; MB: Myoglobin; AST: Aspartate aminotransferase; LDH: Lactate dehydrogenase and ALT: Alanine transaminase.

Significant differences between phases by Bonferroni post hoc test (p < 0.05):

a: vs. April.
b: vs. May.

With respect the haematological parameters, the leukocyte counts were the only parameter showing significant differences (Table 2) in July with respect to April session. The rest of parameters remain in similar levels during the study, without significant differences.
Table 2

<table>
<thead>
<tr>
<th>Haematological parameters at baseline (April), at the end of AM3 treatment (May) and follow-up (July) in elite basketball players (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>RBC (×10¹²·L⁻¹)</td>
</tr>
<tr>
<td>Hct (%)</td>
</tr>
<tr>
<td>Hb (g·L⁻¹)</td>
</tr>
<tr>
<td>Leukocytes (×10⁹·L⁻¹)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of the mean. Red Blood Cells (RBC), hematocrit (Hct), Haemoglobin (Hb).

Significant differences between phases by Bonferroni post hoc test (p < 0.05):

a: vs. April.
b: vs May

Handgrip strength in each period of the study is reported in Figure 1. Significant differences were observed between the different moments (April, May and July). We observed significant higher levels in May and July with respect the previous moment of measurement.
DISCUSSION

A continuous damaging stimuli induced by excessive training and competition bring on inflammation, tissue damage and fatigue, which diminish recovery and performance in athletes (6, 9). In this sense, professional basketball players, due the characteristics of sport (eccentric-concentric) perform intense exercise in training and during competition. In the present study, we observed that consumption of 5 g/day of AM3 (inmunoferón®) for 6 weeks benefited on biochemical markers of muscular damage, because against the competition and the greater physical demands these parameters are maintained stable. Therefore, regular training and competition can be modulated by AM3 treatment reducing the instauration of an inflammatory state.
Basketball is characterized by acyclic exercise, with discontinuous efforts and actions and by the combination of eccentric and concentric muscular concentrations that it required a good physical adaptation. The power for technical and physical movements (jumping to make shots or blockades) is mainly produced during the eccentric contraction of muscles. In this sense, while programming workout out, plyometric exercises are performed to improve muscle response. Specifically, plyometric training aims to reduce the time required between eccentric muscle contraction and the concentric contraction initiation (1). Eccentric muscle action is characterized by the muscle extended under tension activating a cascade that results in metabolic events leading to the muscle fiber damage and degeneration (27). Exercise duration and intensity and adaptation to training can influence the muscle damage degree and thus the leakage of enzymes.

In this study, DOMs degree was maintained along the season, en the different phases of study, in spite of the training and matches accumulation throughout the season. These results were supported by the dynamometric results, since there were not decreases in the maximal handgrip strength. On contrary, along of the season increase the handgrip, due in part by AM3 treatment and in other big part by the training and improvement of performance of the players.

In general, the decrease in serum enzymes markers of muscular damage depends on the rest period after exercise, because short-term physical inactivity may reduce both the lymphatic transport of CK and the release of the enzyme from the muscle fibers (15).

As previously described, AM3 has demonstrated positive outcomes in studies with athletes (10-12) and in the treatment of chronic inflammatory diseases such as EPOC and hepatitis (24, 26). Cordova et al., (2004; 2006, 2015) reported significant reductions in the concentration of inflammatory cytokines and biochemical markers of DOMS muscular damage in cyclists and volleyball players during competition. Similar results were observed in this study with regards to the professional basketball players studied after 6 weeks of AM3 treatment in a high-period competition like the playoffs in the National League and Euroleague. Moreover, and in contrast to AM3 treatment, the anti-inflammatory drugs such as steroids with the ability to prevent muscular damage are associated with significant secondary effects (16, 24, 26). AM3 also shows additional immunomodulatory effects, including macrophages stimulation and NK activity that might be beneficial in elite sportsmen who are more susceptible to infections due to chronic exercise-related immunosuppression and stress (9, 26).

The AM3 administration ameliorated the changes in biochemical parameters, but also improved the handgrip strength. Significant differences were observed after six weeks of treatment and after the end treatment without AM3 consumption. Overall, in the period of the study, no significant differences were showed in CK, CK-MB, Mb, urea and AST. These results showed that the use of AM3 during
periods of maximum competition significantly inhibits the enhanced serum levels of proteins associated with tissue damage. Increased serum urea concentrations are used as a marker of enhanced protein catabolism and stimulated gluconeogenesis resulting from higher training loads, especially longer intensive endurance training (14, 17). Follow-up studies of serum urea and CK concentrations may primarily indicate an acute impairment in exercise tolerance, which may be prophylactic for overtraining syndrome in long term (28).

Cortisol levels are considered an indicative factor of accumulated stress and increased during the season in elite sportsman (9, 21). However, in our case no significant changes were evidenced in cortisol levels. This fact is important because with AM3 treatment in this population cortisol levels can be considered as normal variations. In this way, we have previously observed that the AM3 treatment favoured the stress stabilization in sportsmen, maintaining the cortisol levels (10-12).

When analysing the haematological parameters, no significant differences were evidenced during the studied period, except for leukocyte counts in the last studied point with a slight increase. Resistance training did not have a significant effect on the basal levels of blood cells according with previous studies (3). These data can indicate the good process training of the sportsmen and in some way can confirm the adequate adaptation to training and competition benefited by the AM3 treatment.

A limitation to this study was our small sample size. However, to counter this notion a previous investigation in a similar population (elite basketball athletes) used fewer athletes than the present study. Likewise, other limitation to this study was the absence of a similar population control group who consumed AM3. However our groups achieve to the Euroleague final play-offs, where only achieve the four best teams of Europe. On the other hand, future studies should try to identify the exact mechanism of action of AM3, which results in the clear prevention of muscle damage based on the evidence provided by biochemical markers.

CONCLUSION

In conclusion, the Inmunoferon (AM3) supplementation may reduce muscle damage markers following prolonged and repeated exercise. Our results confirm that the AM3 (5 g/day) use during 6 weeks in periods of maximum competition maintain the serum levels of DOMS biochemical markers, especially in players with high exigency. We also suggest a buffering effect in DOMS biochemical markers during 6 weeks beyond the consumption phase. Thus, evaluation of serum enzymes in athletes is a simple method that trainers and physicians could use to know the training status of athletes.
ACKNOWLEDGEMENTS

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