Release of Heat-Shock Protein Hsp72 After Exercise and Supplementation With an *Opuntia ficus indica* Extract TEX-OE

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During exercise, a number of physiological and metabolic events occur. These include a rise in core and muscle temperature, oxidative stress, altered pH, and structural damage to muscle proteins. Heat-shock proteins (Hsp) are produced in response to different stress involving exercise and heat. Four polo ponies participated in the study. Blood samples were withdrawn 30 and 120 min after the beginning of a chukka. The same group of horses repeated the same test several weeks later; for that test, the ponies ingested 10 g of a powdered cactus-pear extract 6 h before the game. The blood concentration of Hsp72 was determined using a sandwich enzyme-linked immunosorbent assay. Blood Hsp72 significantly increased 120 min after the beginning of the exercise when the animals did not receive the supplement. Administration of the prickly pear cactus (*Opuntia ficus indica*) patented natural extract TEX-OE resulted in a significant increase of Hsp72 only 30 min after the beginning of the exercise. The extract had no effect on the level of Hsp72 before the beginning of the exercise. This study is the first to show that exercise can increase Hsp72 in the peripheral circulation of horses. Accelerating Hsp response time with prickly pear cactus extract will protect proteins in muscles and other tissues as well as foster repair and recovery of stressed cells. The use of the supplement will improve resistance to physical stress and should be included in optimal training regimens for competing horses. Authors’ addresses: Recovery Science Company, 37 Skyline Drive, Groton, CT 06340 (Martinod); Equine Internal Medicine, 4901 Mount Horeb Pike, Lexington, KY 40511 (Bernard); and Texinfine, 60 Rue Duquesclin, 69006 Lyon, France (Serrar, Gutierrez); e-mail: SMartinod@aol.com. © 2007 AAEP.

1. Introduction
The horse is an extraordinary athlete with a highly developed skeletal musculature. The performance of an equine athlete is determined by many interdependent biological processes, which need to be understood to design and manage training and exercise programs for competition.

Exercise is a major stress on muscles, including increased formation of reactive oxygen species (ROSs). ROSs contribute to muscle damage by denaturing proteins, disrupting membranes, lowering pH, and elevating temperature. Heat-shock proteins (Hsp) are a group of highly conserved proteins present in the cells of all living organisms. Hsp families include both constitutive and stress-inducible members whose primary function is to interact with naïve and denatured proteins to prevent the aggregation of aberrantly folded pro-
The information on Hsp-induction patterns in horses is limited. In equine cell lines, temperature and calcium stress have been shown to induce the synthesis of Hsp. Additionally, after exercise and training of horses, Hsp72 mRNA was induced in skeletal muscles with a positive correlation with the peak concentration of blood lactate.

Recently, the up-regulation of Hsp72 has been shown to be of great therapeutic potential in a variety of diseases including cancers and infections as well as tissue protection (cardio protection, neuroprotection, and skeletal muscle protection). This could be achieved by using inducers or co-inducers of Hsps.

The aim of this study was to examine if exercise increases serum Hsp72 in horses and if the use of the prickly pear cactus (Opuntia ficus indica) specific extract TEX-OE before exercise can induce a faster response.

2. Materials and Methods

Prickly Pear Cactus (Opuntia ficus indica) Extract TEX-OE
Naturally grown prickly pear cactus (Opuntia ficus indica) was collected in a pollution-free area and processed under strict manufacturing conditions. The extract was produced by drying the epicarp and specific tissues of the fruit and using a patented extraction process. The biological activity is controlled using standardized in vitro assay titration that measures the quantity of Hsp72 produced by cells in culture after exposure heat stress (43°C for 15 min) in the presence of the extract.

Test Subjects and Experimental Procedures
The polo ponies used in the study were Thoroughbreds and weighed between 430 and 480 kg. Between polo matches, they were subject to light exercise daily.

For the first phase of this experiment, a blood sample was drawn from the jugular vein before the beginning of the match. Blood samples were collected into Vacutainer® heparinized tubes and kept on ice before being frozen at −20°C until analysis. Other samples were taken 30 and 120 min after the beginning of the chukkas in which the ponies were playing. A chukka lasted 7 min and is an acute exercise.

After several weeks of rest, the same horses were used for the second phase of the experiment under similar weather conditions. The procedure was identical, but the animals received 10 g of the Opuntia ficus indica extract TEX-OE as a supplement mixed with the feed ~6 h before the polo match. Each horse acted as its own control.

Statistical Analysis
Hsp72 measures were expressed as a mean ± standard deviation. Data were compared by one-way analysis of variance (ANOVA). Results were regarded as significant at p < 0.05.

3. Results

None of the test subjects experienced any side effects from the Opuntia ficus indica extract TEX-OE or any adverse reaction. The animals did not seem to notice the supplement in their feed while eating.

Effect of Exercise on Hsp72 Levels in Blood
Results are given in Tables 1 and 2.

The exercise significantly increased the blood level of Hsp70 in horses after 120 min (p < 0.05) but not after 30 min.

The administration of the Opuntia ficus indica extract TEX-OE before exercise resulted in a significant increase in Hsp72 blood level after 30 and 120 min (p < 0.05). The levels did not change between 10 and 120 min.

Comparing the Results of No Supplementation Versus Supplementation
The results of Hsp blood levels with and without the supplementation are compared in Fig. 1.
The treatment with *Opuntia ficus indica* extract TEX-OE had no effect on the level of Hsp72 before exercise.

The increase in blood level of Hsp72 was significantly higher 30 min after the beginning of the exercise (p < 0.01) for the animals receiving the extract. After 120 min, the preconditioning with the extract had no effect on the Hsp72 blood levels.

4. Discussion

The major findings of this study can be summarized as (1) exercise induces the release of Hsp72 in blood of horses after 2 h, (2) the release of Hsp72 during exercise can be accelerated by preconditioning the horses with a natural supplement extracted from specific tissues of the fruits of *Opuntia ficus indica* TEX-OE, and (3) the use of the extract TEX-OE did not change the physiological values of Hsp72 in the absence of exercise or 2 h after the beginning of the exercise.

To our knowledge, this is the first study reporting that exercise increases blood Hsp72 in horses. Similar findings that a physiological event like exercise induced the release of Hsp72 in blood of horses 2 h after starting the exercise (p < 0.01) for the animals receiving the extract. After 120 min, the preconditioning with the extract had no effect on the Hsp72 blood levels.

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>T0</th>
<th>T30</th>
<th>T120</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>0.596 ± 0.012</td>
<td>1.081 ± 0.098</td>
<td>1.247 ± 0.217</td>
</tr>
<tr>
<td>H2</td>
<td>0.303 ± 0.005</td>
<td>0.626 ± 0.018</td>
<td>0.583 ± 0.020</td>
</tr>
<tr>
<td>H3</td>
<td>0.244 ± 0.023</td>
<td>0.794 ± 0.015</td>
<td>0.963 ± 0.068</td>
</tr>
<tr>
<td>H4</td>
<td>0.399 ± 0.020</td>
<td>0.853 ± 0.043</td>
<td>0.962 ± 0.104</td>
</tr>
<tr>
<td>Mean</td>
<td>0.3855 ± 0.1562</td>
<td>0.8385 ± 0.1881</td>
<td>0.9388 ± 0.276</td>
</tr>
</tbody>
</table>

Data are shown as mean of ELISA OD triplicates at 450 mm ± SD.
includes limiting protein aggregation, facilitating refolding, and chaperoning proteins to improve cell survival in the face of a broad array of cellular stressors. It has been reported that Hsp72 can protect nerves, heart, kidneys, intestines, liver, and muscles from heat or ischemia-induced apoptosis and cell deaths. Of particular importance in horses, Hsp72 may protect muscle cells against the protein-denaturing effects caused by the physiological consequences of exercise such as increased concentration of lactic acid, heat, and production of reactive oxygen species. A proportion of the muscle fibers that are reversibly damaged after muscle contraction may be repaired more readily. Temperature in the central core of equine superficial digital-flexor tendons can reach 45°C during high-speed locomotion. Expression of Hsp72 has been observed in human fibroblasts as a reaction to heat or mechanical stress in ligaments and tendons that protects the tendon fibroblasts from a high incidence of apoptosis and cell death.

There is also evidence that Hsp72 provided cardio protection by providing protection against myocardial dysfunction and infarction. In the nervous system, neurons exposed to extracellular Hsp are more likely to survive injury than their naïve counterparts. Lastly, it has been proposed that elevated Hsp72 acts as a signal danger to stimulate or enhance the immune function during times of increased risk of pathogenic challenge and tissue damage. It has been shown that super human athletes are exquisitely prone to infections. The ability to stay sound is critical for every equine athlete. Just like human athletes, they have a tendency to push their bodies higher, further, and faster than nature might have intended. The injuries that result, even when not catastrophic, can limit a horse’s ability to perform at the expected level. The balance between training and overtraining or between training and recovery are often very delicate. Because exercise induced Hsp72 only 2 h after the beginning of the competition in our test, it is likely that some cell damage already occurred; therefore, a preconditioning method using pharmacological compounds or natural supplements to accelerate an early induction of stress-protein response in vivo is needed and would be beneficial to the animals.

In this study, we successfully used a proprietary natural extract from the fruits of the prickly pear cactus Opuntia ficus indica TEX-OE to accelerate the release of Hsp72 in the blood. Significant increases in blood levels were observed only 30 min after the beginning of the exercise, and this improved the chance for a better recovery after stress or exercise. Opuntia ficus indica grows in some of the hottest and most arid regions of the world. It is common in deserts of North and South America, Africa, and the Mediterranean. Because of its ability to survive extremes of low and high temperatures and drought, the cactus likely possesses active molecules to protect and restore cells. The Opuntia ficus indica extract TEX-OE has been shown previously to accelerate the production of Hsp during stress in rodents and humans. In patients who have undergone the stress of deep-sea diving or high-altitude climbing, the accelerated Hsp response correlated with a reduction in symptoms.

The extract of Opuntia ficus indica TEX-OE is not a performance-enhancing agent. In the absence of stress, it does not stimulate the release of Hsp72. Furthermore, 2 h after the beginning of the exercise, the blood levels of Hsp72 are the same as the physiological values observed without preconditioning with the extract. The peak values remain the same, but the extract accelerates the release of the Hsp. At the cellular level, Hsp72 synthesis is induced by the activation of the heat-shock factor (HSF-1). In resting cells, Hsp72 are bound to HSF-1 and are kept in an inactive form. Stress activates a molecule, which results in the dissociation of the cytoplasmic Hsp72/HSF-1 complex and therefore, the release of Hsp72. Stress also triggers the trimerization and nuclear translocation of cytoplasmic HSF-1 with subsequent transcription of Hsp72. The extract TEX-OE stimulates the dissociation of the Hsp72/HSF-1 complex, and it is active only in response to stimuli such as stress.

Activation of stress proteins such as Hsp72 by exercise and acceleration of its release by pharmacological agents of natural origin like a specific Opuntia ficus indica extract TEX-OE allow cells such as the muscle cells to withstand a metabolic insult that could otherwise cause significant damage. It also activates the immune system. Such a practice called preconditioning should be included in training programs of competing horses. Other applications for the use of the Opuntia ficus indica extract TEX-OE are under investigation.
The extract TEX-OE was supplied by the manufacturer Icp Ltd Mosta MST 09 Malta (Gilles Gutierrez, Director) and will be commercialized by the Recovery Science Company (Serge Martinod, Managing Partner).

References and Footnotes


*TEX-OE, Icp Ltd. Mosta MST 09 Malta.
*SPA-810, StressGen Biotechnologies, Victoria, British Columbia, V8Z 4B9, Canada.
*SPA-812 StressGen Biotechnologies, Victoria, British Columbia, V8Z 4B9, Canada.
*Jackson ImmunoResearch, West Grove, PA 19390.
*TMB, Sigma Chemical Co., St. Louis, MO 63118.