Effectiveness of oral and topical hydrogen

Effectiveness of oral and topical hydrogen for sport-related soft tissue injuries

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Since hydrogen therapy in humans seems to be beneficial for treating inflammation, ischemia-reperfusion injury and oxidative stress, it seems plausible to evaluate the effects of exogenously administered hydrogen as an element of instant management of sport-related soft tissue injuries. The main aim of the present study was to examine the**effects of two-week hydrogen administration on the biochemical markers of inflammation and functional recovery in professional male athletes** after acute soft-tissue injury. Thirty six professional athletes were recruited and examined by certified sports medicine specialist in the first 24 hours after the sport-related soft tissue injury was sustained. The subjects were allocated to a double-blind design to three randomly assigned trials. During the period of 2 weeks subjects in the control group (CON) received traditional treatment protocol after the soft-tissue injury, consisting of RICE protocol during the first 48 h and sub-acute protocol thereafter. **Subjects in the first experimental group** (HYD1) followed the above procedures with additional administration of oral hydrogen-rich tabletes throughout the study. Subjects in the second experimental group (HYD2) followed the procedures of first experimantal group with additional administration of topical **hydrogen-rick packs 6 times per day for 20 minutes throughout the study.** Participants were evaluated at the time of the injury report, after 7 and 14 days after baseline testing.**Hydrogen intervention augmented plasma viscosity decrease as compared to the control group response (P = 0.04).** Differences were found for the range of motion (ROM) recovery between the three groups, with hydrogen intervention resulted in more superior return to normal joint ROM for both flexion and extension of injured limb as compared to the control intervention ($P < 0.05$).T**hese results support the hypothesis that the hydrogen may be effective as an additional agent to traditional conservative treatment of soft tissue injuries in athletes, with combination of oral and topical administration particularly powerful in this manner.** ***Relations between hydrogen administered and plasma C reactive protein or interleukin, pain scores and limb swelling during recovery were not found (P > 0.05).***

Key words Plasma Viscosity; Interleukin; Range of Motion; RICE protocol; Hydrogen

Introduction

Increase in sports participation in the past two decades has been accompanied by an increase in rates of sports injuries among both professional and recreational athletes, with soft tissue injuries (e.g. muscle sprain, ligament strain, tendonitis, contusion) account for over 75% of all injuries (Giannotti et al. 2011). The acute and effective management of sport-related soft tissue injuries is one of the key factors that contribute to fast recovery from injuries and return to regular training and competition (Chen & Dragoo 2013). The soft-tissue injuries repair is often facilitated by several conservative procedures (e.g. RICE protocol, topical or oral administration of non-steroidal anti-inflammatory drugs) as a way to relieve pain, swelling, bruising and improve functional movement (Leversedge & Srinivasan 2012). It seems that more cell damage can occur from the tissue hypoxia and acute reactive oxygen species produced at site of soft-tissue injury, with this subsequent tissue damage is often referred as the secondary zone of injury, in contrast to the initial damage caused by the actual mechanism of injury (Jackson 2008). **Since hydrogen therapy in humans seems to be beneficial for treating inflammation, ischemia-reperfusion injury and oxidative stress (Ohno et al. 2012), it seems plausible to evaluate the effects of orally and topically administered hydrogen as an element of instant management of sport-related soft tissue injuries.** The main aim of the present study was to examine the effects of two-week oral and/or topical hydrogen-rich administration on the inflammation, recovery, functional ability and the degree of pain intensity in competitive male athletes after **acute soft-tissue injury**.

Methods

Participants

Participants were eligible to participate in the study if they suffered a recent history of acute soft-tissue sports injury and had clinical findings consistent with trauma. Acute soft tissue sports injury was defined as direct or indirect trauma an athlete incurred in any sport-related activity that caused absence from training or from match. During the season 2013 (from March to May) participants (36 professional athletes) were recruited and examined by certified sports medicine specialist in the outpatient clinics of the Center for Health, Exercise and Sport Sciences in the first 24 hours after injury was sustained. Clinical findings were graded according to the amount of pain, weakness, and loss of motion, resulting in grades of I (mild), II (moderate), or III (severe) (Jarvinen et al. 2007). Patients who are not ambulatory or who have clinical findings classed as more severe than grade II were excluded from the study. All patients provided informed consent and volunteered to participate in the study. The protocol was approved by the local IRB in accordance with the Declaration of Helsinki. At the first assessment session, participants were fully informed verbally and in writing about the nature and demands of the study as well as the known health risks. They completed a health history questionnaire, and were informed that they could withdraw from the study at any time, even after giving their written consent. All subjects were in good health (free from diabetes, heart disease, cancer, and smoking), participating in consistent training (average of 12 hours per week) for the past five or more years, and not currently taking a drug or dietary supplement that contained hydrogen (or any similar preparation).

Experimental Procedures

Participants were randomized according to a computer-generated list in a double-blind design to three randomly assigned trials. During the period of 2 weeks subjects in the control group (CON) received traditional treatment protocol after the soft-tissue injury, consisting of RICE

protocol during the first 48 h (e.g. rest, ice packs for 20 minutes every 2 hours, compression with elastic bandage, elevation of the injured area above the level of the heart at all possible times) and sub-acute protocol thereafter (e.g. passive stretching 3 times per day for 90 sec, isometric strength exercise with 3 sets with 15 repetitions, 30 min of pain-free weight-bearing exercise). Subjects in the first experimental group (HYD1) followed the above procedures with additional administration of oral hydrogen-rich tablets (4 tablets three times per day) throughout the study. Subjects in the second experimental group (HYD2) followed the procedures of first experimental group with additional administration of topical hydrogen-rick packs 6 times per day for 20 minutes throughout the study. The oral hydrogen treatment formulation was provided by SevenPoint2TM (7.2 Recovery with HydroFXTM, Neport Beach, CA, USA) in tablet form. The topical hydrogen treatment formulation was provided by NORP Inc. (San Diego, CA, USA) and the participants were instructed to administer the packs directly to the skin above the site of injury with elastic wrap used to secure the hydrogen pack to the body area. During the administration period all subjects refrained from training. No other interventions were made. Participants were evaluated at the beginning of the study (e.g. at the time of injury report), and after 7 and 14 days after injury report. Baseline testing was performed prior to administration. Fasting blood was collected from a radial vein into gel vacutainer for biochemical variables. Serum C-reactive protein was determined using a highly sensitive ELISA procedure (eBioscience, San Diego, CA, USA). **Plasma viscosity at 25oC was measured using capillary viscometer (Coulter Viscometer II, Electronics Ltd, Luton, UK).** Serum interleukin 6 was determined with ELISA protocol (eBioscience, San Diego, CA, USA). Pain intensity was assessed using a visual analogue scale (Flaherty 1996). Participants completed two visual analogue assessments at each visit, one representing pain intensity while at rest, and the other representing pain while walking. Passive joint flexibility of the injured limb in sagittal plane was measured using a modified goniometer with spirit level (Creative Health Inc., Plymouth, CA, USA), with deficit of flexion and extension recorded. The degree of limb swelling at the site of injury was measured with anthropometric tape (Creative Health Inc., Plymouth, CA, USA) and compared with the non-injured limb. In order to assess potential side effects to the treatment regimen, all subjects were instructed to report any adverse effects of administration (e.g. skin irritation, rush) at every visit to the Center.

Statistical Analysis

The primary efficacy outcome was the change in serum CRP level at 2 weeks after the administration (effect size of 1.0) in the HYD1 group over the placebo group. Allowing for > 80% power, it was estimated that 10 participants per group would be required in the final analyses. This was adjusted to 12 subjects per group to account for a predicted 20% dropout. All results were expressed as mean ± standard deviation (SD). For group comparison during intervention at a series of time points we firstly identified and calculated area under the curve (AUC) (Jaki et al. 2009) for all dependent variables for each subject. Second, summary measures (mean AUC) for each group were tested with Shapiro-Wilk test for the normality of distribution, and with Bartlett's test for the homogeneity of the variances. When homogenous variances were verified for normally distributed data, summary measures were compared by ANOVA, with post-hoc Tukey HSD test was employed to identify the differences between individual sample pairs. When non-homogenous variances were identified mean AUC were compared using the three independent samples Kruskal-Wallis test, with Games-Howell post-

hoc test used to evaluate whether differences between any two groups are significant. Significance level was set at 0.05.

Results

A total of 36 participants completed the study, with no participant was lost due to follow-up. Most participants received all interventions regularly but a few omitted some quantity of tablets and/or packs. The total compliance with the hydrogen regimen was 83% for the HYD1 group, and 75% for the HYD2 group. Eighteen participants (8 men from HYD1 group, and 10 men from HYD2 group, respectively) reported different minor subjective side effects of hydrogen administration (Table 1). The frequency of reported adverse effects was similar between two experimental groups. There were no serious adverse events which occurred during the study.

- Table 1 about here -

Changes in plasma inflammatory markers during the study were presented in Table 2, with drop in plasma C reactive protein, interleukin 6 and viscosity noted for all intervention protocols throughout the study.

- Table 2 about here -

Hydrogen intervention augmented **plasma viscosity decrease** as compared to the control group response ($P = 0.04$), while the magnitude of alteration for other markers of inflammation were not different between the control group and hydrogen regimens ($P > 0.05$). However, the small-to-medium effect sizes were found for plasma viscosity and interleukin 6 for both hydrogen protocols ($d > 0.35$) (Table 3).

- Table 3 about here -

In response to the treatment, a substantial drop in pain scores at rest and while walking were observed in all three groups after the first and second week, respectively (Figure 1). No differences were found for pain scores changes between the groups ($P > 0.05$). However, **close-to-large effect size of HYD2 intervention was found for pain scores at walking** as compared to the control group ($d = 0.74$). Injured limb swelling decreased throughout the study (Figure 2), yet no differences were found for the degree of swelling reduction between the groups ($P > 0.05$). **Finally, differences were found for the range of motion (ROM) recovery between the three groups (Figure 3), with hydrogen intervention resulted in more superior return to normal joint ROM for both flexion and extension of injured limb** as compared to the control intervention ($P < 0.05$).

- Figure 1 about here -
- Figure 2 about here -
- Figure 3 about here -

Discussion

In this preliminary study, we showed that ***two-week hydrogen intervention augmented plasma viscosity decrease and speeded-up joint flexibility*** regain in male athletes suffered a sport-related soft tissue injury as compared to the control intervention per se. Relations between hydrogen administered and plasma C reactive protein or interleukin, **pain scores and limb swelling during recovery were not found**. The primary findings of this study provide evidence that hydrogen may be effective as an additional agent to traditional conservative treatment of soft tissue injuries, with combination of oral and topical administration particularly powerful in this manner.

From 1975 and first medical application of hydrogen in humans (Dole et al. 1975), hydrogen has been evaluated in the number of experimental and clinical disease conditions. Although research on health benefits of hydrogen is limited and there is a scant data on long-term effects, hydrogen has been identified as **beneficial in the prevention**and/or treatment of **diabetes mellitus** (Kajiyama et al. 2008), **metabolic syndrome** (Nakao et al. 2010), radiation-induced side-effects for liver tumors (Kang et al. 2010), inflammatory and mitochondrial myopathies (Ito et al. 2011), **exercise-induced acidosis** (Ostojic 2012), and **rheumatoid artritis** (Ishibashi et al. 2012). Effects of hydrogen on various diseases have been attributed to four major molecular mechanisms: a specific scavenging activity of hydroxyl radical, a scavenging activity of peroxynitrite, alterations of gene expressions, and signal-modulating activities (Ohno et al. 2012). Since hydrogen is known to scavenge toxic reactive oxygen species (ROS) and induce a number of antioxidant proteins during inflammation (Hong et al. 2010), **use of hydrogen may have a significant impact especially on oxidative stress-mediated disorders and inflammatory diseases in humans.**

As have been previously reported, oxidative stress is highly involved in the development of cell damage after sport-related soft tissue injuries (Mc Ginley et al. 2009). The acute response to trauma results in a drastic activation of immunocompetent cells and factors, interstitial edema and reduction of the microvascular blood supply, with highly ROS are released during the peroxidation of membrane lipids (van der Vusse et al. 1995). This leads to cell destruction and subsequent pain, swelling, bruising and loss of function (Kannus et al. 2003), with standard medical treatment usually encompass the RICE protocol to decrease swelling and pain. Yet, rapid elimination of ROS and inflammation markers in athletes suffered soft tissue injury may be beneficial for enhanced recovery of clinical markers and functional abilities. Our results suggest that addition of hydrogen to traditional soft-tissue injury treatment positively affected selected clinical and biochemical indicators of post-injury recovery, such as plasma viscosity and flexibility of the injured area. Several inflammatory markers (e.g. C-reactive protein, erythrocyte sedimentation rate, fibrinogen, ferritin, interleukin 6, plasma viscosity) are routinely monitored after the injury and inflammation in musculoskeletal medicine (Kumbhare et al. 2012). These biomarkers are immediately elevated after the soft tissue injury, with levels correlate to the clinical stages of the condition (Gabay & Kushner 1999). Furthermore, evaluation of biomarkers time course after the injury is relevant for monitoring injury management and recovery (Kumbhare et al. 2012). In the present study, we noticed drop in selected blood inflammatory markers for all experimental protocols throughout the study, indicating reduced inflammation during recovery after sport-related soft tissue injury. **However, we found significant difference between groups for changes in plasma viscosity, with athletes additionally supplemented with both topical and oral hydrogen experienced much faster decline in plasma viscosity relative to control**

group. Since plasma viscosity sensitivity and specificity are better than that of erythrocyte sedimentation rate or C-reactive protein in inflammation (Watson et al. 2012), we could assume that hydrogen may positively affect the inflammation process in injured athletes as an additive to conventional treatment. Although oxidative stress is involved in the development of post-injury inflammation, antioxidant effect of hydrogen may not be the only driving factor causing positive anti-inflammatory effects of administration. Possible impact of hydrogen on down-regulation of proinflamatory citokines (Ito et al. 2011) after musculoskeletal injury requires further investigation.

Most sport-related soft tissue injuries recover rapidly, yet different therapy protocols are launched to speed-up the process of return to sport after injury (Nepple & Matava 2009). Provision of aggressive acute and sub-acute treatment protocols while healing will facilitate recovery in athletes (Kannus et al. 2003), with restoring function of injured limb set as a main goal of injury treatment. Traditional medical treatment of soft-tissue injury is designed to **decrease the swelling and pain, and regain the mobility of injured limb.** In this study, we demonstrated similar **positive dynamics of recovery for limb swelling and pain** at rest and while walking among groups. It seems that additional hydrogen did not intensify the reduction of pain or edema during recovery by traditional treatment. Yet, comparison of effect size for pain at walking revealed large effect of treatment between control group and group of athletes supplemented with both oral and topical hydrogen (d = 0.74), which may indicate the more power of HYD2 to affect the pain during recovery. Interestingly, subjects supplemented with hydrogen demonstrated a significant improvement in range of motion of injured limb during recovery. Taken together it is possible to suggest that the improvement in clinical outcomes after hydrogen administration is somewhat noticeable, yet the effects are rather small-to-medium, with combination hydrogen treatment particularly effective in this manner. The incidence of subjective side effects was not different between the two experimental groups, with most side effects reported as rather single episodes, which disappeared during the first week of the intervention. The swallowing problems can be clearly attributed to the number of ingested tablets (12 per day) and could be reduced by the application of a different dosage form. On the other hand, abdominal adverse effects (e.g. heartburn, intestinal cramping) may be due to the effects of molecular hydrogen on gut mucosae or peristalsis after ingestion, which requires further investigation.**No previous study reported side effects after topical administration of hydrogen,** with side effects found in the present study having possible relationship to the topical administration were classified as mild in intensity. Previous studies found no serious adverse events of oral hydrogen administration, with this method of hydrogen delivery to humans reported to be safe and simple (Nakao et al. 2010; Ostojic 2012). Nevertheless, the pharmacokinetics of oral and/or topical hydrogen administration have not been studied in depth.

Despite the evidence that hydrogen has relevant effects on soft tissue injury recovery in professional male athletes, the present study has several limitations. Firstly, we did not consider other possible factors that could be responsible for the changes of injury recovery outcomes between the groups, such as the site, mechanism and type of injury, age and professional experience of participants, previous injury etc. Second, the size of the experimental samples could be considered partly limited, in particular considering that compliance with the protocol is not perfect. Consequently, although of small-to-moderate magnitude, the observed differences between the groups in several outcomes (e.g. pain at rest and while walking) could not reach the statistically significant level. During this study we

assessed only a few important biochemical components related to the soft tissue injury recovery, neglecting further parameters that might be directly or indirectly connected to hydrogen intake, such as creatine kinase (CK), endothelial leukocyte adherence and mean protein content. Since hydrogen affects derivates of reactive oxidative metabolites (dROMs), biological antixodant power (BAP) and superoxide dismutase (SOD) in healthy subjects (Aoki et al. 2012), it will be interesting for future studies to assess several antioxidant parameters during hydrogen administration in athletes suffered a soft-tissue injury.

In conclusion, administration of hydrogen for 2 weeks demonstrated an improvement in the level of plasma viscosity associated with acute sport-related soft tissue injury and boosted the injured limb ROM recovery. Use of oral and topical hydrogen represents a potentially novel and safe therapeutic strategy for the treatment of the soft tissue injury in male professional athletes.

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Table 1. Incidence of subjective side effects reported during the study.

Symptoms	HYD1 (n = 12)	HYD2 (n = 12)	P *
Swallowing problems	8	9	0.99
Heartburn	7	7	1.00
Intestinal cramping	3	3	1.00
Diarrhea	3	2	0.99
Nausea	1	-	0.99
Skin irritation	-	2	0.48
Rash	-	3	0.22

* P value from Fisher exact probability test.

Table 2. Changes in plasma inflammatory markers during the study. Values are mean ± SD (n = 36).

	Baseline	Week 1	Week 2	AUC	P	Post-hoc differences *
C-reactive protein (mg/L)						
CON	60.2 ± 38.3	34.3 ± 21.3	18.7 ± 10.4	73.7 ± 44.4	0.97 †	-
HYD 1	75.0 ± 71.1	47.5 ± 44.5	29.4 ± 29.8	99.7 ± 93.4		
HYD 2	62.6 ± 36.0	37.0 ± 24.3	21.9 ± 12.1	79.2 ± 47.1		
Interleukin 6 (pg/mL)						
CON	92.5 ± 24.3	72.3 ± 10.5	68.6 ± 6.9	152.9 ± 24.4	0.45 †	-
HYD 1	105.7 ± 35.7	77.2 ± 11.5	68.1 ± 8.6	164.1 ± 31.2		
HYD 2	101.0 ± 22.8	74.1 ± 12.5	67.3 ± 7.7	158.3 ± 25.4		
Viscosity (mPa•s)						
CON	1.45 ± 0.12	1.34 ± 0.10	1.26 ± 0.10	2.70 ± 0.20	0.04 ‡	a
HYD 1	1.42 ± 0.15	1.26 ± 0.08	1.19 ± 0.07	2.57 ± 0.17		
HYD 2	1.39 ± 0.14	1.25 ± 0.07	1.16 ± 0.06	2.52 ± 0.15		

Note. AUC - area under the curve, is defined as the area under the plot of serum/urine concentration of selected outcome (not logarithm of the concentration) against time after intervention administration; CON – control group; HYD 1 – group supplemented with oral hydrogen; HYD 2 – group supplemented with oral hydrogen and topical hydrogen packs; † P value from independent samples Kruskal-Wallis test. ‡ P value from three-sample unpaired ANOVA test. * Significant difference at $P < 0.05$. a, CON vs. HYD 2.

Table 3. Effect size between groups for mean gain scores during the study

	HYD1 vs. CON	HYD2 vs. CON	HYD1 vs. HYD2
C-reactive protein	0.03	0.01	0.03
Interleukin 6	0.38	0.41	0.12
Viscosity	0.32	0.44	0.08
Pain at rest	0.20	0.09	0.29
Pain at walking	0.35	0.74	0.46
Degree of swelling	0.18	0.05	0.03
ROM deficit in flexion	0.09	0.08	0.05
ROM deficit in extension	0.27	0.14	0.31

Note Effect sizes are indicated as small (d = 0.20), medium (d = 0.50), and large effect size (d = 0.80).

Figure 1. Pain at rest and while walking during the study. CON – control group; HYD1 – group supplemented with oral hydrogen; HYD2 – group supplemented with oral hydrogen and administered with topical hydrogen-rich packs.

Figure 2. Degree of swelling during the study. CON – control group; HYD1 – group supplemented with oral hydrogen; HYD2 – group supplemented with oral hydrogen and administered with topical hydrogen-rich packs.

Figure 3. ROM deficit in flexion and extension during the study. CON – control group; HYD1 – group supplemented with oral hydrogen; HYD2 – group supplemented with oral hydrogen and administered with topical hydrogen-rich packs. * Indicates significant difference between groups at $P < 0.05$.