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Article in Complementary Therapies in Medicine · October 2014
Impact Factor: 1.55 · DOI: 10.1016/j.ctim.2014.10.008

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Wet-cupping removes oxidants and decreases oxidative stress

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Summary

Objectives: Wet-cupping therapy is one of the oldest known medical techniques. Although it is widely used in various conditions such as acute, chronic inflammation, infectious diseases, and immune system disorders, its mechanism of action is not fully known. In this study, we investigated the oxidative status as the first step to elucidate possible mechanisms of action of wet cupping.

Material and methods: Wet cupping therapy is implemented to 31 healthy volunteers. Venous blood samples and Wet cupping blood samples were taken concurrently. Serum nitric oxide, malondialdehyde levels and activity of superoxide dismutase and myeloperoxidase were measured spectrophotometrically.

Results: Wet cupping blood had higher activity of myeloperoxidase, lower activity of superoxide dismutase, higher levels of malondialdehyde and nitric oxide compared to the venous blood.

Conclusion: Wet cupping removes oxidants and decreases oxidative stress.

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Abbreviations: MDA, malondialdehyde; iNOS, inducible nitric oxide synthase; SOD, superoxide dismutase; MPO, myeloperoxidase; HRV, heart rate variability; LP, lipid peroxidation; TBA, thiobarbituric acid; NBT, nitroblue tetrazolium; H2O2, hydrogen peroxide; NOx, nitrite + nitrate.

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http://dx.doi.org/10.1016/j.ctim.2014.10.008
0965-2299/© 2014 Published by Elsevier Ltd.
Introduction

Cupping is a traditional therapy dating back at least 2000 years. There are a lot of cupping applications in practice such as needle cupping, moving cupping, retained cupping, medicinal (herbal) cupping and bleeding cupping (wet cupping). The last one is the most commonly used cupping type. Each kind of cupping therapy may be used for different diseases or different purposes of treatment. In general, a glass cup is applied on the skin over an acupuncture point, painful area, or a reflex zone. This treatment creates a vacuum over certain points on the skin. Some researchers hypothesize that implementation of cups on selected acupoints on the skin results in a therapeutic effect by hyperemia.

The most conditions in which wet cupping therapy is commonly employed were pain related conditions including chronic muscle pain, fibromyalgia, herpes zoster pain and neuralgias such as headache and sciatica. Also, cupping therapy is used in many other abnormalities such as cough or asthma, acne, common cold, urticaria, facial paralysis, soft tissue injury, arthritis, neurodermatitis.

The main purpose of this therapy is to precipitate the circulation of blood and to reduce blood-stasis and waste from the body. Local damage of the skin and capillary vessels may act as a noiceptive stimulus. Cupping is thought to remove noxious materials from skin microcirculation and interstitial compartment.

Wet cupping has been claimed to drain excess fluids and toxins, loosen adhesions and lift connective tissue, bring blood flow to skin and muscles, and to stimulate the peripheral nervous system. Also, cupping is said to reduce pain and high blood pressure as well as modulate neurohormones and the immune system. Cupping therapy is also used to improve subcutaneous blood flow and to stimulate the autonomic nervous system.

Free oxygen radicals formed during physiological and pathophysiological metabolism are balanced by a similar rate of their consumption by antioxidants. Although their excess production may cause oxidative damage on biological molecules, cell membranes and tissues, their generation is inevitable for some metabolic processes. Free radical-mediated oxidative damage has been implicated in the pathogenesis of a large number of diseases, including autoimmune diseases of endocrine glands, cancer, inflammatory diseases, cardiovascular disease, (atherosclerosis, hypertension, ischemia/reperfusion injury), diabetes mellitus, neurodegenerative diseases (Alzheimer’s disease and Parkinson’s disease), rheumatoid arthritis, and ageing.

A recent study showed cupping had therapeutic effects on myocardial infarctions and cardiac arrhythmias in rats. Also, another recent study investigated the possible useful effects of cupping therapy on cardiac rhythm in terms of heart rate variability (HRV). All HRV parameters increased after cupping therapy compared to before cupping therapy in healthy persons. They suggested that cupping might be cardio protective. It can be stated that cupping therapy restored sympathovagal imbalances by stimulating the peripheral nervous system.

As seen, wet cupping is widely used in many cases mentioned above which are associated with oxidative damage.

We wondered if wet cupping therapy affects oxidative balance or not.

The aim of this study was to investigate the dynamics of oxidative stress and antioxidant status markers in both venous blood and wet cupping blood of healthy volunteers by measuring the levels of malondialdehyde (MDA), nitrite, and the activities of superoxide dismutase (SOD) and myeloperoxidase (MPO). The originality of our study is that we simultaneously measured all these parameters in wet cupping and venous blood samples of healthy people for the first time in the literature.

Materials and methods

Subjects and study design

Our study population consisted of 31 healthy volunteers, 15 females and 16 males; aged 21-40 years (mean age 30.24 ± 9.53 years). Venous blood samples were collected after overnight fasting and just before wet cupping employment and placed into no-additives-containing tubes. Wet cupping blood samples were taken from the cups after bleeding and vacuum applications and placed into the test tubes. Serum fraction was obtained by centrifugation (2000 × g, 10 min, and 4 °C) after storing the whole blood at room temperature (approximately 10 min) and stored at −80 °C until analysis.

Participants who had serious conditions of the spine and spinal cord (e.g., Ankylosing spondylitis), infectious disease, malignancy or immune disorder were excluded. Cupping therapy involves an invasive procedure, so participants who had blood-borne diseases or hemostatic abnormalities, such as AIDS, active hepatitis, syphilis, hemophilia and anemia, or took anticoagulants or antiplatelet agents, such as warfarin, aspirin were excluded. Written informed consent was obtained from each participant and study protocol was accepted by local ethic committee.

Wet cupping

All cupping procedures were applied by two physicians certificated by British Cupping Society and Natural Health Institute. For the cupping therapy, sterile disposable cups of 5 cm in diameter were used. Five points of the posterior neck, bilateral perispinal areas of the neck and thoracic spine were selected for treatment (Fig. 1). Same points were applied to all participants. Application areas were cleaned with antiseptic solutions. Cups were placed to these points and negative pressure applied by cupping pump. Two-three minutes were waited and cups removed. Then, the skin was punctured to a 2-mm depth within the cupping sites with 26-gage disposable lancets. After this, the pumping with vacuum was applied for the second time and 3 to 5 cm² of blood was drained per cupping site. Application sites were covered the sterile pads.

We did not experience any adverse reaction but fainting due to intolerance to pain might have been possible, a doctor, a nurse and emergency response kit—stretcher were kept ready in application room.
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Determination of serum nitric oxide (NO\textsubscript{x}) levels

NO\textsubscript{x} levels in plasma were determined spectrophotometrically, based on the reduction of NO\textsuperscript{−−} to NO\textsuperscript{−} by VaCl\textsubscript{2}. Nitric oxide level was measured by the Griess reaction. Sodium nitrite and nitrate solutions (1, 10, 50, 100 \textmu M) were used as standards. Serum samples were deproteinized prior to assay. Samples were added to 96% cold ethanol (1/2 v/v) and then vortexed for 5 min. After incubation for 30 min. at +4 °C, the mixture was centrifuged at 14000 rpm for 5 min and the supernatants were used for the Griess reaction.\textsuperscript{19}

Determination of serum MDA levels

As an index of lipid peroxidation (LP), MDA were used for the assessment of superoxide (O\textsuperscript{2−}) radical production and determined by thiobarbituric acid (TBA) reaction according to the Uchiyama.\textsuperscript{19} The principle of the method depends on the measurement of the pink color produced by interaction of TBA with MDA. The pink color absorbance is measured at 532 and 520 nm. MDA levels are expressed as nmol/L.

Determination of SOD activity

As an index of the antioxidative defense system, we measured the activities of serum SOD, a specific O\textsubscript{2−} scavenger. Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al.\textsuperscript{20} The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a O\textsubscript{2−} generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The enzyme activity is expressed as U/L.

Determination of MPO activity

MPO (EC 1.11.1.7) activity was assayed by measuring the hydrogen peroxide (H\textsubscript{2}O\textsubscript{2})-dependent oxidation of o-dianisidine. In its oxidized form, o-dianisidine has a brown color. This was measured spectrophotometrically at 410 nm. The results are given as U/mL. One unit of MPO activity was defined as the amount of enzyme that caused absorbance change in 1 min at 410 nm and 37 °C.\textsuperscript{21}

Statistical analysis

Results are expressed as mean ± standard error mean. Distributions were evaluated by using One Sample Kolmogorov Smirnov test. A two-tailed paired t-test or Wilcoxon test was used to compare as appropriate. Pearson or Spearman Rho correlation test was used to indicate relationships between variables. Differences were considered statistically significant at p < 0.05. The SPSS statistical software package (SPSS, version 16.0 for windows; SPSS Inc., Chicago, Illinois, USA) was used to perform all statistical calculations.

Results

Study population consisted of 31 healthy volunteers. Wet cupping blood had higher activity of MPO, higher levels of MDA, and NO\textsubscript{x} compared to the venous blood (see Table 1). In addition, wet cupping blood had lower activity of SOD. After correlations analysis, we found positive correlations between NO\textsubscript{x} levels of venous blood and NO\textsubscript{x} levels of wet cupping blood (r = 0.64, p = 0.001). Except for NO\textsubscript{x} levels, there was no correlation for the other parameters.

Discussion

The mechanism of action of wet cupping therapy is not fully known despite its common use. Wet cupping therapy might act through a lot of different mechanisms. We hypothesize that one of the mechanisms of action of wet cupping may be through oxidative balance. The cardinal findings of this study include that: (i) compared with the venous blood, wet cupping blood had higher activity of MPO (ii) lower activity of SOD (iii) higher levels of MDA (iv) and higher levels of NO\textsubscript{x} (v) and there was no correlation for the oxidative status parameters between venous and wet cupping blood except for NO\textsubscript{x} levels. This correlation may be used to determine optimal wet cupping time. Of course, further studies are required.

Nitratating oxidants are implicated in host defense mechanisms and the pathogenesis of many diseases. Nitrite, a stable end product of NO metabolism, converts hypochlorous acid (HOCl) into the more potent chlorinating species.\textsuperscript{21} It has been reported that MPO and inducible nitric oxide synthase (iNOS) are co-localized in the primary granules of leukocytes. During oxidative stress, activated MPO and iNOS generate HOCl and NO, respectively. As a result of this, the MPO-dependent oxidation of nitrite increases reactive nitrogen species forming. The measurement of MPO activity has not been investigated in both wet cupping and venous serum of healthy ones simultaneously.

NO is one of the most abundant free radicals in the body. Both cytotoxic and cytotoxic effects of NO have been reported. Interaction of O\textsubscript{2−} and NO leads to the production of more reactive oxidant.\textsuperscript{22} We observed that in wet cupping blood the level of nitrite was significantly higher than the venous one.

The organism can defend itself against the effects of oxidative stress by increasing SOD activity as a protection
mechanism. Our results indicate that the activity of SOD was lower in wet cupping serum. It should be further investigated why SOD activity decreased while oxidant parameters were increasing.

LP was monitored by measuring MDA, which results from free radical damage to membrane components of the cells. LP modifies the functional characteristics of the cellular membranes, changing their permeability and causing inactivation of membrane-bound receptors and enzymes. MDA can also deactivate membrane transporters, by forming intramolecular and intermolecular crosslinks. In the present study, we found that the MDA level was higher significantly in wet cupping blood than it was in venous blood.

Niasari et al. tested wet cupping therapy on serum lipid concentrations. They concluded that wet cupping may be an effective method of reducing LDL cholesterol in men and consequently may have a preventive effect against atherosclerosis. These results support the present study and drainage or excretion hypothesis of excess fluids and toxins from the body by wet cupping.

The results of this study showed that venous blood and wet cupping blood did not have the same characteristics. Wet cupping blood had obviously higher oxidants compared to venous samples. The therapeutic effects of wet cupping in various conditions might be due to the excretion of these oxidants from the body. We hope that the relationships between wet cupping and other harmful substances in the body will be investigated in the future and this preliminary study would be a guide for these studies.

In conclusion, wet cupping removes oxidants and decreases oxidative stress.

Limitations

We could not evaluate the levels of oxidative stress parameters in venous blood after WCT employment because of not taking venous samples for the second time. Furthermore, all volunteers in our study group were healthy so we could not assess the effect of WCT on the diseases related to oxidative stress. We emphasize that more extensive studies with a broader study population should be carried out to further determine the effect of WCT on oxidative status.

Conflict of interest statement

There is no conflict of interest to declare.

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