Complementary Therapies in Medicine 36 (2018) 25–29

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Is cupping blister harmful?—A proteomical analysis of blister fluid induced by cupping therapy and scald

Zhidan Liu[a,1], Chunlan Chen[a,1], Xiaoyan Li[a], Chuang Zhao[a], Zunyuan Li[a], Wei Liang[a], Yufang Lin[b,*]

[a] Department of Acupuncture, Shanghai Baoshan Hospital of Integrated Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, 201999, China
[b] Department of Acupuncture, Zhejiang Hospital, Hangzhou, 310013, China

ARTICLE INFO

Keywords:
Proteomics
Cupping therapy
Blister fluid

ABSTRACT

Objective: Cupping therapy has a long history in traditional medicine especially in Asian countries. It was controversial whether cupping induced blisters are beneficial to healing effects, and the formation and content in the blisters remain unexplored. We aimed to identify and compare the molecular components of the blister fluid from the cupping therapy and the scalds to explore the necessary of inducing cupping induced blisters.
Methods: Fluid sample of blisters from fifteen patients receiving cupping therapy (Cupping group) and scald burns (Scald group) were collected in this study. Proteins from the blisters were separated by two-dimensional electrophoresis (2D-gel) and further analyzed by mass spectrometry. In addition, the changes in particular proteins were confirmed by Western blotting.
Results: The protein components are significantly different between blister from cupping therapy and scalds. The immune responses, oxidative stress and metabolic related proteins (Ig lambda-2 chain C regions, Ig gamma-1 chain C region, hemopexin, prdx2, calmodulin, succinyl-CoA ligase and tetranectin) were increased, whereas the hemoglobin subunit beta was decreased in the Cupping group compared with the Scald group.
Conclusions: Cupping induced blisters contain several proteins which relate to the activation of certain immune pathways including anti-oxidation, anti-apoptosis, tissue repairing and metabolic regulation. This proteomic analysis may indicate a significant clue to the mechanism study of cupping.

1. Introduction

Cupping therapy which utilize a glass or bamboo cup to create suction on the skin has a long history in the traditional medicine in Asia, especially in China.[1–3] The efficacy has been shown in treating dysmenorrheal, pain, osteoarthritis, et al. [4–6]. The skin where cupping therapy applied would usually be marked with a spot in pink, dark purple or red, as we can see on the skin of Olympic athletes, and sometimes be accompanied by the appearance of small blisters on the skin due to the generation of negative pressure by combustion (Fig. 1).

Some opinion holds that cupping-induced blisters are embodiment of “damp evil” suction to excrete the “toxin” in vivo,[7] so it’s beneficial to recovery, and symptoms including edema, crepitation and stiffness were significantly relieved after blister formation. But some others argue that cupping-induced blisters might be similar to blisters caused by scalds which manifest the production of severe skin damage, and the blisters could leave skin scars and will affect the patient’s appearance, or induce erythematous based vesiculobullous plaque.[8] So practitioners should try to avoid blisters when applying cupping therapy.

Thus it’s unclear whether the composition of the cupping-induced blisters will promote therapy efficiency; and if it is, what is the molecular mechanisms underline. Therefore, it should be answered that is there something different be produced in cupping-induced blisters? So it is necessary to firstly compare the protein components between blister fluid samples caused by cupping therapy and scalds. We characterized the molecular elements of the blister fluid by proteomic analysis.

Abbreveations: MS, mass spectrometry; TCM, Traditional Chinese Medicine; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; GTP, guanosine triohosphte; ATP, adenosine triphosphate; CaMKII, Calcium/calmodulin-dependent protein kinase II; GABAA R, γ-aminobutyric acid type A receptor

* Corresponding authors.
E-mail address: springvividly@sina.com (Y. Lin).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ctim.2017.11.002
Received 2 August 2017; Received in revised form 2 October 2017; Accepted 1 November 2017
Available online 14 November 2017

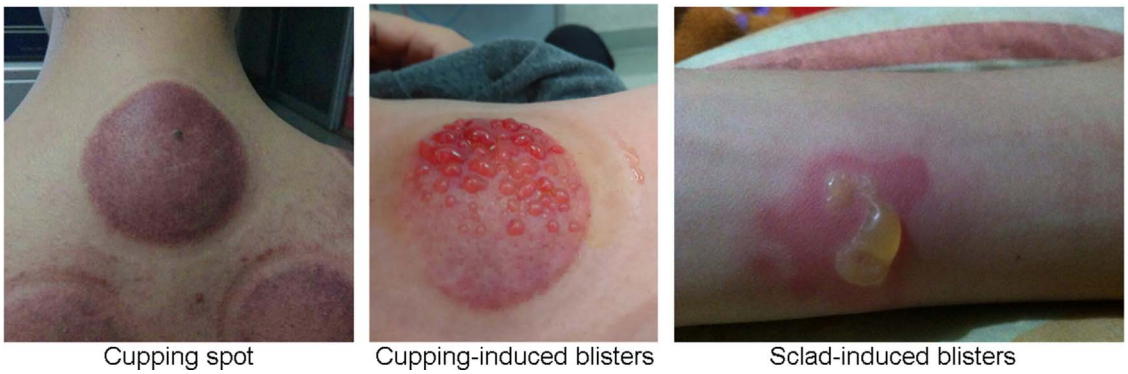

Fig. 1. Cupping spot, cupping-induced and scald-induced blisters.
Cupping spot usually shows in pink, dark purple or red color related to the maintaining time and intensity of negative pressure. Cupping-induced blisters appears when cups retains a long time more than fifteen minutes or in other conditions of some sick individuals, and its' exterior are similar to scald-induced blisters.

2. Materials and methods

2.1. Subjects

This study was conducted from January 2013 to December 2015. Fluid sample of cupping group were collected from fifteen patients suffered from shoulder and back pain who were given cupping therapy in the Department of Acupuncture, Shanghai Baoshan Integrated Hospital of Traditional Chinese Medicine and Western Medicine, Shanghai University of Traditional Chinese Medicine (TCM). And fluid sample of scald group were collected from twenty burned patients in the Department of Burn and Plastic Surgery, Ninth People's Hospital of Shanghai Jiaotong University.

All patients were between 20 to 60 years old with males and females. This study was reviewed and approved by the ethical committee in Shanghai Baoshan Integrated Hospital of Traditional Chinese and Western Medicine, Shanghai University of TCM. Participants with the following conditions were excluded: (1) serious comorbid conditions (e.g., life-threatening condition or severe neurological defects); (2) patients who cannot communicate reliably with the investigator or who are not likely to obey the instructions of the trial; (3) severe infection; (4) pregnancy.

Patients in Cupping group received dry cupping therapy with glass pot (5 cm caliber) on related acupuncture points or regions like DU14(Da-zhui), LI15(Jian-yu), SJ14(Jian-liao), BL11(Da-zhu), BL12(Feng-men), BL13(Fei-shu) and Ashi points. The patient was in prone position. Routine disinfection of back skin was applied. The sucked glass pots were kept for ten minutes. Once blisters appeared, the glass pots were removed and blister liquid was collected immediately. For patients in Scald group, routine clinical care was applied at the Department of Burn and Plastic Surgery. Skin blister liquid was collected prior to appropriate treatment.

2.2. Sample preparation and protein extraction

Blister liquid of all participants was extracted using 2 ml clean syringe and was stored at room temperature for 30 min. Samples were centrifuged at 2000xg for 20 min at room temperature, and supernatant was collected and stored at −70 °C for analysis. Supernatant (to remove serum albumin and IgG) was cleaned using ProteoExtract™ Albumin/IgG Removal Kit (CALBIOCHEM, USA), and was centrifuged at 5000xg at 4 °C for 30 min. Pellets were resuspended in lysis buffer containing protease inhibitors at 30 °C for 1 h. Extracts were centrifuged at 15000 × *g* for 15 min to remove debris. Protein concentrations were determined using the Bradford assay. 10 μg total protein was loaded on the gel and subject to western blot analysis.

2.3. Two-dimensional (2-D) PAGE

300 μg samples were loaded into IPG Runner cassettes and a dry strip (GE Healthcare , 24cm, pH = 3–10 isoelectric focusing gel) was placed into the well. Rehydration was carried out for 8 h, after which the strips were transferred. Isoelectric focusing was carried out at 20 °C as follows: 100 V, 1 h; 200 V, 1 h; 500 V, 1 h; 1000 V, 1 h; 1000–10000 V-h, 108000 V, 12 h; 500 V, 12 h. After focusing, the strips were placed into gels and sealed in place with agarose with bromophenol blue to be fractionated. Signals were visualized by silver staining. The difference between group B and group S was calculated as fold ratio. A threshold of $p \leq 0.05$ and fold change ≥ 2 or ≤ 0.5 was set to select differentially protein spots.

2.4. Mass spectrometry analysis

Gels were stained, destained and subjected to tryptic digestion, followed by MALDI-TOF/TOF Plus mass spectrometer analysis (Applied Biosystems, Foster City, USA). Data were acquired in a positive MS reflector using a CalMix5 standard to calibrate the instrument (ABI5800 Calibration Mixture). Data were integrated and processed by using the GPS Explorer V3.6 software (Applied Biosystems, USA) with default parameters. Proteins were successfully identified based on 95% or higher confidence interval of their scores in the MASCOT V2.3 search engine (Matrix Science Ltd., London, U.K.). We used NCBInr-Homo Sapiens database, Carbamidomethyl (C) was set as fixed modification, variable modifications included protein N-terminal acetylation (Protein N-term), Deamidated (NQ), Dioxidation (W), Oxidation (M), 100 ppm for precursor ion tolerance and 0.3 Da for fragment ion tolerance.

2.5. Western blot

Protein samples were resolved by 10% sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS–PAGE), and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Uppsala, Sweden). For western hybridization, the membranes were preincubated with 0.5% skim milk in TTBS (0.1% Tween 20 in Tris-buffered saline) at room temperature for 2 h. The primary antibody to phospho-Src, or phospho-PKC (Cell Signaling Technology Inc., Danvers, MA) was diluted 1:1000 in TTBS containing 5% bovine serum albumin (BSA), and incubated overnight at 4 °C. The membranes were washed four times with TTBS, horseradish peroxidase-conjugated secondary antibody was added, and the membranes were incubated for 1 h at room temperature. After washing with TTBS, the hybridized bands were detected using an ECL detection kit and Hyperfilm-ECL reagents (Amersham Pharmacia).