European Journal of Integrative Medicine 8 (2016) 204–212

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/eujim

Acupuncture inhibits liver injury induced by morphine plus acetaminophen through antioxidant system

Young Joon Lee[a], Rong Jie Zhao[b], Young Woo Kim[c], Su Jin Kang[a], Eun Kyung Lee[a], Nam Jun Kim[d], Suchan Chang[e], Jin Mook Kim[e], Ji Eun Lee[f], Sae Kwang Ku[f,**], Bong Hyo Lee[d,*]

[a] Department of Preventive Medicine, College of Korean Medicine, Daegu Haany University, Gyeongsan 712-715, Republic of Korea
[b] Department of Pharmacology, Jining Medical University, 272000, China
[c] Department of Prescription, College of Korean Medicine, Daegu Haany University, Gyeongsan 712-715, Republic of Korea
[d] Department of Acupuncture, Moxibustion and Acupoint, College of Korean Medicine, Daegu Haany University, Daegu 706-828, Republic of Korea
[e] Department of Physiology, College of Korean Medicine, Daegu Haany University, Daegu 706-828, Republic of Korea
[f] Department of Anatomy and Histology, College of Korean Medicine, Daegu Haany University, Gyeongsan 712-715, Republic of Korea

ARTICLE INFO

Article history:
Received 19 June 2015
Received in revised form 21 December 2015
Accepted 22 December 2015

Keywords:
Hepatotoxicity
Oxidative stress
Fibrosis
Hepatic damage
Morphine
Acupuncture

ABSTRACT

Introduction: Morphine (MP) and acetaminophen (APAP), widely used-pain relievers and antipyretics, are known to induce hepatotoxicity. Acupuncture, a traditional therapy in Asia, is used for various reasons including detoxification. This study aimed to examine whether acupuncture exerts protective effects against MP + APAP-induced hepatic damage.
Methods: Male Sprague-Dawley rats received chronic MP, withdrawal, and APAP. Thereafter, blood and liver were taken. Acupuncture was performed once daily. Asparte aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured, and percentages of abnormally decreased-hepatocyte regions, mean liver cell counts, and mean inflammatory cell numbers infiltrated on hepatic parenchyma were examined. In addition, antioxidant effects were evaluated based on liver lipid peroxidation malondialdehyde (MDA) and glutathione (GSH) contents, superoxide dismutase (SOD) and catalase (CAT) activities with the number of immunopositive hepatocytes against nitrotyrosine (NT) as a marker of inducible nitric oxide synthase (iNOS) related-oxidative stresses and 4-hydroxynonenal (4HNE) as a marker of lipid peroxidation.
Results: Significant elevations of AST and ALT were noted of MP or APAP. They also induced an increase in MDA contents as well as decreases in GSH levels and activities of SOD and CAT activity. A centrolobular decrease in hepatocytes along with degenerative changes of hepatocytes were also observed, and increases of NT and 4HNE immunoreactive hepatocytes were shown. These hepatocellular damages were more severe with the combination of MP + APAP. However, these MP + APAP-induced hepatic damages were significantly inhibited by acupuncture.
Conclusion: This study suggests that acupuncture may have a protective effect against the MP + APAP-induced hepatic damage through the amelioration of antioxidant defense systems.

1. Introduction

Morphine (MP), a representative analgesic [1,2], can cause a variety of adverse effects when not used properly [3]. Most importantly, MP has been demonstrated to produce a potential oxidative stress [3], inducing hepatic damage [4,5]. Opioid-induced hepatotoxicity may be related to the generation of reactive oxygen species (ROS), which further damage hepatic biomolecules. Additionally, MP has been shown to deplete reduced-glutathione (GSH) levels in rodents [5], resulting in the

* Corresponding author at: Department of Acupuncture, Moxibustion and Acupoint, College of Korean Medicine, Daegu Haany University, 165 Sang-Dong, Suseong-Gu, Daegu 706-828, Republic of Korea. Fax: +82 53 768 6340.
** Corresponding author at: Department of Anatomy and Histology, College of Korean Medicine, Daegu Haany University, and The Medical Research Center for Globalization of Herbal Formulation, Daegu Haany University, Gyeongsan 712 715, Republic of Korea.
E-mail addresses: gucci200@hanmail.net, dlqhdgy@dhu.ac.kr (B.H. Lee).

http://dx.doi.org/10.1016/j.eujim.2015.12.009
1876-3820/

cell death [6]. These impacts pose additional concerns to the problems of dependence and addiction [7,8].

Acetaminophen (APAP), a ubiquitous pain reliever and antipyretic, is also well known to result in liver injury through oxidative stress [9] when used excessively.

However, there may be cases when patients treated for chronic pain with MP wish to use APAP as an antipyretic, or wish to substitute MP with APAP to avoid dependence. In such cases, risk of liver injury could be elevated. Nevertheless, the severity of hepatic damage has been poorly reported. Therefore, we investigated the hepatotoxicity induced by a combination of MP and APAP.

Acupuncture, a well-known healing part of complementary and alternative medicine, has long been used in Asia for diverse functions, including detoxification. Indeed, acupuncture has been gaining interest as a useful therapy for drug addiction. Most of all, acupuncture has been demonstrated to suppress several problems induced by MP.

However, the effect of acupuncture on the hepatotoxicity induced by MP and APAP has not been reported.

Therefore, we investigated whether acupuncture plays a role in the MP + APAP-induced hepatic damage, and also, explored the possible underlying mechanisms.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Daehan Animal, Seoul, Korea) weighing 270–300 g at the beginning of the study were used. Animals were housed under conditions of temperature (22 ± 2 °C) and humidity (60 ± 5%) with a 12 h light-dark cycle (turn on at 7:00 am). They were allowed free access to food and water and were adapted to the experimental environment for at least 7 days. Experimental procedures were approved by the Institutional Animal Care and Use Committee at Daegu Haany University and followed the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) and Guide for the Care and Use of Laboratory Animals (NIH publication, 86-23).

2.2. MP and APAP treatment

MP hydrochloride (10 mg/kg, JEIL Pharmaceutical Co., Daegu, Korea) or saline was given subcutaneously for 9 consecutive days [10]. Thereafter, animals underwent a withdrawal period of 48 h after the last MP treatment. APAP (5 g/kg, Sigma–Aldrich, St. Louis, MO, USA) dissolved in 50% polyethylene glycol 400 (Yakury Pure Chemical Co., Kyoto, Japan) [9] or saline was given orally at the end of withdrawal, and animals were fasted for 24 h prior to APAP.

2.3. Collection of liver and serum

After 12 h from APAP, rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and the liver and blood were collected. Serum samples were prepared using a centrifuge at 3000 rpm for 15 min. Livers were divided into two parts of left lateral lobe and the remainder.

2.4. Experimental design

Animals were assigned into the following 8 groups: A (saline + salinetreated-normal group, $n = 3$); B (saline + APAP treated-control group, $n = 3$); C (MP + salinetreated-control group, $n = 3$); D (MP + APAP treated-control group, $n = 6$); E (MP + APAP with acupuncture at HT7, $n = 3$); F (MP + APAP with acupuncture at SI5, $n = 3$); G (MP + APAP with acupuncture at ST36, $n = 3$); H (MP + APAP with acupuncture at LI5, $n = 3$).

2.5. Acupuncture

Each group of E–H received acupuncture stimulation at each bilateral acupoint (Fig. 1). HT7 is located on the transverse crease of the wrist of the forepaw, radial to the tendon of the muscle flexor carpi ulnaris [11,12]. SI5 is located on the posteromedial aspect of the wrist in the depression between the triquetral bone and styloid process of the ulna [11,13,14]. ST36 is located on the anterior aspect of the leg on the line connecting ST35 with ST41 on the tibialis anterior muscle [11,13]. LI5 is located on the posterolateral aspect of the wrist, at the radial side of the dorsal wrist crease, distal to the radial styloid process, in the depression of the anatomical snuffbox [11,12]. The locations of acupoints were found according to the anatomical structures and were equivalent to those in human as described in the previous studies [12,14].

Acupuncture was performed once a day for 1 min across all experiments, immediately after MP and APAP, during withdrawal, and immediately before sacrifice, by one researcher (Lee, BH). A stainless-steel needle (diameter 0.18 mm, length 8 mm, Dongbang Acupuncture Inc., Chingdao, China) was inserted vertically into a depth of 2–3 mm, and stimulation was produced by bidirectional twisting of needles, as described in our previous studies. Rats received acupuncture in awakened state with a slight movement restriction. Daily handling was given for 2–3 min to minimize the stress from the movement restriction. The normal (A) and control groups (B–D) received the same treatment without needle stimulation.

2.6. Serum analysis

Serum AST and ALT levels were detected using a blood biochemical autoanalyzer (Hemagen Analyst, Hemagen Diagnostics, Columbia, MD, USA).

2.7. Measurement of liver lipid peroxidation

Separated liver tissues were weighed and homogenized in ice-cold 0.01 M Tris–HCl (pH 7.4) and then centrifuged at 12,000 × *g* for 15 min [15]. The concentrations of liver lipid peroxidation were determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test at an absorbance of 525 nm as nM of MDA/mg protein [16]. Total protein contents were measured using bovine serum albumin (Invitrogen, Carlsbad, CA, USA) as an internal standard [17].

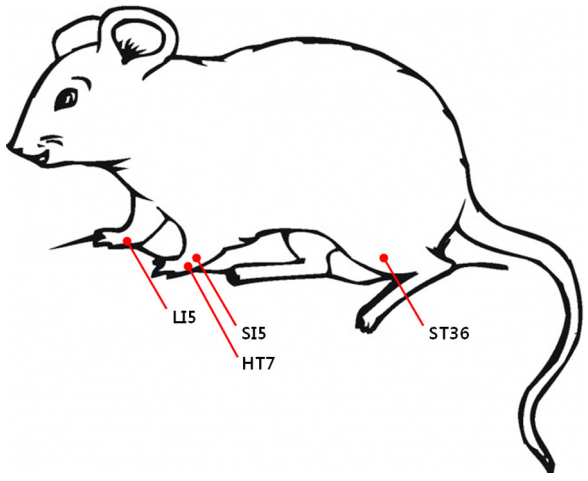

Fig. 1. Schematic diagram of acupuncture points.