Journal of Ethnopharmacology 143 (2012) 905–913

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Topical treatment with *Tong-Luo-San-Jie* gel alleviates bone cancer pain in rats

Juyong Wang [a,1], Ruixin Zhang [b,1], Changsheng Dong [a], Liying Jiao [a], Ling Xu [a,**], Jiyong Liu [c], Zhengtao Wang [c], Qi Liang Mao Ying [d], Harry Fong [e], Lixing Lao [b,*]

[a] Tumor Institute of Traditional Chinese Medicine, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, No. 725, South Wanping Rd, Shanghai 200032, China
[b] Center for Integrative Medicine, University of Maryland, 520 West Lombard Street, Suite 204, Baltimore, MD, 21201, USA
[c] The Ministry of Education Key Laboratory for Standardization of Chinese Medicines, Shanghai University of Traditional Chinese Medicine, Shanghai 201210, China
[d] Department of Integrative Medicine and Neurobiology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[e] Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612, USA

ARTICLE INFO

Article history:
Received 14 May 2012
Received in revised form
6 August 2012
Accepted 17 August 2012
Available online 30 August 2012

Keywords:
Chinese herbs
Bone cancer pain
Tong-Luo-San-Jie (TLSJ) gel
Type I collagen carboxy-terminal telopeptide
Bone-specific alkaline phosphatase
Osteoclast

ABSTRACT

Ethnopharmacological relevance: The herbal analgesic gel *Tong-Luo-San-Jie* (TLSJ) and its modifications are used in traditional Chinese medicine to manage cancer pain. However, its mechanisms are still unknown.

Aim of the study: To investigate the effects and mechanisms of TLSJ gel on bone cancer pain in a rat model.

Materials and methods: A bone cancer pain rat model was established by inoculating Walker 256 rat carcinoma cells directly into the right tibial medullary cavity of Sprague–Dawley rats (150–170 g); Phosphate buffered saline (PBS) tibial inoculation was used as control. Cancer-bearing rats were treated twice a day with external TLSJ gel (0.5 g/cm^2/day) or inert gel control for 21 day (n=10/group). Behavioral tests such as mechanical threshold and paw withdrawal latency (PWL) were carried out. Osteoclastic activities were determined and carboxyterminal pyridinoline cross-linked type I collagen telopeptides (ICTP) and bone-specific alkaline phosphatase (BAP) concentrations were detected with ELISA after treatment. Adverse effects were monitored, and biochemical and histological tests were performed in naïve rats treated with local TLSJ gel for six weeks.

Results: TLSJ treatment significantly restored bone cancer-induced decrease of PWL and mechanical threshold compared to inert gel. It also decreased the level of blood serum ICTP and BAP and inhibited osteoclast activities. No adverse effects or abnormal biochemical and histological changes were detected after TLSJ treatment.

Conclusion: The present study shows that TLSJ significantly inhibits bone cancer-induced thermal and mechanical sensitization. It suggests that the gel may be useful in managing cancer pain and that it may act by inhibiting osteoclastic activity.

1. Introduction

Cancer pain, the most common symptom of cancer, can result both from the direct effects of malignancy and from the treatment. In 56–79% of cases, the pain is tumor related; in 5–62% of cases it is related to the treatment (Banning et al., 1991; Gutgsell et al., 2003). A recent systematic review reports that pain was documented in 64% of patients with metastatic, advanced, or terminal disease, in 59% of those on anticancer treatment, and in 33% of individuals who had been cured of cancer (van den Beuken-van Everdingen et al., 2007).

Opioids are the main pharmacological therapy for managing cancer pain (Pargeon and Hailey, 1999; Radha Krishna et al., 2010; Virik and Glare, 2000). However, their adverse effects, including nausea, constipation, urinary retention, dizziness, and

Abbreviations: ALT, Alanine aminotransferae; ALB, Albumin; AKP, Alkaline phosphatase; AST, Aspartate aminotransferae; BUN, Blood urea nitrogen; BAP, Bone-specific alkaline phosphatase; CREA, Creatinine; CK, Creatine kinase; CK-MB, Creatine kinase-MB (M, muscle subunit, B, Brain type subunit); CysC, Cystatin C; ELISA, Enzyme-linked immunosorbent assay; EDTA, Ethylenediaminetetraacetic acid; FDA, Food and Drug Administration; HE, Hematoxylin and eosin; HBDH, Hydroxybutyrate dehydrogenase; ICTP, Type I collagen telopeptides; LDH, Lactate dehydrogenase; PWL, Paw withdrawal latency; PBS, Phosphate buffered saline; SD, Standard deviation; TBIL, Total bilirubin; TCM, Traditional Chinese medicine; TLSJ, Tong-Luo-San-Jie; TP, Total protein; TRAP, Tartarate-resistant acid phosphatase; TRPV1, Transient receptor potential cation channel subfamily V member 1; UA, Uric acid; UPLC, Ultra performance liquid chromatography

* Corresponding author. Tel.: +1 410 706 6187; fax: +1 410 706 6210.
** Corresponding author. Tel.: +86 21 6438 5700 1303; fax: +86 21 6439 8310.
E-mail addresses: xulq67@gmail.com (L. Xu), llao@compmed.umm.edu (L. Lao).
[1] They made equal contributions to this work.

0378-8741/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jep.2012.08.026

dysphoria, markedly limit their use (Bowdle, 1998; Cherny et al., 2001; Inturrisi, 1989; Pasternak, 1988). Non-steroidal anti-inflammatory drugs are used to manage painful metastases but also have severe side effects such as gastrointestinal disturbances (Schaffer et al., 2006; Scheiman, 2001) and cardiovascular risks (Davies and Jamali, 2004).

In recent years, Chinese herbal medicine has played an important role in the treatment of cancer pain. It is reported that 41–62% of cancer patients use herbs as a complementary or alternative medical therapy (Gozum et al., 2003; Richardson et al., 2000). Because oral ingestion of herbal medicine may cause nausea, vomiting, or diarrhea, external treatment might be an acceptable alternative route for treating localized, cancer-related pain such as bone pain. Clinical observations showed that Chinese herbal medicine may be useful for managing cancer pain in patients (Chen et al., 2006; Xu et al., 2007). In a previous study, we demonstrated that an herbal analgesic gel, Xiaotan Tongluo (XTTL), prepared from four herbs, Tiannanxing (*Arisaema heterophyllum* Blume, rhizome), Banxia (*Pinellia ternate* (Thunb) Makino, tuber) Shancigu (*Cremastra appendiculate* (D. Don) Makino, peudobulb) and Weilingxian (*Clematis chinensis* Osbeck, root), significantly alleviated the mechanical pain in a bone cancer pain animal model (Yu et al., 2009). However, the mechanisms of action of topically used herbs are largely unknown.

Osteoclastic activity is involved in bone metastasis-induced cancer pain, and osteolytic cancers produce significant proliferation of osteoclasts (Adami, 1997; Clohisy et al., 2000). Recent experiments in a bone cancer pain model show that osteoclasts have an essential role in cancer-induced bone loss and contribute to the etiology of bone cancer pain (Honore et al., 2000; Luger et al., 2001). Carboxyterminal pyridinoline cross-linked type I collagen telopeptides (ICTP) and bone alkaline phosphatase (BAP) are respectively the best characterized markers of osteoclastic (bone resorption) and osteoblastic (bone formation) activity (Smith, 2006). ICTP and BAP are significantly elevated in cancer patients (Koizumi et al., 1995; Tamada et al., 2001), which, when used in combination, may be reliable serum markers for detecting bone metastases. In our previous study, measurements on day 21 post-cancer cell inoculation demonstrated that herbal analgesic gel decreased serum ICTP and BAP (Yu et al., 2009).

We recently developed another herbal analgesic gel, *Tong-Luo-San-Jie* (TLSJ), based on a classical formula for pain management, *San Qi He Bi Zhen*. Our TLSJ formula is prepared from seven herbs, different from those used in the XTTL gel. In traditional Chinese medicine (TCM), TLSJ is used to activate Qi and Blood circulation and to warm the channels to alleviate pain (Li et al., 2003; Sheu et al., 2009; Su et al., 2012; Yuan et al., 2004). According to TCM theory, qi is defined as the vital energy necessary for growth, development, and the maintenance of healthy physiological functions in the human body. In addition, qi promotes the formation of blood and drives its circulation (Lao, 1999).

The aim of this study was to evaluate the effects and safety of TLSJ gel and to explore the mechanisms of its action on cancer pain in a bone cancer pain rat model by assessing both mechanical and thermal sensitivity, the time course of serum ICTP and BAP, and the activity of osteoclasts and osteoblasts in the tibia. We hypothesized that our TLSJ gel alleviates bone cancer-induced pain by inhibiting osteoclastic activity.

2. Materials and methods

2.1. Herbal preparation and extraction

TLSJ gel is composed of extracts prepared from seven herbs: *Haifengteng* (*Piper kadsura* (Choisy) Ohwi, stem) 30 g, *Luoshiteng* (*Trachelospermum jasminoides* (Lindl.) Lem., stem) 30 g, *Chuanshanlong* (*Dioscorea nipponica Makino*, *rhizome*) 50 g, *Yanhusuo* (*Corydalis yanhuso W.T. Wang*, *rhizome*) 30 g, *Chuanlianzi* (*Melia toosendan* Sieb. et Zucc, fruit) 10 g, *Ruxiang* (*Boswellia carterii* Birdw., defatted gum resin) 10 g, and *Moyao* (*Commiphora myrrha* Engl, resin) 10 g. The herbs, processed with traditional methods after harvest (Bensky and Gamble, 1993), were purchased from the Huayu Pharmaceutical Company (Shanghai, China), which has retained a complete set of samples of the authenticated herbs. Each extract was monitored for contaminants (heavy metals, pesticides, and mycotoxins) prior to formulation. The herbs were pulverized, admixed in the proper ratio, and extracted twice with 70% ethanol. The extract was subjected to decompression ethanol recovery (50 °C, 0.08 MPa). Carbopol® 940 was used as the binding agent in the preparation of the gel, the formulation of which consists of Carbopol® 940, 0.8%; distilled water, 84.2%; herbal extract, 7.0%; propylene glycol, 4.0%; glycerol, 2.0%; and azone 2.0%. The pH of the gel was 6.5. Each g of gel contains 5 g equivalent of raw herbs (Liu et al., 2009; Sareen et al., 2011).

The chemical profile of the formulated gel was established by ultra performance liquid chromatography (UPLC) analysis (He et al., 2011; Wang et al., 2011) employing gradient elution with acetonitrile and 0.1% formic acid as mobile phases A and B, respectively. The ESI source was operated in the positive ionization mode. Data were collected between minutes 1 and 50; the mass-scan range was 100–800. Characteristic UPLC peaks (profile) for quality control/quality assurance are presented in Fig. 1.

2.2. Animals

Female Sprague-Dawley rats weighing 150–170 g were obtained from the Shanghai Slac Laboratory Animal Co., Ltd. The animals were kept under controlled environmental conditions (24 °C ± 0.5 °C relative humidity 50–60%, 7am to 7pm alternate light-dark cycles, food and water ad libitum). The cage floors were covered with wood shavings to minimize the possibility of painful contact with a hard surface. The animal protocol was approved by the institutional animal care and use committees of the Shanghai University of Traditional Chinese Medicine (China) and the University of Maryland School of Medicine (USA). The research was conducted in accordance to the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2010).

2.3. Experimental design

Four sets of experiments were carried out for: adverse effects, pain assessment, serum levels of ICTP and BAP, and osteoclastic and osteoblastic activity.

In experiment 1, 30 normal rats were used to assess TLSJ toxicity/adverse effects for six weeks. Behavioral observation, blood biochemistry tests, and histopathological examinations were performed.

In experiment 2, rats weighing 150–170 g were randomly divided into three groups ($n=10$ per group): (1) sham: vehicle (PBS) inoculation plus topical administration of inert gel; (2) vehicle: Walker 256 cell inoculation plus topical administration of inert gel; (3) treatment: Walker 256 cell inoculation plus topical administration of TLSJ gel at a dosage of 0.5 g/cm^2/day, which effectively alleviated thermally evoked pain in our pilot study. To investigate the effects of the gel on cancer-induced pain, TLSJ gel (treatment group) or control inert gel (vehicle and sham groups) were evenly applied on the skin of tumor-bearing tibias twice a day at 8:00 AM and 20:00 PM for a total of 21 day beginning one day after Walker 256 cell implantation.

In experiment 3 (determination of serum levels of ICTP and BAP), rats were divided into sham cancer, cancer + vehicle, and cancer + TLSJ