Journal of Ethnopharmacology 142 (2012) 168–174

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

Sanguis Draconis resin stimulates osteoblast alkaline phosphatase activity and mineralization in MC3T3-E1 cells

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ARTICLE INFO

Article history:
Received 8 December 2011
Received in revised form
19 February 2012
Accepted 10 April 2012
Available online 21 April 2012

Keywords:
Sanguis Draconis
Osteoporosis
Bone
MC3T3-E1

ABSTRACT

Ethnopharmacological relevance: *Sanguis Draconis* (SD), "Dragon's Blood", is a resin that is obtained from *Daemonorops draco* (Palmae). Used in traditional medicine, it has shown activity in the prevention of osteoporosis as well as promoting the healing of bone fractures.

Materials and methods: In this study, the effects of Sanguis Dranonis ethanol extract on β-glycerolphosphate and ascorbic acid induced differentiation using mouse calvaria origin MC3T3-E1 osteoblastic cells was examined. We looked at osteoblast differentiation, proliferation, and mineralization by measuring alkaline phosphatase (ALP) and specific bone marker activities. Osteoblast-like MC3T3-E1 cells were cultured in various concentrations of SD ethanol extract (0.005-1 mg/mL) during the osteoblast differentiation period (1, 5, 15, and 25 days).

Results: As measured by 3-[4,5-dimethylthiazol-2-y]-2,5-diphenyltetrazolium bromide assay, SD extracts increased cell proliferation as compared to control. The most pronounced effect was observed at the concentration range between 0.01 and 0.1 mg/mL ($P < 0.01$). This SD stimulatory effect for cell proliferation was observed during the whole osteogenic period. Cellular (synthesized) ALP activity was increased during 15 days of culture, and was confirmed by the staining of ALP activity on cell matrix layers for matrix calcification. SD stimulatory effect for cell mineralization we observed in 14 and 21 days. Elevated mRNA or protein levels of bone morphogenetic protein-2(BMP 2), the differentiation marker osteocalcin, osteopontin, collgen I, and a master osteogenic transcription factor, Runx2, were observed in SD-treated cells.

Conclusions: These results suggest that SD may increase osteogenic effect by stimulating cell ALP activity and affect the BMP signaling pathway cascades in osteoblastic cells, then promotes osteoblast differentiation, mineralization, and bone formation.

1. Introduction

Osteoporosis is an emerging medical and socioeconomic threat characterized by a systemic impairment of bone mass, strength, and microarchitecture, which increases the propensity of fragility fractures. It can occur at any age and in any racial or ethnic group, however there is an increased incidence in post-menopausal women (Leboime et al., 2010). As the most common metabolic bone disease, the cause is an imbalance between the formation and resorption of the bone. This process depends on the interactions between osteoblasts, which are unique bone-forming cells derived from mesenchymal stem cells, and osteoclasts (Rachner et al., 2011; Wang et al., 2008). The formation of bone involves a complex series of events, which include the proliferation and differentiation of osteoprogenitor cells resulting eventually in the formation of a mineralized extracellular matrix. At the cellular level, communication and coupling between the main bone-cell types, the bone-forming osteoblasts and the bone-degrading osteoclasts, constitute the smallest functional unit. Since new bone formation is primarily a function of the osteoblasts, agents that regulate bone formation act by either increasing the proliferation of cells of the osteoblastic lineage, or inducing differentiation of the osteoblast progenitors. Understanding this, it is possible that early intervention, with the help of specific and targeted medications, may reduce the risk of first as well as recurrent fractures.

For centuries plants have been used all over the world for the treatment of illness and disease, and novel drugs continue to be developed through botanical research. Sanguis Draconis (SD),

Abbreviations: SD, Sanguis Draconis; SDEE, Sanguis Draconis ethanol extracts; ALP, Alkaline phosphatase; BMP, Bone morphogenetic protein; OCN, Osteocalcin; OPN, Osteopontin; Col1, Collgen type 1

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0378-8741/$ - see front matter © 2012 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.jep.2012.04.033

which is the pharmaceutical name for "Dragon's Blood", is one such example that has been touted to have numerous medical applications. This red resin obtained from Daemonorops draco (Palmae) as well as over a dozen other species of distinct plant genera, is used in traditional medicines offering several therapeutic benefits: hemostatic, antidiarrhetic, antiulcer, antimicrobial, antiviral, wound healing, antitumor, anti-inflammatory, antioxidant, etc. (Ahn et al., 2004; Gupta et al., 2008; Ichikawa et al., 1997). In China, Japan, and Korea, women who have had lower back pain during climacteric and senescent periods, have been traditionally treated with "Dragon's Blood" medicines. Additionally, in China, the red resin of Dracaena cochinchinensis, is used for treatment of fractures and osteoporosis (Chen and Liu, 2006; Yi et al., 2011). The chemical elements of SD, which include dracorubin, dracorhodin, dracoresinotannol, and abietic acid (Zhu et al., 2007), have been investigated in detail (Edwards et al., 2004). However, no data has yet been provided regarding the recovery of bone mass through the treatment with SD. In this present study, the in vitro effect of SD on the function and mineralization of osteoblastic MC3T3-E1 cells was investigated in order to determine the possible bioactivities of SD on bone metabolism.

2. Materials and methods

2.1. SD preparation

SD was prepared as reported (Choy et al., 2008). Briefly, commercially available plant material was purchased from a traditional Chinese medicine drugstore. The botanical source was Daemonorops draco (Palmae) from Malaysia. SD (10 g) was soaked in 200 mL of absolute alcohol at room temperature for 48 h under shade and then was filtered. Ethanolic extract was passed through a 0.22 m sterile filter (Millipore; Billerica, MA) and first concentrated using a Yamato vacuum rotary evaporator (Japan) at 40 °C and then freeze-dried at −80 °C in a vacuum freeze-dryer (MicroModulyo, Savant Instruments, USA). Normally, 1.57 g of dried powder can be obtained from 10 g of SD. Its main constituents were reported by Edwards et al. (2004), as shown in Fig. 1(A).

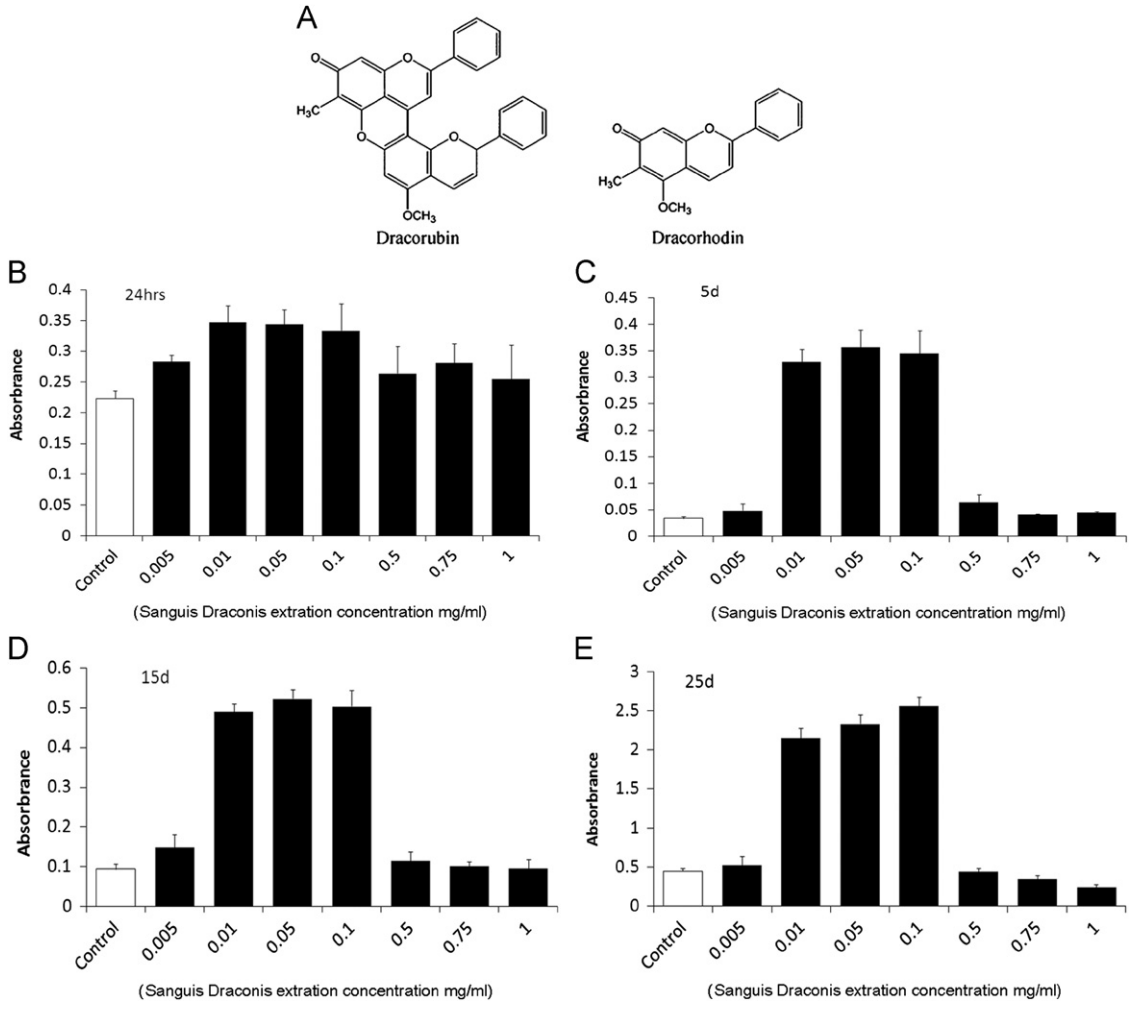

Fig. 1. Effect of Sanguis Draconis ethanol extract (SDEE) on the proliferation of osteoblastic MC3T3-E1 cells over the short-term (first 24 h) (B) and the whole of osteoblast differentiation period (5–25 days) (C),(D),(E). (A) Chemical structures of dracorubin and dracorhodin, the main chemical components of Sanguis Draconis (dragon blood resins) from *Daemonorops draco*. Note: The two structural pictures were acquired from Edwards et al. (2004). Panel. MC3T3-E1 cells were seeded at a density of 1×10^4cells/well and were equilibrated for 1–2 days. At confluence, the cells were treated with various concentrations of SDEE (0–1.0 mg/mL). DMSO was used as the vehicle control (0 mg/mL SDEE). Cell proliferation was determined using the MTT assay. There was a significant increase in cell proliferation between SDEE and Control in both short-term (B) and the whole of osteoblast differentiation period (C),(D),(E) with SDEE treatments at lower concentrations (0.05–0.1 mg/mL). However, this significance was lost at higher concentration (0.5–1 mg/mL). Osteoblastic MC3T3-E1 cell proliferation was increased by SDEE at lower concentration (0.05–0.1 mg/mL). Data represent mean ± SE and statistical comparisons were made using 1-way ANOVA and *t* tests. NS, nonsignificant. a $P < 0.05$ and b $P < 0.01$ compared with vehicle control.