Toxicology and Applied Pharmacology 333 (2017) 1–9

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

High doses of baicalin induces kidney injury and fibrosis through regulating TGF-β/Smad signaling pathway

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ARTICLE INFO

Keywords:
Baicalin
Toxicity
TGF-β/Smad signaling pathway
Kidney injury
Fibrosis

ABSTRACT

Baicalin is a major flavonoid compound purified from Scutellariae radix, which has been described as an herb in the Chinese Pharmacopoeia. Previous studies have suggested baicalin possessed extensive anti-inflammatory, anti-cancer, anti-viral properties. However, up to known, there have been no reports of safety and toxicity in the rats following oral administration of baicalin. In this present study, we showed the first evidence that treatment of baicalin (400, 800 and 1600 mg/kg/day) induced significantly kidney injury and fibrosis. The collagen synthesis and fibrosis-related protein expression were increased in the kidney of Sprague-Dawley (SD) rats after treatment with high doses of baicalin. We further investigated the potential molecular mechanism of baicalin-mediated renal fibrosis and revealed that baicalin activated the transforming growth factor-β (TGF-β)/Smad signaling pathway in a dose-dependent manner. Moreover, we also observed that baicalin induced Smad3 interaction with transcriptional coactivator p300 accompanying with increment of Smad3 acetylation. Our results may contribute to better understanding of the future pharmacological and toxicological studies of Scutellaria baicalensis Georgi and its active compounds on the human disease.

1. Introduction

"Skullcap", the root of *Scutellaria baicalensis Georgi*, is a well-known and widely used traditional herb medicine in China (Huang et al., 2006; Wei et al., 2017). It was confirmed that the active components of which are flavonoids, the four major active flavonoid components are baicalin, baicalein, wogonoside, and wogonin (Gong et al., 2014). Over the past few years, accumulating evidence indicated that skullcap extract and its active compounds have diverse pharmacological effects such as anti-inflammatory, anti-cancer, Anti-thrombotic, and anti-viral activities (Ku and Bae, 2014; Li et al., 2014; Shi et al., 2016; Huang et al., 2017).

Despite favorable data in previous studies, few researchers have found the potential toxicity of skullcap and its active compounds. Recently, cases of hepatotoxicity have been associated with consumption of Move Free Advanced (an over the counter arthritis remedy comprised of glucosamine, chondroitin, skullcap and black catechu) (Linnebur et al., 2010; Yang et al., 2012; Dhanasekaran et al., 2013). In nearly all cases, researcher showed that herbal hepatotoxicity from Skullcap was most likely the causative agent of liver injury based upon liver histology and re-challenge. Moreover, Laboratory studies of skullcap's active compounds in rodents and dogs have also revealed toxic effects. Qi et al. (2009) investigated the acute and subchronic toxicity of wogonin using albino mice and Sprague-Dawley (SD) rats, respectively. They revealed that a long period of treatment with high doses of wogonin (120 mg/kg i.v) could induce reversible heart injury in rats. Additionally, this research group further studied the subchronic toxicity of wogonin using Beagle dogs which were treated with wogonin via intravenous infusion for 90 days (Peng et al., 2009). Typical toxicological responses such as emesia, hyperptyalism, somatasthenia, swollen snout accompanied with the scratching and the discontinuity urine dripping were observed among animals in treatment groups, especially in group I (60 mg/kg i.v). These data suggested that wogonin could induce toxicity in rats when treated with high dose. However, to the best of our knowledge, no safety studies about other active

Abbreviation: SD, Sprague-Dawley; TGF-β, transforming growth factor-β; FN, fibronectin; CTGF, connective tissue growth factor; α-SMA, alpha-smooth muscle actin; CK, Creatine Kinase; LDH, Lactate Dehydrogenase; ALT, Alanine aminotransferase; AST, Aspartate transaminase; BUN, Blood urea nitrogen; CR, Creatinine; CKD, chronic kidney diseases

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http://dx.doi.org/10.1016/j.taap.2017.08.003
Received 20 May 2017; Received in revised form 18 July 2017; Accepted 9 August 2017
Available online 10 August 2017
0041-008X/

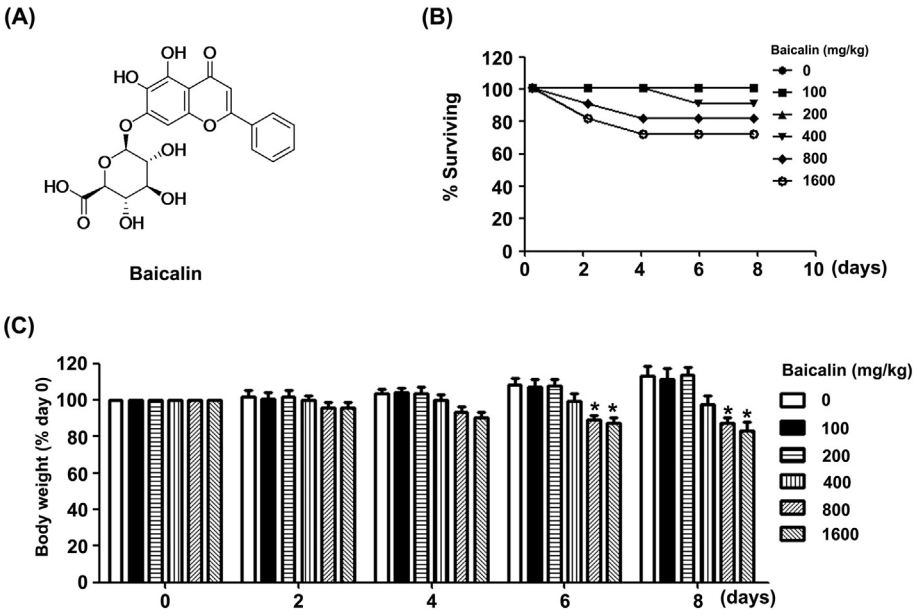

Fig. 1. (A) The chemical structure of baicalin. Effect of oral high dose of baicalin on the survival (B) and body weight (C) of SD rats. *$p < 0.05$ vs. the group treated with CMC-Na only, $n = 10$.

compounds of Skullcap have been conducted to date.

Baicalin (Fig. 1A) is one of the active flavonoid originated from the *Scutellaria baicalensis Georgi* radix. It is also the major active component found in 'DuZhong' (Eucommiaulmoides) that is widely used for promoting bone fracture healing in Chinese traditional medicine (Lu et al., 2017). Baicalin has attracted considerable attention because it has a variety of interesting properties such as anti-bacterial, anti-viral, anti-tumour and anti-inflammatory effects (Fouad et al., 2017; Wang et al., 2017). However, the side effects and toxicity of baicalin has not been well defined. In this present study, we give the first evidence that high doses of baicalin induced kidney injury and fibrosis in SD rats. Collagen content and fibrosis-related gene expression were upregulated in the kidney of rats after treatment with baicalin at high dose. Moreover, we investigated the potential mechanisms involved and revealed that baicalin induced renal fibrosis which may be attributed to activation of transforming growth factor-β (TGF-β)/Smad signaling pathway.

2. Materials and methods

2.1. Reagents

Baicalin (purity ≥ 95%) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Dosing solutions of baicalin were prepared in 0.05% CMC-Na in water. Antibodies against fibronectin (FN), connective tissue growth factor (CTGF) and p300 were purchased from Abclonal (Guangzhou, China), TGF-β, Smad2, CollagenI, CollagenIV and alpha-smooth muscle actin (α-SMA) were purchased from Proteintech (Wuhan, China), p-Smad3, Smad3, Smad4, Acetylated lysine (Ac-Lys), p-AMPK, AMPK and β-actin were purchased from Company CST (cell signaling technology).

2.2. Animals

SD rats (weighing 180–200 g, SPF grade) were achieved from the Guangdong Medical Laboratory Animal Center. They were housed in plastic cages with wood shavings as bedding and maintained on a 12 h light/12 h dark cycle at 24 ± 1 °C with 55% ± 10% humidity. All the animals had ad libitum access to tap water and standard diet pellets. All procedures were performed in accordance with the current Chinese legislation on the care and use of laboratory animals and approved by Department of Scientific Management of the institute.

2.3. Treatment and sample collection

Rats were randomly divided into six groups. These comprised the control group and five experimental groups, each of which were intragastrically administered with different doses of baicalin (0, 100, 200, 400, 800, 1600 mg/kg/day). Animals were observed for signs of abnormalities during whole treatment. General health, body weight and morbidity/mortality were monitored especially after administering. At the end of the observation period, animals were fasted overnight, but with ad libitum access to water. Then, all surviving animals were euthanized at the end of the 8-day treatment period. Plasma was isolated by centrifugation at 3000 rpm for 10 min and stored at − 80 °C for later analysis. Heart, liver and kidney were collected and quickly washed with PBS. One lobe of tissue (heart, liver and kidney) was trimmed and fixed in 10% buffered formalin for histopathological analysis. The remaining tissues were frozed at − 80 °C for next experiment.

2.4. Biochemical analysis

The content of Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Alanine aminotransferase (ALT), Aspartate transaminase (AST), Blood urea nitrogen (BUN) and Creatinine (CR) were assayed by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.5. Histology analysis

For morphometric measures, the vital organs (hearts, liver and kidney) were carefully excised to detect gross lesions, and preserved in 10% neutral formalin for histopathological assessment. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin-eosin (HE) and Masson's trichrome. The remaining tissue was stored in the refrigerator at − 80 °C.

2.6. Western blotting and co-immunoprecipitation (co-IP)

Western blotting and co-IP analyses were performed as previously described (Cai et al., 2012; Yu et al., 2013). Briefly, protein was separated by SDS–PAGE gel electrophoresis, and then transferred to PVDF membranes (Millipore). After blocking with 5% nonfat milk, the membranes were incubated with primary antibodies, followed by incubation with appropriate horseradish peroxidase (HRP)-labeled