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In vitro metabolism, permeation, and brain availability of six major boswellic acids from *Boswellia serrata* gum resins

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ABSTRACT

Boswellia serrata gum resin extracts (BSE) revealed potent anti-inflammatory actions in preclinical and clinical studies. In 2002 BSE was assigned an orphan drug status by the European Medicines Agency (EMA) for the treatment of peritumoral edema. In the past pharmacological effects of BSE were mainly attributed to 11-keto-β-boswellic acid (KBA) and 3-acetyl-11-keto-β-boswellic acid (AKBA). Therefore pharmacokinetic and pharmacodynamic studies focused mainly on these two boswellic acids (BAs). However, other BAs, like β-boswellic acid (βBA), might also contribute to the anti-inflammatory actions of BSE. Here, we determined the metabolic stability, permeability and brain availability of six major BAs, that is, KBA, AKBA, βBA, 3-acetyl-β-boswellic acid (AβBA), α-boswellic acid (αBA), and 3-acetyl-α-boswellic acid (AαBA). For permeability studies, the Caco-2 model was adapted to physiological conditions by the addition of bovine serum albumin (BSA) to the basolateral side and the use of modified fasted state simulated intestinal fluid (FaSSIF) on the apical side. Under these conditions the four BAs lacking the 11-keto moiety revealed moderate permeability. Furthermore the permeability of AKBA and KBA was improved compared to earlier studies. In contrast to Aα- and AβBA, βBA and αBA were intensively metabolized after incubation with human and rat liver microsomes. Finally, the availability of all six major BAs could be confirmed in rat brain 8 h after oral administration of 240 mg/kg BSE to rats showing mean concentrations of 11.6 ng/g for KBA, 37.5 ng/g for AKBA, 485.1 ng/g for αBA, 1066.6 ng/g for βBA, 43.0 ng/g for AαBA and 163.7 ng/g for AβBA.

1. Introduction

Boswellia serrata resin dry extracts (BSE) are traditionally used for the treatment of various inflammatory diseases in ayurvedic and folk medicine [1]. The pharmacological effects of BSE are mainly attributed to boswellic acids (BAs), which were proposed to act as inhibitors of 5-lipoxygenase (5-LO) [2], nuclear factor kappa-B (NFκB)-pathway [3], human leukocyte elastase [4], cathepsin G (catG) [5], and microsomal prostaglandin E_2 synthase (mPGES)-1 [6]. Several pilot clinical trials investigating the efficacy of BSE in the treatment of inflammatory disorders like Crohn's disease [7], ulcerative colitis [1,8], inflammatory bowel disease, rheumatoid arthritis,

Abbreviations: AαBA, 3-acetyl-α-boswellic acid; AβBA, 3-acetyl-β-boswellic acid; AKBA, 3-acetyl-11-keto-β-boswellic acid; BA, boswellic acid; αBA, α-boswellic acid; βBA, β-boswellic acid; BSE, *Boswellia serrata* gum resin extract; HLM, human liver microsomes; KBA, 11-keto-β-boswellic acid; LC, liquid chromatography; MS, mass spectrometry; P_{app}, apparent permeability coefficient; Pgp, P-glycoprotein; RLM, rat liver microsomes; TEER, transepithelial electrical resistance.

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osteoarthritis [9], and asthma [10] as well as phase I toxicity studies [11] suggest promising beneficial therapeutic effects with no serious, long-term or irreversible adverse effects. Moreover, BSE was assigned the orphan drug status for the reduction of peritumoral edema by the European Medicines Agency (EMA) in 2002 [12,13].

Until recently, the pharmacological effects were mainly attributed to the suppression of 5-LO by 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-ß-boswellic acid (AKBA) [2]. For that reason pharmacokinetic studies carried out so far focused primarily on these two 11-keto-BAs [14–16]. However, KBA and AKBA levels in plasma and brain were very low following oral administration of BSE and in most cases, AKBA could not be even detected [5,16–20]. Recent studies have shown that other more abundant BAs, like β-boswellic acid (βBA), acetyl-β-boswellic acid (AβBA), α-boswellic acid (αBA), and acetyl-α-boswellic acid (AαBA) could also play an important role [21,22] (Fig. 1). For these other four BAs, several fold higher C_{max} values were observed in plasma after oral administration of BSE to humans, paralleling partly pharmacological relevant concentrations for catG and m-PGES-1 inhibition [5,6,23]. In order to better estimate the factors affecting the bioavailability of these BAs, the present study determined the metabolic stability of βBA, αBA, AαBA, and AβBA in human and rat liver microsomes (HLM, RLM) and their permeability in the Caco-2 model.

Previous experiments on the intestinal absorption of KBA and AKBA in the Caco-2 model revealed moderate and poor permeability for KBA and AKBA, respectively [15]. However, a strong retention of both BAs in Caco-2 cells was reported, which is common for lipophilic drugs [24]. For better prediction of the absorption *in vivo*, the Caco-2 experiments in this study were more adapted to physiological conditions of the gastrointestinal tract by the addition of 4% bovine serum albumin (BSA) to the receiver side [24], and the use of physiologically relevant media like modified FaSSIF (fasted state simulating intestinal fluid) in the donor side [25].

So far, only KBA and AKBA have been determined in the brain [16]. In light of the increasing relevance of the other BAs, it lends itself to measure the brain levels of βBA, αBA, AαBA, and AβBA too. This helps to better evaluate the role of these BAs in the cerebral anti-inflammatory activity of *B. serrata*. In addition, the insights gained in this study may be also of importance in several other diseases, which have been associated with inflammation only recently, e.g. psychic disorders [26–29].

2. Material and methods

2.1. Standards and reagents

Boswellic acids (KBA, AKBA, αBA, βBA, AαBA and AβBA) with a purity of >99.0% were kindly provided by Dr. Johann Jauch, Univ. Saarland, Germany. Fluoxymesterone used as internal standard (I.S.) was supplied by Sigma Aldrich (purity >98.0%). The BSE extract available in H15® capsules (Hecht Pharma, Stinstedt, Germany) was used for the transport experiments, and a BSE dry extract, kindly donated by Indena (Milano, Italy) was applied in the rat study. The reagents used were tetrahydrofurane, n-hexane, dimethylsulfoxide (p.a. or gradient grade, Roth, Karlsruhe, Germany), 2-propanol, meth-

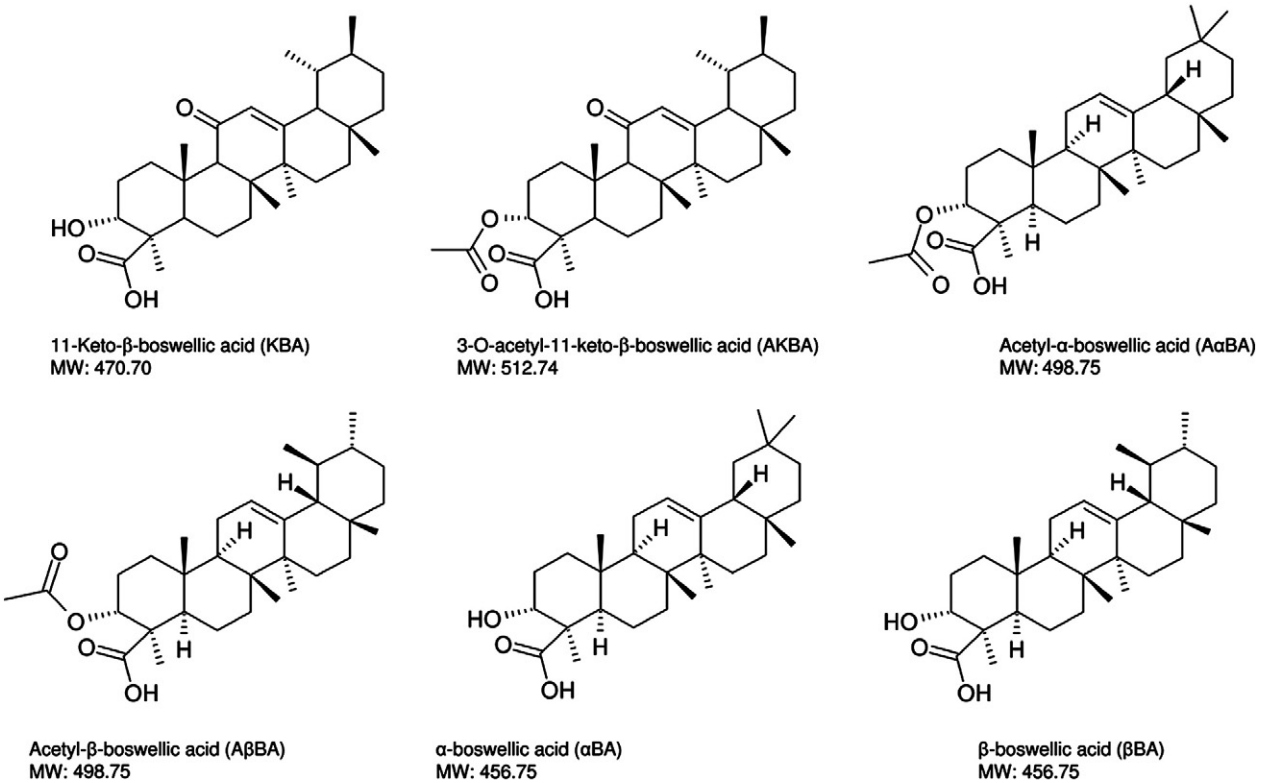

Fig. 1. Structures of major BAs.