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Original article

Enhanced absorption of boswellic acids by a micellar solubilized delivery form of Boswellia extract

Jürgen Meins[a], Dariush Behnam[b], Mona Abdel-Tawab[a,*]

[a] Zentrallaboratorium Deutscher Apotheker, Carl-Mannich-Str. 20, Eschborn 65760, Germany
[b] AQUANOVA AG, Birkenweg 8-10, Darmstadt 64295, Germany

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ABSTRACT

Background: Boswellic acids (BAs) the pharmacologically active ingredients of the gum resin extract of *Boswellia serrata* are known for their anti-inflammatory effects. However they suffer from poor bioavailability because of their hydrophobicity and poor water solubility.

Purpose: The present study aimed at investigating the effect of AQUANOVA micellation technology on the bioavailability of Boswellia extract in rats compared to its native form.

Study design.

Female albino wistar rats (n = 6) weighing around 250 g were orally administered solubilized (Sol-BE) and native (Nat-BE) Boswellia extract at an equimolar dosage of 128 mg/kg. Plasma samples collected at defined time points (0, 0.5, 1, 2, 3, 4, 6 and 8 h) were analyzed for the content of the six major boswellic acids (KBA, AKBA, αBA, βBA, AαBA and AβBA - 11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), α-boswellic acid (αBA), β-boswellic acid (βBA), acetyl-α-boswellic acid (AαBA) and acetyl-β-boswellic acid (AβBA)) using a sensitive LC-MS/MS method.

Results: **The oral administration of Sol-BE led to a remarkable increase in the AUC and** C_{max} of all BAs in plasma compared to Nat-BE. Whereas no KBA could be detected after the administration of Nat-BE, KBA could be detected at a maximal plasma concentration of 439.21 ng/mL and an AUC_{last} of 1185.37 ng/mL*h following the administration of Sol-BE. The highest increase was observed in the case of AKBA where a 56-fold increase in the AUC_{last} and a 25-fold increase in the C_{max} was determined compared to Nat-BE.

Conclusions: Micellar solubilisation represents a promising approach for enhancing the bioavailability of poorly soluble substances.

1. Introduction

The gum resin of *Boswellia serrata* also known as Indian frankincense or Salai guggal has been used for centuries in the Ayurvedic medicine for its anti-inflammatory properties and is now counted among the well-established plant food supplements in Europe and the USA. Hence in 2015 Boswellia achieved a 674% increase in sales over 2014 in the USA alone [1]. In fact, it was shown that a number of pivotal enzymes in inflammation like 5-lipoxygenase (5-LO), cathepsin G (catG), and microsomal prostaglandin-E synthase (mPGES)-1 as well as nuclear transcription factor κB (NF-κB) and several pro-inflammatory cytokines like tumor necrosis factor (TNFα), interleukin (IL)-1β, IL-2, and IL-6 are inhibited by boswellic acids (BAs), the main active ingredients of *Boswellia serrata*. The structure of the six most important BAs (11-keto-β-boswellic acid, acetyl-11-keto-β-boswellic acid, α-boswellic acid, β-boswellic acid, acetyl-α-boswellic acid and acetyl-β-boswellic acid) is presented in Fig. 1. In addition several preliminary pilot clinical trials support the potential of Boswellia to treat a variety of chronic inflammatory diseases like rheumatoid arthritis, osteoarthritis, chronic colitis, ulcerative colitis, collagenous colitis, Crohn's disease and bronchial asthma [2,3]. However, due to their hydrophobicity and low water solubility only negligible amounts of BAs are absorbed after oral ingestion [2]. This could be verified in the Caco-2 *in vitro* model revealing only moderate to poor permeability for BAs [4,5]. The resulting poor bioavailability represents thus the major limitation to the efficacy of these promising herbal substances. Therefore strategies to improve the bioavailability of BAs are urgently needed, in order to be able to benefit more from the therapeutic potential of Boswellia.

A promising approach for the efficient delivery of poorly soluble substances is the preparation of micellar formulations based on Tween 20. In fact, the application of this micellation technology on other herbal substances resulted for example in a tremendous increase in the

* Corresponding author at: Central Laboratory of German Pharmacists, Carl-Mannich-Str. 20, Eschborn D-65760, Germany.
E-mail addresses: j.meins@zentrallabor.com (J. Meins), dariush.behnam@aquanova.de (D. Behnam), m.tawab@zentrallabor.com (M. Abdel-Tawab).

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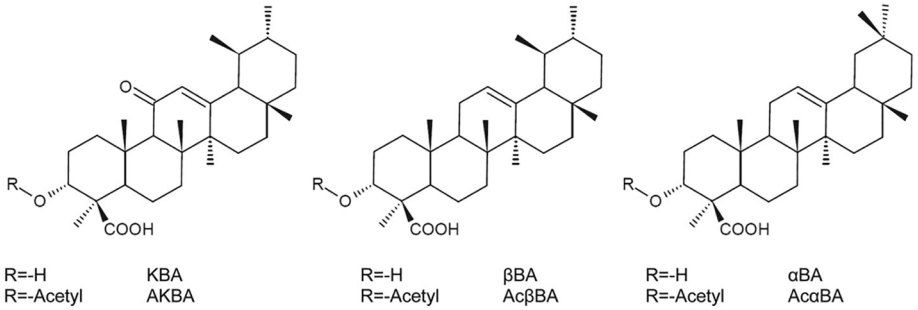

Fig. 1. Structures of the investigated boswellic acids. KBA: 11-keto-β-boswellic acid, AKBA: acetyl-11-keto-β-boswellic acid, αBA: α-boswellic acid, βBA: β-boswellic acid, AαBA: acetyl-α-boswellic acid, AβBA: acetyl-β-boswellic acid.

Table 1
Content of the individual six major BAs in native (Nat-BE) and solubilized (Sol-BE) Boswellia extract.

	Nat-BE		Sol-BE	
	[%]	Absolute amount [mg] in 128 mg total BAs/kg administered to rats	[%]	Absolute amount [mg] in 128 mg total BAs/kg administered to rats
KBA	4.2	5.4	0.4	5.4
AKBA	4.4	5.6	0.5	6.0
αBA	4.5	5.8	0.3	4.0
βBA	11.6	14.9	0.7	9.3
AαBA	2.7	3.4	0.2	2.9
AβBA	9.0	11.5	1.0	12.3
Total	36,4	46.6	3.1	39.9

bioavailability of curcumin, known for its poor absorption because of low solubility. Hence a 3.5-fold increase in the apparent permeability coefficient for micellar over native curcumin was observed in the Caco-2 *in vitro* model [6] and a 185-fold larger AUC for micellar curcumin compared to its native form was reported in human trials [7]. Encouraged by this tremendous increase in the oral bioavailability observed for curcumin, the present study was devoted to investigate the effect of micellation technology on the bioavailability of Boswellia extract in rats compared to its native form.

2. Materials and methods

2.1. Chemicals and reagents

Boswellic acids (11-keto-β-boswellic acid (KBA), acetyl-11-keto-β-boswellic acid (AKBA), α-boswellic acid (αBA), β-boswellic acid (βBA), acetyl-α-boswellic acid (AαBA) and acetyl-β-boswellic acid (AβBA)) used as reference substances with purity > 99% were obtained from Phytoplan GmbH, Heidelberg, Germany. Native *Boswellia serrata* extract (N.: 1509020–01, Code N.: 10115/246) (Nat-BE) standardized to 80% total BAs and solubilized *Boswellia serrata* extract (Batch N.: L124.16.LM.02.01, Code N.: EW0123/1) (Sol-BE) containing 10% *Boswellia serrata* extract were kindly donated by AQUANOVA AG (Darmstadt, Germany). The internal standard fluoxymesterone was purchased from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany, content > 98.0%). Ammonium formate was obtained from VWR (Leuven, Belgium). All solvents used were of analytical grade or better quality. Methanol, tetrahydrofuran, ethyl acetate and n-hexane were purchased from Roth GmbH (Karlsruhe, Germany). 2-Propanol, water and Extrelut® NT from Merck (Darmstadt, Germany). Plasma samples of rats administered the solubilized and native Boswellia extract were received from the Institute of Pharmacy and Molecular Biotechnology, Heidelberg, Germany. Blanc pooled rat plasma were received from Dunn Labortechnik, Asbach, Germany.

2.2. Animal study

All experiments were carried out according to the guidelines of German Protection of Animal act (Deutsches Tierschutzgesetz, BGBI 1998, Part I, No. 30, S.1105 ff.) and approved by the local ethical committee (AZ: 35.9185.81/G-17/11).

Female albino wistar rats weighing around 250 g were administered Sol-BE ($n = 6$) or Nat-BE (n = 6). For that purpose 300 mg of Nat-BE were weighed in Falcon tubes, filled up to 15 mL with water while shaking vigorously before 2 mL of that solution was administered orally to rats. This corresponds to a dose of 128 mg total BAs/kg. In case of Sol-BE, with a total BA fraction of 3.12%, 20 g solubilisate were weighed into a test tube and rehydrated with 80 mL water while shaking vigorously. Afterwards 2 mL of the respective solution, corresponding to 128 mg total BAs/kg were administered orally to rats by gavage *via* a pharyngeal tube. Blood samples for plasma analysis were collected from the retrobulbar venous plexus of the anesthetized animals after defined time points (0, 0.5, 1, 2, 3, 4, 6 and 8 h), centrifuged and stored at −20 °C.

2.3. Sample preparation

Concentrated stock solutions of all used BAs (AKBA, KBA, αBA, βBA, Aα- and AβBA) for standards and quality controls as well as the internal standard fluoxymesterone were prepared at a concentration of 1 mg/mL diluted in methanol. Different working solutions containing all BAs as well as an internal standard solution at 4 µg/mL were prepared by diluting the stock solutions with methanol. Calibration standards were prepared daily by spiking 1 mL of blank plasma with 25 µL of the internal standard solution and 40 µL of the corresponding working solution resulting in concentrations of 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 1500.0 and 3000.0 ng/mL plasma for βBA, AβBA, αBA and AαBA and 5.0, 10.0, 50.0, 100.0, 500.0, 1000.0, 1500.0, 3000.0 ng/mL plasma for KBA and AKBA. QC pools at different concentration levels (15.0, 800.0 and 2500.0 ng/mL plasma for AKBA and KBA and 1.5, 15.0, 800.0, 800.0 and 2500.0 ng/mL plasma for βBA, AβBA, αBA and AαBA) were prepared by spiking blank plasma with the corresponding spike solution. Afterward the QC samples were aliquoted and stored at −20 °C until analysis. Based on the method described by Buechele and Simmet [8] and Reising et al. [9], the rat samples and QC's were carefully thawed and 1 mL of the plasma homogenate was spiked with 40 µL pure methanol corresponding to the volume of the spike solution for calibration samples and 25 µL internal standard solution containing 1 µg fluoxymesterone in methanol. All samples were mixed briefly using a vortex while 0.8 g of Extrelut® NT was filled into an 8 mL glass column for each sample. The plasma homogenates were transferred onto the columns for a matrix-based liquid-liquid-extraction. After 15 min the BAs were eluted with 8 mL of an elution mixture consisting of tetrahydrofuran - n-hexane - ethyl acetate - 2-propanol (160,160:160:15, v/v/v/v) into clean centrifuge tubes. After that the solvent was evaporated to dryness using a nitrogen stream at 40 °C. The