Phytomedicine 23 (2016) 1375–1382

Contents lists available at ScienceDirect

Phytomedicine

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

Original article

A single-dose, randomized, cross-over, two-way, open-label study for comparing the absorption of boswellic acids and its lecithin formulation

Antonella Riva[a], Paolo Morazzoni[a], Christian Artaria[a], Pietro Allegrini[a], Jürgen Meins[b], Daniele Savio[c], Giovanni Appendino[d], Manfred Schubert-Zsilavecz[e], Mona Abdel-Tawab[b,*]

[a] Indena S.p.A., Viale Ortles 12, Milano, Italy
[b] Central Laboratory of German Pharmacists, Carl-Mannich-Str. 20, 65760 Eschborn, Germany
[c] R&D Solution s.r.l., Via Luigi Perna, 51 00142 Roma, Italy
[d] Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Via Bovio 6, 28100, Novara, Italy
[e] Department of Pharmaceutical Chemistry, Goethe-University Frankfurt, Max-von-Laue Strasse 9, 60438 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 12 October 2015
Revised 24 May 2016
Accepted 26 July 2016

Keywords:
Boswellia serrata
Frankincense
Boswellic acids
Triterpenoids
Lecithin
Absorption

ABSTRACT

Background: The oral administration of the gum resin extracts of Indian frankincense (*Boswellia serrata* Roxb. ex Colebr) results in very low plasma concentrations of boswellic acids (BAs), being far below the pharmacologically active concentrations required *in vitro* for anti-inflammatory activity. For that reason the use of Indian frankincense in clinical practice and pharmaceutical development has substantially lagged behind. Recently the application of new formulation technologies resulted in a formulation of frankincense extract with lecithin, which revealed improved absorption and tissue penetration of BAs in a rodent study, leading for the first time to plasma concentrations of BAs in the range of their anti-inflammatory activity.

Purpose: In order to verify these encouraging results in humans, the absorption of a standardized *Boswellia serrata* extract (BE) and its lecithin formulation (CSP) was comparatively investigated in healthy volunteers.

Study design: According to a randomized cross-over design with two treatments, two sequences and two periods, 12 volunteers alternatively received the lecithin-formulated *Boswellia* extract (CSP) or the non-formulated *Boswellia* extract (BE) at a dosage of 2×250 mg capsules.

Methods: The plasma concentrations of the six major BAs (KBA, AKBA, βBA, αBA, AβBA, AαBA) were determined using LC/MS.

Results: With the exception of KBA, a significantly higher (both in terms of weight-to-weight and molar comparison) and quicker absorption of BAs from the lecithin formulation was observed, leading to C_{max} in the range required for the interaction with their molecular targets.

Conclusion: These findings pave the way to further studies evaluating the clinical potential of BAs, and verify the beneficial effect of lecithin formulation to improve the absorption of poorly soluble phytochemicals.

Introduction

The belief that natural medicines are safer than synthetic drugs led to a tremendous growth of phytopharmaceuticals, reaching a

Abbreviations: αBA, Alpha-boswellic acid; AαBA, Acetyl- alpha-boswellic acid; AβBA, Acetyl- beta-boswellic acid; AKBA, Acetyl-11-keto-boswellic acid; βBA, Beta-boswellic acid; BAs, Boswellic acids; BE, *Boswellia* extract; catG, Cathepsin G; CI, Confidence interval; COX-1, Cyclooxygenase-1; ECG, Electrocardiogram; ESI, Electro Spray Ionization; HBSAg, hepatitis B surface antigen; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HLE, Human leuokocyte elastase; IL, Interleukin; KBA, 11-keto-boswellic acid; LSM, Least squares means; 5-LO, 5-lipoxygenase; mPGES-1, microsomal prostaglandin E synthase-1; NF-κB, nuclear factor-kappaB; S.A.S., Société Anonyme Simplifiée (form of organization for companies); SIM, single ion mode; SAS®, Statistical Analysis System; THF, tetrahydrofurane; TNFα, tumor necrosis factor alpha.

* Corresponding author: Fax: +49-6196-937-810.
E-mail address: m.tawab@zentrallabor.com (M. Abdel-Tawab).

http://dx.doi.org/10.1016/j.phymed.2016.07.009

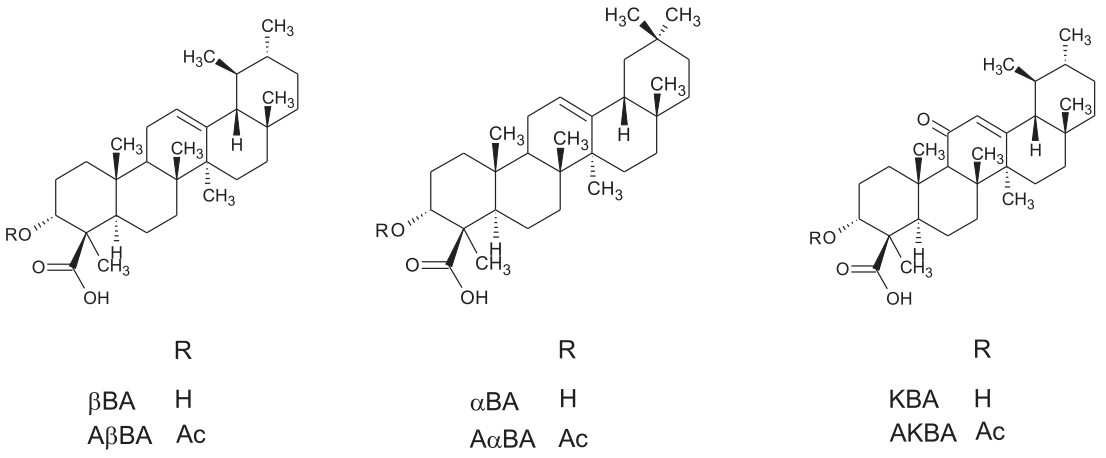

Fig. 1. Chemical structures of the six characteristic boswellic acids of *Boswellia serrata*.

worldwide 25% share of the pharmaceutical arsenal (Bhattaram et al., 2002). Especially the interest in alternative, well-tolerated anti-inflammatory herbal remedies has re-emerged in the last decades, as all efforts aiming to develop safe synthetic anti-inflammatory drugs are still far from achieving a real breakthrough. Gum resin extracts of *Boswellia serrata* Roxb. ex. Colebr. (Indian frankincense) have been found to represent a promising anti-inflammatory herbal remedy. Numerous experimental data from *in vitro* studies and animal models support the potential of boswellic acids (BAs) (Fig. 1), a class of pentacyclic triterpenoids representing the major active principles of *B. serrata*, for the treatment of various diseases. Hence it was shown that a number of pivotal enzymes in inflammation like 5-lipoxygenase (5-LO), cyclooxygenase-1 (COX-1), human leuokocyte elastase (HLE), cathepsin G (catG) and the microsomal prostaglandin E synthase-1 (mPGES-1) as well as the nuclear factor-kappaB (NF-κB) and several cytokines like TNFα, IL-1β and IL-6 are inhibited by BAs with IC_{50} values in the range of 0.6 µm to 24 µm (Abdel-Tawab et al., 2011; Poeckel and Werz, 2006). Modern research has highlighted the relevance of the whole fraction of triterpenoid acids for the anti-inflammatory activity of *Boswellia* extracts, since all BAs show significant *in vivo* activity in animal models of inflammation (Bannoa et al., 2006). This represents a dramatic change compared to the early studies, that emphasized the relevance of a single constituent (AKBA, a minor constituent of the BAs bouquet) and a single target (5-lipoxygenase, 5-LO) (Safayhi et al., 1992).

However, various pharmacokinetic studies in human and animals revealed very low plasma concentrations of BAs, far below the pharmacologically active concentrations determined *in vitro*, in spite of administering very high doses of *Boswellia* extract reaching up to 3000 mg/day (Abdel-Tawab et el., 2011; Du et al., 2015). This limited the use of *B. serrata* in clinical practice and pharmaceutical development. Consequently the anti-inflammatory potential of BAs could as yet not been translated into an approved clinical application, (Ernst, 2008). The dismally low oral absorption of BAs is not surprising, since these compounds are very poorly soluble in water, suggesting a strong tendency to self-aggregation. This hypothesis is supported by the marked increase of absorption observed when *B. serrata* extracts are administered with food, as expected from the disruption of self-aggregates by biliary salts (Skarke et al., 2012). These considerations provided a rationale for the development of a lecithin formulation of BAs, which revealed a significantly improved absorption of BAs accompanied with enhanced tissue penetration in rats, leading for the first time to plasma concentrations of BAs in the range of their anti-inflammatory activity (Huesch et al., 2013).

In the wake of these promising animal studies, we have carried out a comparative pharmacokinetic study in healthy volunteers on weight-equivalent (500 mg) dosages of the lecithin-formulated *Boswellia* extract (CSP) and the non-formulated *Boswellia* extract (BE) used for its preparation. Since CSP contains only 33% *Boswellia* extract on molar basis, this involved comparison between 150 mg of extract formulated with lecithin and 500 mg of non-formulated extract.

Materials and methods

Products

Gum resin extracts of *Boswellia serrata* Roxb. ex. Colebr. (plant name checked with http://www.theplantlist.org) (Batch N.: 11239, Code # 36BW60090) (BE) and its lecithin formulation Casperome® (Batch N.: 12146, Code # 36BWP0090) (CSP) used in the study were produced by Indena S.A.S. (Tours, France). Both were assigned voucher numbers and representative voucher specimen has been deposited in the Central Laboratory of German Pharmacists, Eschborn, Germany. CSP is composed of *B. serrata* extract and soy lecithin in a 1:1 ratio, with about half part of microcrystalline cellulose being added to improve the physical state and to standardize the product to a content of total triterpenoid acids by HPLC of at least 25%. For this study, 250 mg of BE or CSP were formulated by Indena S.p.A. (Milan, Italy) into Swedish orange size "0" hard gelatin capsules containing: 54.0 mg of corn starch and pregelatinized starch (StarCap 1500®, Colorcon, UK), 50.0 mg of Citric Acid Anhydrous, 9.0 mg of Croscarmellose Sodium (Solutab®, Blanver, San Paolo, Brazil), 4.0 mg of Silicon Dioxide (Syloid® 244 FP, W. R. Grace & Co., Conn., Columbia, USA), 9.0 mg of magnesium stearate, and 4.0 mg of talc. Before releasing the hard gelatin capsules containing BE (Batch # 89035) or CSP (Batch # 89036), the appearance, average mass, uniformity of mass (Eur. Pharm. 2.9.1.), HPLC content of BAs, disintegration time (< 10 min according to Eur. Pharm 2.9.1) and microbiological quality were tested. For the detailed quantification of the six major BAs contained in the capsules see Table 1.

Subjects

In order to standardize all variables that may affect the pharmacokinetics of BAs, twelve healthy non-smoking subjects aged between 20 to 51 years of Caucasian race were recruited and considered eligible for enrolment as per protocol inclusion and exclusion criteria. The screening procedures included collection of anamnesis and demographic data (gender, age, race, body weight [kg], height