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Short communication

High-performance liquid chromatographic determination of acetyl-11-keto-α-boswellic acid, a novel pentacyclic triterpenoid, in plasma using a fluorinated stationary phase and photodiode array detection: Application in pharmacokinetic studies

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Abstract

A rapid, sensitive and selective HPLC separation with photodiode array detection was developed for the analysis of the novel pentacyclic triterpenoid acetyl-11-keto-α-boswellic acid. Complete baseline separation of acetyl-11-keto-α-boswellic acid from the corresponding isomer acetyl-11-keto-β-boswellic acid was achieved on a fluorinated stationary phase. The standard curve was linear from 0.98 nmol/l to 196 nmol/l acetyl-11-keto-α-boswellic acid. The compound was isolated from chick embryonic plasma using extraction on diatomaceous earth with an overall average extraction yield of 82%. This method was applied in a kinetic study on the chick chorioallantoic membrane model (CAM) and showed unequivocal separation between acetyl-11-keto-α-boswellic acid and acetyl-11-keto-β-boswellic acid unachievable so far.

Keywords: Pentacyclic triterpenoids; Acetyl-11-keto-α-boswellic acid; Chick chorioallantoic membrane; Pharmacokinetics; Pentafluorophenylpropyl-silica

1. Introduction

The gum resins from various *Boswellia* species contain boswellic acids, pharmacologically active compounds belonging to the family of pentacyclic triterpenoids. Distinct boswellic acids have been reported to possess both anti-inflammatory and anti-tumor activity. These effects might be due to pleiotropic effects including inhibition of human leukocyte elastase and/or of 5-lipoxygenase [1,2], as well as to topoisomerase inhibition [3] resulting in apoptosis-related tumor cell death [4]. Only recently, we were able to demonstrate that boswellic acids also inhibit the activity of the nuclear transcription factor, NF-κB, that is crucial for the expression of proinflammatory and other genes related to cancer cell survival and chemoresistance [5,6]. For the first time, these findings provided a molecular pharmacological basis for the dual efficacy of these triterpenoids in inflammatory and neoplastic disorders. To evaluate the putative pharmacological and therapeutic potential of various boswellic acid derivatives, we initiated several lines of investigations. Thus, we isolated and structurally characterized chemically pure standard compounds from frankincense gum resin [7], and analyzed their contents in phytopharmaceutical preparations [8]. Except for acetyl-11-keto-β-boswellic acid, most of the boswellic acid derivatives are known to exist either in an α- or a β-configuration. To clarify this exception, we synthesized and structurally elucidated acetyl-11-keto-α-boswellic acid from the corresponding acetyl-α-boswellic acid (submitted). Apart from pharmacological studies aiming at molecular mechanisms such as their antitumor efficacy [3,9], it is necessary to analyze the plasma levels of these compounds to gain insight into their pharmacokinetic parameters. So far, most studies dealing with pharmacokinetics used mammalian animal models or human clinical studies [10,11]. The analytical methods usually combine solid phase or liquid–liquid plasma extraction with HPLC-UV or GC–MS detection [12,13]. Here we show that a very efficient plasma extraction method on diatomaceous earth can be successfully combined with HPLC analysis on a fluorinated stationary phase that shows a high selectivity for acetyl-11-keto-α-boswellic; this

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method allowed to analyze the test compound in very small plasma samples collected from chick chorioallantoic membrane vessels. Specifically with regard to human cancer xenografts, this testing system offers several advantages such as: (i) reduction of mammalian animal experimentation due to pre-selection; (ii) consumption of only small amounts of limited and precious compounds; (iii) good reproducibility when used at a constant embryonic development [14]; and (iv) economic efficiency.

2. Experimental

2.1. Chemicals, animals

All chemicals were of analytical reagent grade unless stated otherwise. Reverse-osmosis type quality water, pureAqua (Schnaitsee, Germany) combined with a Milli-Q station from Millipore (Eschborn, Germany) was used throughout. The standard compounds acetyl-11-keto-α-boswellic acid and acetyl-11-keto-β-boswellic acid (Fig. 1) were obtained and characterized as described [7,8]. Methanol, ethyl acetate, tetrahydrofuran, isopropanol, dimethylsulfoxide, Extrelut® NT and acetic acid 96% were purchased from Merck (Darmstadt, Germany). The test substance acetyl-11-keto-α-boswellic was solubilized in physiological sodium chloride solution after complexation with γ-cyclodextrin from Wacker-Chemie (Burghausen, Germany). Fertilized chicken eggs from Hysex brown hens were obtained from a local supplier, Schuhmacher (Sinningen, Germany).

2.2. Instrumentation and software

The HPLC system consisted of a low pressure gradient LC-9A Shimadzu pump (Kyoto, Japan), an automatic sample injector Aspec XL (Abimed, Langenfeld, Germany), a column oven IWN CH100 (Junedis, Gröbenzell, Germany) and a photodiode array detector UVD 340U (Dionex, Idstein, Germany) connected to a personal computer equipped with Chromeleon Software version 6.6 (Dionex, Idstein, Germany). Statistical calculations were carried out with the software package Valoo (Applica, Bremen, Germany). The separation was performed on a Discovery HS F5 column (150 mm × 4.0 mm I.D., particle size 5 μm; Sigma–Aldrich, Taufkirchen, Germany). Solid phase extraction was performed with a Lichrolut extraction manifold (VWR International, Darmstadt, Germany).

2.3. Standards and sample solutions

Standard stock solutions were prepared by dissolving 1 mg substance in 1 ml dimethylsulfoxide. For further preparation of standard solutions, the stock solutions were diluted with a mixture of methanol–water (80:20, v/v) yielding concentrations from 9.8 nmol/l to 1.96 μmol/l. Standard solutions of acetyl-11-keto-α-boswellic acid for the validation of the method in chick embryonic plasma were prepared in the same range. For the pharmacokinetic study, acetyl-11-keto-α-boswellic acid was first complexed with γ-cyclodextrin according to standard procedures [6], which allowed it to be dissolved in appropriate concentrations in 0.9% sodium chloride solution.

2.4. Sample preparation

The test compounds were applied to the CAM as described previously [6,14]. Blood samples anticoagulated with EDTA 5 mmol/l (final concentration) were collected on day 10 after fertilization from the embryonic vessels using 0.3 mm × 13 mm

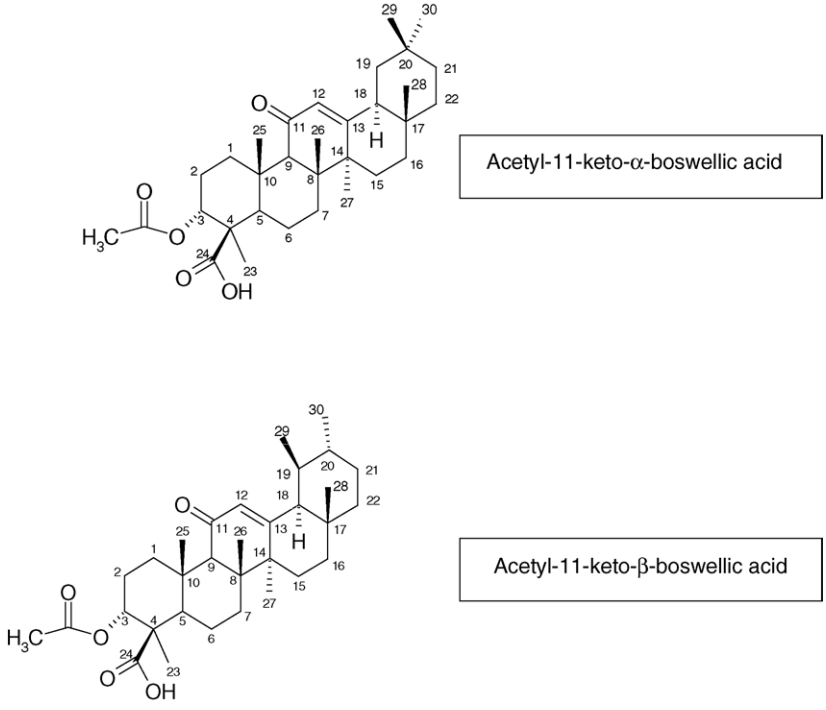

Fig. 1. Structures of the acetyl-11-keto-boswellic acids analyzed.