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Mouse tissue distribution and persistence of the food-born fusariotoxins Enniatin B and Beauvericin

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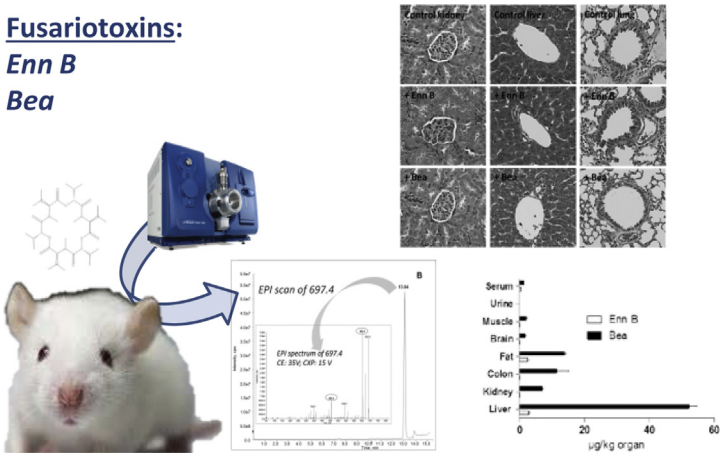
HIGHLIGHTS

- Development of a LC–MS/MS method for Enn B and Bea quantification in mice tissue.
- Nine days i.p. treatment of Enn B or Bea showed no systemic toxicity in mice.
- Contribution of hepatic/intestinal metabolism for Enn B but not Bea was suggested.
- Both substances showed distinct tissue accumulation with Bea being more potent.
- Tumor accumulation of Enn B and Bea emphasizes their anticancer potential

GRAPHICAL ABSTRACT



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ABSTRACT

The fusariotoxins Enniatin B (Enn B) and Beauvericin (Bea) have recently aroused interest as food contaminants and as potential anticancer drugs. However, limited data are available about their toxic profile. Aim of this study was to investigate their pharmacological behavior *in vivo* and their persistence in mice. Therefore, liquid chromatography tandem mass spectrometry (LC–MS/MS) was used to analyze

Abbreviations: ABC, ATP-binding cassette; Bea, beauvericin; b.w., bodyweight; DMSO, dimethyl sulfoxide; Enn, Enniatin; ESI, Electrospray ionisation; LC-MS/MS, liquid chromatography-tandem mass spectrometry; p.a., per analysis; sSRM, scheduled selected reaction monitoring; SSE, signal enhancement or suppression; LODs, limits of detection; LOQs, limits of quantitation; RSD_r, relative standard deviation; SCID, severe combined immunodeficiency.

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the distribution of Enn B and Bea in selected tissue samples and biological fluids originating from mice treated intraperitoneally with these cyclohexadepsipeptides. Overall, no toxicological signs during life time or pathological changes were observed. Moreover, both fusariotoxins were found in all tissues and serum but not in urine. Highest amounts were measured in liver and fat demonstrating the molecules' tendency to bioaccumulate in lipophilic tissues. While for Bea no metabolites could be detected, for Enn B three phase I metabolites (dioxygenated-Enn B, mono- and di-demethylated-Enn B) were found in liver and colon, with dioxygenated-Enn B being most prominent. Consequently, contribution of hepatic as well as intestinal metabolism seems to be involved in the overall metabolism of Enn B. Thus, despite their structural similarity, the metabolism of Enn B and Bea shows distinct discrepancies which might affect long-term effects and tolerability in humans.

1. Introduction

Enniatins (Enns) and Beauvericin (Bea) belong to a group of cyclic hexadepsipetides which are produced mainly by *Fusarium* fungi that invade and grow on crops. They are resistant to heat, acids as well as digestion and are stable during commercial processing like brewing, melting, hot drying or ensiling. Consequently, these cyclodepsipeptides play an important role as contaminants of grain and grain-based products (Faeste et al., 2011; Jestoi, 2008). The most important contributors to chronic dietary exposure to Enns and Bea are especially bread and rolls, fine bakery wares and pasta. Usually, toddlers are the population group with the highest dietary chronic and acute exposure to both fusariotoxins (Panel, 2014). Recently, both fusariotoxins were shown to be capable of crossing the human skin barrier and reaching the viable epidermis and dermis (Taevernier et al., 2015b). Moreover, immunological disorders were suggested in humans after ingestion of these alimentary toxins (Ficheux et al., 2013; Juan et al., 2014).

Interestingly, Enns and Bea also evoked interest because of their pharmacological properties. In 1953 a mixture of Enn A, A1, B and B1 (called fusafungine) was originally patented as local antibiotic for the topically treatment of nose and throat infections. Fusafungine is currently marketed under the trade names Locabiotal®, Bioparox®, Locabiosol® and Fusaloyos®. However, only limited data are available about its bioavailability. Recently, Taevernier et al. (2015a) demonstrated that Enns are capable of permeating porcine buccal mucosa and suggested that Enn-based solutions for oromucosal use in the treatment of innocent upper respiratory tract infections should be questioned, because Enns potentially will reach significant systemic concentrations. Additionally, Bea is used in traditional Chinese medicine as a constituent in preparations with reputed anticonvulsant and antineoplastic actions. Moreover, a patent has been issued for a Bea tablet (containing 5 mg Bea) to lower blood cholesterol levels (Jestoi, 2008).

Nevertheless, data regarding the toxicology, toxicokinetics and toxicodynamics of Enns and Bea are still fragmentary. So far, no reports of adverse events in humans or animals due to contaminated food or feed exist (Jestoi, 2008; Panel, 2014). Moreover, subchronic (28 days) feeding experiments with Wistar rats using a repeated dose of 20.91 mg Enn A/kg b.w./day showed no adverse effects (Manyes et al., 2014). Acute toxicity was reported in the literature after oral administration with an $LD_{50} \geq 100$ mg/kg b.w. for Bea and 350 mg/kg b.w. for fusafungine (Panel, 2014).

On the contrary, despite their low *in vivo* toxicity, both fusariotoxins evoked considerable toxicity in diverse *in vitro* assays (Behm et al., 2012; Dornetshuber et al., 2009a; Fornelli et al., 2004; Ivanova et al., 2006). Noteworthy, their cytotoxic potential was especially prominent in malignant cells derived from a wide range of cancer types as compared to non-malignant cells (Dornetshuber et al., 2007). Thus, both cyclic hexadepsipeptides came into focus of interest as potential anticancer drugs and for Enn B *in vivo* anticancer activities against cervical cancer were recently reported by our research group (Dornetshuber-Fleiss et al., 2015).

Consequently, the observed difference of *in vitro* and *in vivo* toxicity might have its origin in the low bioavailability. On the one hand, peptides are generally not considered to be orally well absorbed because of significant enzyme degradation in the digestive tract (Chan et al., 1997). Low oral bioavailability may be caused by impaired uptake from the gastrointestinal tract because of low compound water solubility (Blais et al., 1992) and interaction with efflux pumps (Dornetshuber et al., 2009b; Ivanova et al., 2011). On the other hand, oral bioavailability of N-methyl peptides like cyclosporine A is known, which is structurally related to Enns and Bea (Cooney et al., 1997). Hence, the apparent mean bioavailability in CaCo-2 cells for Enn A, A1, B, and B1 was assessed by an average of 80% (Meca et al., 2012). Moreover, *in vivo* trials using pigs demonstrated even a higher bioavailability of 91% for Enn B (Devreese et al., 2014). Therefore, other mechanisms like rapid elimination from the systemic circulation because of metabolic reactions might explain the low acute oral *in vivo* toxicity (Faeste et al., 2011). Accordingly, recent *in vitro* metabolism studies of Enn B with rat, dog and human liver microsomes reported twelve biotransformation products suggesting that the reduced *in vivo* toxicity is based on an extensive hepatic metabolism (Faeste et al., 2011; Ivanova et al., 2011). Additionally, an *in vivo* study by Manyes et al. reported intestinal degradation products and adducts for Enn A in Wistar rats (Manyes et al., 2014). For Bea, less data are available in this regard. However, at least inhibitory effects on cytochrome P450 enzymes were shown in human and rat liver microsomes (Mei et al., 2009).

Therefore, to fill gaps in toxicology-related knowledge and considering the evaluation of the two fusariotoxins as potential anticancer drug candidates and as emerging food-born toxins, this *in vivo* study aims: (i) to investigate the pharmacological behavior of Enn B and Bea *in vivo* by histochemistry studies and, (ii) to evaluate their persistence in selected tissues and biological fluids of mice after intraperitoneal administration by using a sensitive and specific liquid chromatography tandem mass spectrometric (LC-MS/MS) analytical method.

2. Experimental procedures

2.1. Chemical and reagents

Bea, Cremophor, DMSO, LC gradient grade methanol, acetonitrile as well as MS grade ammonium acetate and glacial acetic acid (p.a.) were purchased from Sigma–Aldrich (Vienna, Austria). A Purelab Ultra system (ELGA LabWater, Celle, Germany) was used for further purification of reverse osmosis water. Enn B for animal studies was purified from *Fusarium oxysporum* ETH 1536/9 as described previously (Dornetshuber-Fleiss et al., 2015). Additionally, the cytotoxic potential was compared to Enn B obtained from