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In vitro myelotoxicity assessment of the emerging mycotoxins Beauvericin, Enniatin b and Moniliformin on human hematopoietic progenitors

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ABSTRACT

The aim of this study was to screen potential myelotoxicity of the emerging mycotoxins Beauvericin, Enniatin b and Moniliformin using human hematopoietic progenitor clonogenic assays.

Depending on mycotoxins, inhibitory effects on proliferation of white blood cells progenitors (CFU-GM), platelet progenitors (CFU-MK) and red blood cells progenitors (BFU-E) have been detected at various concentrations. Beauvericin was cytotoxic at 32 μM, 3.2 μM and 6.4 μM, had no effect on proliferation in the presence of 0.032 μM, 0.16 μM and 0.064 μM, and the IC_{50} was equal to 3.4 μM, 0.7 μM and 3.7 μM for CFU-GM, CFU-MK and BFU-E, respectively. Enniatin b was cytotoxic at 6 μM, 1.8 μM and 5 μM, had no effect on proliferation in the presence of 1 μM, 1.1 μM and 1.2 μM and the IC_{50} was equal to 4.4 μM, 1.3 μM and 3.3 μM for CFU-GM, CFU-MK and BFU-E, respectively. Moniliformin was not cytotoxic at tested concentrations for CFU-GM and CFU-MK and cytotoxic at 10 μM for BFU-E, had no effect on proliferation in the presence of 5 μM, 0.1 μM and 0.1 μM and the IC_{50} was equal to 31 μM, 39 μM and 4.1 μM for CFU-GM, CFU-MK and BFU-E, respectively. Inhibition of the BFU-E differentiation has been observed in the presence of Enniatin b or Moniliformin. For the three mycotoxins, variation of distribution of CFU-MK colonies according to their size has been observed. These *in vitro* effects may be responsible for *in vivo* hematological troubles in case of consumption of contaminated commodities. *In vivo* studies have to be performed to test this hypothesis.

1. Introduction

Mycotoxins are secondary metabolites produced under appropriate environmental conditions by filamentous fungi, mainly *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp in various commodities. Some of these mycotoxins, such as Aflatoxins, Ochratoxin A, Fumonisin B1 and trichothecenes Deoxynivalenol or T-2 toxin, are known to be hepatotoxic, genotoxic, immunosuppressive, nephrotoxic, teratogenic, and carcinogenic (Bennett and Klich, 2003; Paterson and Lima, 2010).

Some of mycotoxins, especially trichothecenes, have been proven to induce hematological troubles (leukopenia, thrombocytopenia, anemia or agranulocytosis) in human after contaminated food ingestion. Hematotoxicity is clinically characterized by abnormal blood cell counts and/or dysfunction of the blood cells of different lineages. Abnormal blood cell counts can have two distinct origins. It could be due to either their destruction in the circulatory system and/or to insufficient production by the bone marrow during hematopoiesis (myelotoxicity).

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Emerging mycotoxins, including Beauvericin, Enniatin b and Moniliformin, are a group of lesser-known toxins. These mycotoxins are principally produced by many species of *Fusarium*, and are found in cereals in various countries (Jestoi, 2008).

Beauvericin is a cyclic hexadepsipeptide consisting of alternating D-α-hydroxy-isovaleryl and aromatic N-methyl-phenylalanine (Fig. 1). This toxin is produced by various *Fusarium* species such as *Fusarium avenaceum*, *Fusarium poae*, *Fusarium oxysporum* and *Fusarium proliferatum*, and naturally occurs on maize, wheat, barley, rice and oat (Logrieco et al., 1998; Uhlig et al., 2006; Jestoi, 2008; Sorensen et al., 2008; Kokkonen et al., 2010; Waskiewicz et al., 2010). Beauvericin has been detected in grains throughout the world under different climates (South Africa, Poland, Norway, Spain, Croatia…), with concentrations ranging from trace level up to 520 mg/kg in maize in Italy (Ritieni et al., 1997). Meca et al. have shown that Beauvericin was present in cereals (barley, corn and rice) purchased on Spanish markets, with levels ranging from 0.51 to 11.78 mg/kg (Meca et al., 2010a).

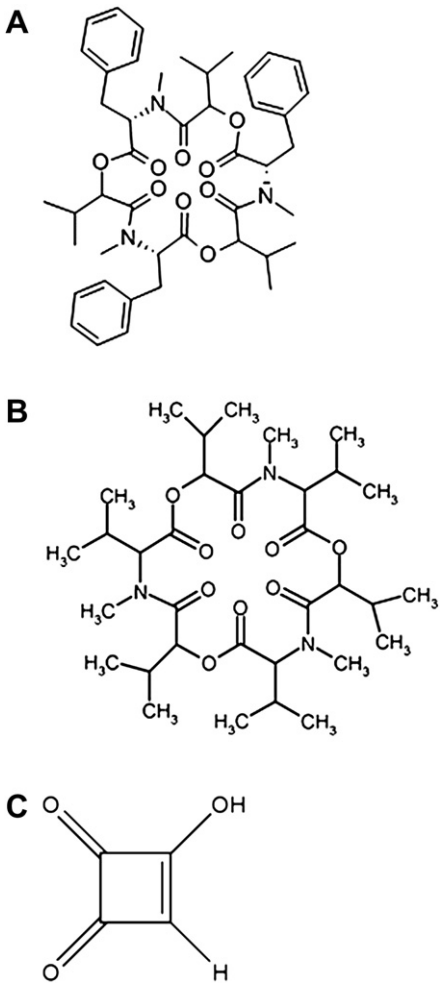

Fig. 1. The chemical structures of Beauvericin (A), Enniatin b (B) and Moniliformin (C).

An *in vivo* study has shown that mice orally exposed to Beauvericin presented an increase of mortality with a Lethal Dose 50 (LD_{50}) superior to 100 mg/kg bw (Jestoi, 2008).

The cytotoxicity of Beauvericin has been demonstrated *in vitro* in several cell line models, including human leukemia cells CCRF-CEM, human monocytic lymphoma cells U-937 and promyelocytic leukemia cells HL-60, monkey kidney epithelial cells Vero, Chinese hamster ovary cells CHO-K1 and murine macrophage J774 (Tomoda et al., 1992; Calo et al., 2004; Jow et al., 2004; Ruiz et al., 2011a,b).

Group of Enniatins represents cyclic hexadepsipeptides, which are commonly composed of three D-α-hydroxyisovaleric acid residues linked alternatively to three L-N-methyl amino acid residues (Fig. 1). Individual Enniatins are primarily distinguished by the nature of the N-methyl amino acid residue and more than 15 distinct Enniatins have been isolated (Ivanova et al., 2006). Presence of Enniatins in field has been studied in Northern Europe. Enniatin b was detected in wheat, oat and barley contaminated by *F. avenaceum* in Norway and Finland (Logrieco et al., 2002; Uhlig et al., 2006). Enniatin b was detected in Finnish wheat with concentrations ranging from trace level up to 18 mg/kg (Jestoi et al., 2004). *F. avenaceum*, *F. poae*, *Fusarium langsethiae*, *Fusarium tricinctum* and *Fusarium sporotrichoides* grown *in vitro* are also shown to produce Enniatin b (Thrane et al., 2004; Vogelgsang et al., 2008; Meca et al., 2010a). Meca et al. have shown that Enniatin b was present in cereals (barley, corn and rice) purchased on Spanish markets with level ranging between 2.23 and 21.37 mg/kg (Meca et al., 2010b).

In vitro, Enniatin b has been shown to be cytotoxic on cell lines including human hepatocellular carcinoma-line HepG2, human colon carcinoma HT-29, human epithelial colorectal adenocarcinoma Caco-2, human fibroblast-like fetal lung cell line MRC-5, Chinese hamster lung fibroblast V79, and murine macrophage J774 (Tomoda et al., 1992; Ivanova et al., 2006; Behm et al., 2009; Meca et al., 2011).

Moniliformin (Fig. 1) is structurally characterized as 3-hydroxycyclobut-3-ene-1,2-dione and occurs naturally as a sodium or potassium salt. Moniliformin is produced by several *Fusarium* species including *F. avenaceum*, *F. proliferatum*, *Fusarium subglutinans*, *F. tricinctum*, *F. oxysporum* and *Fusarium verticillioides*, and frequently occurs in maize, oat, barley, rice and wheat (Sharman et al., 1991; Sorensen et al., 2007; Jestoi, 2008; Waskiewicz et al., 2010). Moniliformin has been detected in various regions of the world (South Africa, Northern Europe). Oat and wheat from Norway have been found to contain up to 0.21 mg/kg and 0.95 mg/kg Moniliformin, respectively (Uhlig et al., 2004). Maize from Gambia has been found to contain 3.2 mg/kg Moniliformin (Sharman et al., 1991).

In human, Moniliformin is suspected to be associated with an endemic disease called Keshan disease, a myocardial impairment reported in rural areas of China and South Africa with large consumption of Moniliformin-contaminated maize even if a clear correlation is lacking (Yu et al., 1995).

The main symptoms of *in vivo* acute Moniliformin toxicity are muscular weakness, respiratory stress, myocardial degeneration, followed by coma and death in ducklings, rats, broiler chickens and mice. LD_{50} is equal to 41.6 or 50 mg/kg bw for female or male rat, and 47.6 mg/kg