Contents lists available at ScienceDirect

Toxicology Letters

Repeated dose 28-day oral toxicity study of moniliformin in rats

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HIGHLIGHTS

- The subacute oral toxicity of synthetic moniliformin in rats was assessed according to an adaption of OECD guideline 407.
- The clinical signs included death and acute cardiac distress in 2 out of 5 rats in the highest dose group (15 mg/kg b.w.).
- Moniliformin reduced the phagocytic activity of the rat neutrophils indicating an adverse effect on innate immunity.
- Excretion of moniliformin into urine was rapid, with no indication of accumulation post-exposure.
- Based on our findings, we suggest a LOAEL of 3 mg/kg b.w.

ARTICLE INFO

ABSTRACT

Moniliformin is a *Fusarium* mycotoxin mainly produced by several species infecting grains in different climatic conditions. According to our previous studies, it is acutely toxic to rats, with an LD_{50} cut-off value of 25 mg/kg b.w. To further assess the possible health risks of low dose exposure to moniliformin, a subacute oral toxicity study was conducted in Sprague-Dawley rats, adapting OECD guideline 407. Five dose groups and two satellite groups, each consisting of five male rats, were daily exposed to moniliformin by gavage. Two rats in the highest dose group, showed decreased activity followed by acute heart failure and death. The rats of the lower doses (<9 mg/kg b.w.) showed no signs of toxicity. The daily intake of moniliformin strongly reduced the phagocytic activity of neutrophils in all dose groups. The decrease continued in the satellite group during the follow-up period, indicating a severe impact on the immune system and a LOAEL value of 3 mg/kg b.w. for moniliformin. Moniliformin was rapidly excreted into urine, ranging between 20.2 and 31.5% daily and showed no signs of accumulation. The concentration of moniliformin in faeces was less than 2%, which suggests efficient absorption from the gastrointestinal tract.

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1. Introduction

The filamentous fungi *Fusarium* globally infect agriculturally important plants and cause common plant diseases such as root, stem and ear rot of maize, as well as *Fusarium* head blight in cereal grains. Additionally, most *Fusarium* species produce an arsenal of secondary metabolites, mycotoxins, which evoke a broad range of adverse effects on animal and human health, including neurotoxicity, immunotoxicity, reproductive and developmental toxicity, and carcinogenicity (Desjardins, 2006). However, disease outbreaks caused by *Fusarium* mycotoxins are infrequent due to the well-established risk management systems in the developed countries.

Climatic and environmental conditions strongly impact mycotoxin production, and most *Fusarium* species are able to concurrently produce several different mycotoxins. The most studied *Fusaria* toxins include trichothecenes, zearalenone and fumonisins. Nonetheless, the majority of *Fusarium* species infecting grains are also capable of producing other, less studied toxic metabolites such as moniliformin (MON), produced by a number of *Fusarium* species belonging to the sexual state *Gibberella* (Schütt et al., 1998). MON is frequently found in maize and small-grain cereals, with high concentrations detected in maize (>400 mg/kg in Poland, Logrieco et al., 1993) and markedly lower levels present in small grain cereals (810 µg/kg wheat in Finland, 950 µg/kg wheat in Norway (Jestoi, 2005; Uhlig et al., 2004). Due to natural MON contamination of grains and grain products, humans and livestock are at risk of daily, low-level exposure to MON, the consequences of which are unknown, as information on the toxicity or absorption, distribution, metabolism and excretion (ADME) of MON after oral administration is very scarce.

MON has not been involved in any natural animal or human disease outbreaks, but has shown comparable toxicity to the most toxic trichothecenes, T-2 toxin and HT-2 toxin, in laboratory experiments involving birds and rodents (Abbas et al., 1990; Allen et al., 1981; Burmeister et al., 1979; Kriek et al., 1977; Nagaraj et al., 1996; Ueno, 1983).

MON is a small ionic molecule appearing as a sodium or potassium salt of semisquaric acid. It is formed from 2 acetate units involving oxidation and dehydration reactions (ApSimon, 1994; Franck and Breipohl, 1984). However, the enzymes and genes involved in the biosynthesis of MON remain unknown.

The toxic mode of action of MON is unclear and only a limited amount of data on its toxicokinetics is available. MON and pyruvate show structural similarity, and it has been proposed that MON substitutes pyruvate and inhibits the function of thiamine pyrophosphate-dependent enzymes, the incorporation of pyruvate into the tricarboxylic acid (TCA) cycle and the oxidation of the TCA cycle intermediates (Pirrung and Nauhaus, 1996). This could lead to cellular energy deprivation and may partially explain the respiratory stress, myocardial effects, and even the mortality of test animals caused by MON (Burka et al., 1982; Engelhardt et al., 1989; Gathercole et al., 1986; Kriek et al., 1977; Morgan et al., 1999; Nagaraj et al., 1996); MON may also interfere with carbohydrate metabolism through the inhibition of gluconeogenesis and aldose reductase (Deruiter et al., 1993). Furthermore, oxidative damage in myoblasts has been reported, possibly due to the inhibition of glutathione peroxidase and glutathione reductase by MON (Chen et al., 1990).

The toxic responses of test animals to MON exposure warrant further assessment of its toxicity. Studies on ADME and information on chronic or long-term exposure are scarce. Values for no observed adverse effect levels (NOAEL) and tolerable daily intake (TDI) for MON have not yet been established. Consequently, there is a need for further assessment of the toxic effects of this mycotoxin. In this study, the subacute toxic effects of a repeated, low-dose exposure to MON on Sprague-Dawley rats was assessed according to the Organisation for Economic Cooperation and Development (OECD) guideline 407. This provides information on the effects of a repeated, low-dose exposure to MON on excretion kinetics and on innate immunity, and it evaluates possible target organs and clinical signs in the test animals.

2. Materials and methods

2.1. Chemicals and reagents

2.1.1. Chemicals for animal experiments and excretion studies

A synthetic potassium salt of MON, used in *in vivo* settings, was provided by Sigma–Aldrich (Batch Q43844-pr-032, Bangalore, India). The structure of MON was verified with NMR and MS and the purity of the mycotoxin was accordingly demonstrated to be >99.8%. Acetonitrile, methanol and formic acid of HPLC grade and potassium chloride (KCl) were purchased from J.T. Baker (Deventer, the Netherlands). Ammonium formate (NH_4HCO_2) of p.a. quality and leucine enkephalin were obtained from Sigma–Aldrich (Munich, Germany). Water was purified using a Milli-Q Plus system (Millipore, Espoo, Finland). The analytical MON standard was purchased from Sigma (St. Louis, MO, USA; Cat. No. M5269).

The laboratories of the Finnish Food Safety Authority, Evira are accredited according to the ISO 17025 standard.

2.1.2. 2 Chemicals for chemiluminescence

Hanks balanced salt solution, pH 7.4, with 0.1% gelatin (Sigma–Aldrich, St. Louis, USA) (gHBSS) was prepared for the reaction buffer. A stock solution of 10 mM luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma–Aldrich, St. Louis, USA) was prepared in 0.2 mol/L sodium borate buffer, pH 9.0 (1.24 g of H_3BO_3 and 7.63 g of $Na_2B_2O_7·10H_2O$ in 1 L of redistilled water). Zymosan (zymozan A from *Saccharomyces cerevisiae*, Sigma–Aldrich, St. Louis, USA) was suspended (20 mg/mL) in phosphate buffer (pH 7.6), boiled for 20 min and washed three times in HBSS by centrifugation at $250 \times g$ for 7 min (Sorvall TC-6, Sorvall, Newton Ct., US). For opsonization, the suspension was incubated for 60 min at 38 °C in 10% Sprague-Dawley rat serum, obtained from the Central Animal Laboratory of the University of Turku, and washed twice with gHBSS.

2.2. Experimental procedure

The *in vivo* toxicological properties of MON were investigated with an adaptation of OECD guideline 407. The purpose of this study was to provide information on possible health effects arising from repeated exposure to MON over a limited period of time (OECD guideline for the testing of chemicals). All experiments were conducted under a permit from the National Animal Experiment Board of Finland.

In this experiment, 5 dose groups (3, 6, 9, 12 and 15 mg/kg b.w.) of test animals were exposed to MON for 28 days. Each group consisted of 5 male Sprague-Dawley rats. The dose groups were determined based on our acute toxicity study of MON in rats (guideline OECD 423, Jonsson et al., 2013). In addition, a control group administered with filtered tap water and two satellite groups (dosed 12 and 15 mg/kg b.w. MON) were used. The two satellite groups were kept alive for an additional 14 days without treatment to detect possible delayed toxic effects and to follow up recovery.

The Sprague-Dawley rats (Harlan Laboratories Inc., Horst, the Netherlands) used as test animals were 9–10 weeks old and weighed 217–307 g at the beginning of the experiments. The rats were acclimatised to the laboratory conditions and the metabolic cages for one week prior to the onset of the experiment. All animals