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Developmental and reproduction toxicity studies of glycolipids from *Dacryopinax spathularia*

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ABSTRACT

The developmental and reproduction toxicity potential of jelly mushroom glycolipids from *Dacryopinax spathularia* was studied in Crl:CD (SD) rats by daily oral gavage administration at doses of 150, 500 or 1000 mg/kg/day. Pregnant female rats in the developmental study received the test article from Gestation Days 6–19. F_0 and F_1 parental animals in the 2-generation reproduction toxicity study were dosed for a minimum of 70 days prior to mating and throughout mating, gestation, and lactation, until the day prior to euthanasia (following weaning of litters on postnatal day 21). The offspring of the F_0 and F_1 generations were potentially exposed to the test article in utero and via the milk while nursing. In the developmental study, there were no adverse effects on intrauterine growth and survival, or fetal morphology. In the 2-generation reproduction toxicity study, there were no adverse effects on observed parameters including macroscopic or microscopic findings, or organ weights for F_0 or F_1 animals, no effects on reproductive performance, and no test article-related effects on F_1 and F_2 postnatal survival, development, or growth. Therefore, the no-observed-adverse-effect level (NOAEL) for parental systemic toxicity, parental reproductive toxicity, and developmental/neonatal toxicity, was considered to be 1000 mg/kg/day, the highest dosage tested.

1. Introduction

A mixture of glycolipids with antimicrobial properties, herein referred to as “AM-1” or jelly mushroom glycolipids, was obtained via natural fermentation of the edible jelly mushroom *Dacryopinax spathularia* (Schwein.) (Martin, 1948), also known as *Cantharellus spathularius* (Schwein.) and “sweet osmanthus ear mushroom” in China. The components of jelly mushroom glycolipids (CAS RN 2205009-17-0) are structurally-related glycolipid congeners, all sharing a long chain fatty acid (LCFA) backbone and the same trisaccharide moiety. The three main components are depicted in Fig. 1. Components i) and ii) together account for ca. 30–40% of the glycolipids, and component iii) accounts for 15–25% as determined by HPLC-MS analysis. The balance of other glycolipids present in the fermentation product mixture are congeners of the parent components, sharing the same fatty acid and trisaccharide moiety but differing in the acylation pattern.

As reported in Bitzer et al. (2017a,b,c), a series of studies and scientific assessments have been conducted to evaluate the safety of jelly mushroom glycolipids (AM-1) for use as a food ingredient. AM-1 and its ultimate hydrolysis product, LCFA, are poorly absorbed by the oral route and are primarily eliminated in the feces without absorption (Bitzer et al., 2017a). Absorbed components to a large extent appear to be completely metabolized to CO_2 and expired. There were no metabolites of safety concern identified for AM-1 or LCFA and no accumulation of these compounds in tissues (Bitzer et al., 2017a). AM-1 has low potential for systemic toxicity with oral repeated dose (90-day) no-observed-adverse-effect-levels (NOAELs) of ≥1200 mg/kg bw/day in rats (oral drinking water administration) and ≥1000 mg/kg bw/day in dogs (oral capsule administration), the highest dose levels tested (Bitzer et al., 2017b; c). AM-1 was determined to be non-genotoxic based on the results of a complete battery of *in vitro* genetic toxicity assays in bacteria as well as in mammalian cells including human lymphocytes (Bitzer et al., 2018).

The present research was conducted at Charles River Laboratories

Abbreviations: Analysis of variance, ANOVA; Association for Assessment and Accreditation of Laboratory Animal Care, AAALAC; Gestation Day, GD; Institutional Animal Care and Use Committee, IACUC; Lactation Day, LD; long chain fatty acid, LCFA; no-observed-adverse-effect level, NOAEL; Organisation for Economic Co-operation and Development, OECD; Postnatal Day, PND; U.S. Food and Drug Administration, FDA

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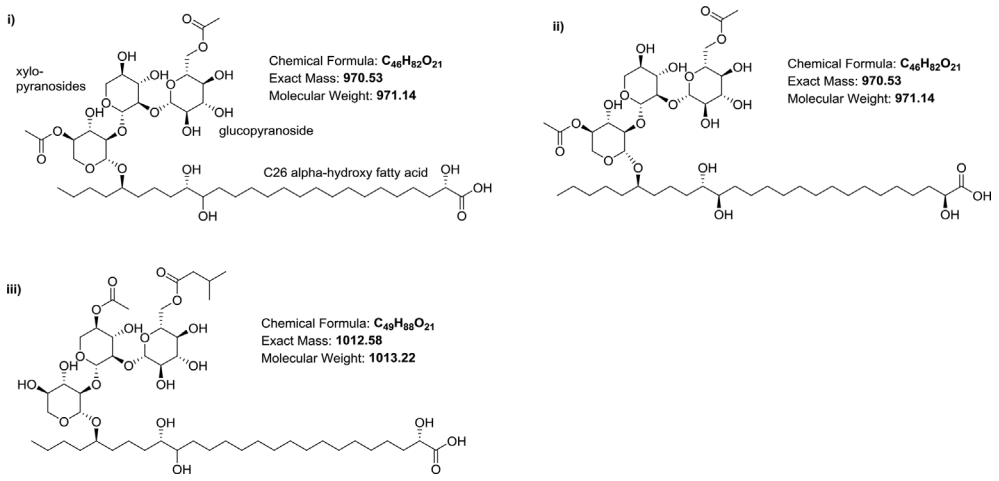

Fig. 1. Representative structure diagrams for main components of jelly mushroom glycolipids (AM-1).

Ashland (Ashland, Ohio) during September–October 2016 for the developmental toxicity study (Study No. WIL-294507) and during 2016–2017 for the reproduction toxicity study (Study No. WIL-294508). The studies were performed in compliance with the U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations (21 CFR Part 58) (FDA, 1987). The protocol for the reproduction toxicity study was in general accordance with the Organisation for Economic Co-operation and Development (OECD) Testing Guideline 416 (Two-Generation Reproduction Toxicity Study) (OECD, 2001a) and the US FDA Redbook Testing Guideline IV.C.9.a (FDA, 2000a). The protocol for the developmental toxicity study was in general accordance with OECD Testing Guideline 414 (Prenatal Developmental Toxicity Study) (OECD, 2001b) and the U.S. FDA Redbook Testing Guideline IV.C.9.b (FDA, 2000b).

2. Materials and methods

2.1. Test article and dose preparation

Jelly mushroom glycolipids (AM-1) was supplied by IMD Natural Solutions, GmbH (INS) (Dortmund, Germany) as a beige powder with 95% glycolipid content as determined by qNMR (the remaining ca. 5% was comprised of residual water, protein, and lipids). Additional details regarding the characterization and composition of the test article are reported in Bitzer et al. (2017c).

2.2. Administration, vehicle, and analysis of dose formulations

The vehicle used in preparation of the test article formulations and for administration to the control group was reverse osmosis-treated water (prepared on-site). The test article AM-1 was orally administered to the test animals via gavage at target concentrations of 150, 500, and 1000 mg/kg/day. The selected route of administration for this study was oral because this is a potential route of exposure for humans.

Dosage levels for the developmental toxicity study were selected based on the results of a previous range-finding study in which pregnant female rats were administered the test article at dosage levels of 150, 500, and 1000 mg/kg/day from Gestation Day (GD) 6–19. In the previous study, a single female in the 1000 mg/kg/day group was found dead on GD 11 following dosing. In the surviving animals, there were no significant effects on body weights, food consumption, reproductive parameters, or fetal weights noted in any dosage group tested. As a result, the same dosage levels of 150, 500, and 1000 mg/kg/day were used for this current definitive developmental toxicity study.

The dose levels for the reproduction toxicity study were selected based on the results of a dose range-finding study in which administration of AM-1 to F_0 male and female rats via oral gavage (500 and 1000 mg/kg/day) or drinking water (1650 and 5500 ppm) resulted in no reproductive toxicity at any dosage/exposure level. At the initial high AM-1 dose level of 11,000 ppm in drinking water, excessive body weight losses correlating to reduced food and water consumption were noted during the first seven days of test article exposure. Reduced consumption of the treated water was attributed to lower palatability likely due to the surfactant properties of the test article at higher concentrations and increased viscosity of the test article solution (Bitzer et al., 2017b). Animals in this group were given a 3-day dosing holiday before dosing was restarted via oral gavage at 500 mg/kg/day for the remainder of the study. Due to the technical challenges and potential indirect effects of drinking water administration, the definitive 2-generation reproduction toxicity study used only oral gavage as the route of test article administration.

The stability of the test article in the vehicle, over the range of concentrations used on each study, was confirmed in a separate study for at least 10 days at room temperature and refrigerated (2 °C–8 °C) storage (data not presented). Concentrations and homogeneity were analytically confirmed as being within the laboratory's acceptance criteria of ± 15% of target concentrations in samples taken from the dosing formulations (data not presented). The test article was not detected or quantifiable in the analyzed vehicle formulations that were administered to the control groups.

2.3. Animals, housing, and environmental conditions

For the developmental toxicity study, sexually mature, virgin female Crl:CD (SD) rats (120), approximately 71–89 days of age, were obtained from Charles River Laboratories, Inc., Raleigh, NC and were acclimated for at least 7 days. Upon arrival, all female rats were housed 2–3 per cage in solid bottom caging with ground corn-cob bedding material. Resident males were untreated, sexually mature rats utilized exclusively for breeding and these rats were maintained under similar laboratory conditions as the females. The rats were paired for mating in the home cage of the male. Following positive evidence of mating, the females were individually housed in clean, solid-bottom cages with bedding material.

For the reproduction toxicity study, sexually mature male and virgin female Crl:CD (SD) rats (110/sex), approximately 30 days of age, were obtained from Charles River Laboratories, Inc., Raleigh, NC and were acclimated for 14 days. All F_0 and F_1 parental animals were housed 2–3 per cage, by sex, from receipt (F_0) or selection (F_1) in solid bottom caging with ground corn-cob bedding material. During cohabitation, the rats were paired (1 female to 1 male) in solid bottom caging with bedding material. Following breeding, F_0 and F_1 parental animals were