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Effects of dietary alpha-linolenic acid-enriched diacylglycerol oil on embryo/fetal development in rats

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ABSTRACT

Recent studies suggest that diets supplemented with alpha-linolenic acid (ALA)-enriched diacylglycerol (DAG) oil provide potential health benefits in preventing or managing obesity. However, available safety information about reproductive and developmental toxicities of ALA-DAG oil is limited. This study was conducted to clarify the effect, if any, of ALA-DAG oil on embryo-fetal development, following maternal exposure during the critical period of major organogenesis. ALA-DAG oil was administered via gavage to pre-mated female Sprague Dawley rats from gestation day 6 through 19, at dose levels of 0, 1.25, 2.5, and 5.0 mL/kg/day (equivalent to 0, 1149, 2325, and 4715 mg/kg/day, respectively), with total volume adjusted to 5 mL/kg/day with rapeseed oil. All females survived to the scheduled necropsy. There were no treatment-related changes in clinical or internal findings, maternal body weights, feed consumption, intrauterine growth, survival, and number of implantations. No ALA-DAG oil-related fetal malformations or developmental variations were noted. A maternal maximum tolerated dose for ALA-DAG oil could not be achieved in this study. Based on these results, a dose level of 5.0 mL/kg (4715 mg/kg/day), the highest dose tested, was considered as the no-observed-adverse-effect level (NOAEL) for both maternal and developmental toxicity.

1. Introduction

Diacylglycerol (DAG) is a natural component found in various edible oils and has been widely consumed for numerous years in the human diet. Furthermore, a high-purity DAG oil (DAG content > 80%) has been enzymatically prepared from plant oils for commercial use (Von Der Haar et al., 2015).

The uniqueness of DAG oil lies in its fatty acid composition and position. As compared to the commonly consumed dietary fat and oils that primarily contains triacylglycerols (TAG) consisting of three fatty acids (D'Alonzo et al., 1982), DAG oil has two fatty acids ester-linked to a glycerol backbone (Yanai et al., 2007). The available evidence from clinical studies suggest that DAG oil has health benefits, such as preventing body fat accumulation (Nagao et al., 2000; Watanabe et al., 1998).

The various fatty acids that attach to the glycerol backbone of the TAG in oil also influence fat utilization. Among the fatty acids, alpha-linolenic acid (ALA) is easier to be metabolized for energy by beta-oxidation as compared to palmitic, stearic, oleic, or linoleic acids in animals (Bessesen et al., 2000; Leyton et al., 1987) and humans (DeLany et al., 2000; Saito et al., 2016). ALA is an essential fatty acid for humans and is naturally present in seeds, vegetable oils, green leafy vegetables, nuts, and beans (Kim et al., 2014). Flaxseed oil, which contains ALA-rich TAG oil (ALA-TAG oil), is one of the main dietary sources of the vegetable, *n*-3 fatty acid, ALA (C18:3). Flaxseed oil, which contains ALA-rich TAG oil (ALA-TAG oil), is a major dietary source of vegetable n-3 fatty acid ALA (18:3) and has been listed as the Generally Recognized As Safe (GRAS) substance by the Food and Drug Administraion (FDA, 2009).

DAG oil consisting mainly of ALA (ALA-DAG oil) has various

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; DAG, diacylglycerol; EPA, eicosapentaenoic acid; FDA, Food and Drug Administration; GD, gestation day; GRAS, Generally Recognized as Safe; NaClO, sodium hypochlorite; NOAEL, no-observed-adverse-effect level; OECD, Organization for Economic Co-operation and Development; S.D., standard deviation; SDG, secoisolariciresinol diglycoside; TAG, triacylglycerol

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beneficial effects attributable to both the DAG oil and ALA. ALA-DAG oil reduced body weight gain in mice (Murase et al., 2002) and suppressed fatty liver formation in Zucker fatty rats (Murase et al., 2005). In humans, ALA-DAG oil was more effective than oleic and linoleic acid-rich DAG oil in terms of postprandial fat oxidation (Ando et al., 2016), reduced visceral fat accumulation without any adverse effects (Saito et al., 2016). In addition to its beneficial effects on human health, the safety of DAG oils (including ALA-DAG), which mainly consist of oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, has been well established in multiple animal and human studies (Morita and Soni, 2009), and also has been listed as a GRAS substance by FDA (FDA, 2000). In the series of safety evaluation of ALA-DAG oil, genotoxic- or carcinogenic-potentials have not been identified (Honda et al., 2016, 2017; Mori et al., 2017). Also, no general toxicity were observed in a 90-day repeated-dose toxicity study (Bushita et al., 2018). However, available safety information about reproductive and developmental toxicities of ALA-DAG oil is limited. Although Morita et al. (2008) reported that DAG oil had no reproductive toxicity, the fatty acid composition of dietary oils could affect the physiologic actions of these oils. This suggests that the mechanisms of action, disposition, and adverse effects may differ between ALA-DAG oil and DAG oil. In addition, there have been conflicting reports of whether fetal numbers and weights are altered by feeding pregnant rats flaxseed (or flaxseed oil) (Collins et al., 2003; Rao et al., 2007; Tou et al., 1998). Therefore, it is important to clarify the effects of ALA-DAG oil on embryo-fetal development in a controlled, developmental toxicity study.

The objective of this study was to investigate the potential adverse effects, if any, on embryo-fetal development in pregnant rats that were fed ALA-DAG oil during the period of major embryonic organogenesis.

2. Materials and methods

2.1. Study design

The study was performed according to a well-designed protocol and in compliance with OECD Principles of Good Laboratory Practice (as revised in 1997) (OECD, 1997). The protocol was designed to be in general accordance with OECD Guidelines for the Testing of Chemicals (No. 414, 2001) (OECD, 2001).

2.2. Preparation of ALA-DAG oil

ALA-DAG oil was prepared by esterifying glycerol with fatty acids obtained from flaxseed oil (ALA-TAG oil) using immobilized lipase, as previously described (Watanabe et al., 2003). The fatty acid and glycerol composition of the test oils are summarized in Table 1. The level of glycidyl fatty acid esters, which are detected as impurities in other DAG oils (Bundesinstitut für Risikobewertung, 2009), was comparable to that in other edible oils (< 0.3 μg/g) (MacMahon et al., 2013). The test oils (Lot no.: 150803) were stored frozen (−22.2 °C to −20.4 °C) and shielded from light in a tight container. After thawing, the oil was stored in a cold place (3.6 °C–6.1 °C) in a tight container, shielded from light.

Table 1
Composition of rapeseed oil, ALA-TAG oil and ALA-DAG oil used for embryo/fetal developmental toxicity study in rats.

	Rapeseed oil	ALA-TAG oil	ALA-DAG oil
Monoglycerols (%)	0.0	0.0	0.7
Diacylglycerols (%)	1.8	2.7	83.1
Triacylglycerols (%)	98.2	97.3	16.2
Saturated fatty acids (%)			
Palmitic C16:0	4.1	4.9	2.2
Stearic C18:0	2.0	3.4	1.2
Unsaturated fatty acids (%)			
Oleic C18:1	62.1	19.0	21.4
Linoleic C18:2	19.7	15.1	16.5
Linolenic C18:3 n-3	9.1	56.7	57.6

2.3. Animals and husbandry

For the prenatal developmental toxicity study, sexually mature Sprague Dawley rats (Crl:CD) were obtained from the Hino Breeding Center, Charles River Laboratories Japan, Inc. The animals were maintained in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Research Council, 2011) and satisfies the criteria of “ARRIVE guidelines” (National Centre for the Replacement Refinement & Reduction of Animals in Research, 2010). The animals were 10–11 weeks old upon receipt and were allowed a 2-week acclimation period (a 6-day quarantine period was included) prior to the start of the study. After acclimation, the rats were individually housed in stainless-steel cages suspended above cage-board. Food (Oriental Yeast Co., Ltd., CRF-1) and water (well water mixed with NaClO, free residual chlorine concentration: approximately 2 ppm) were supplied *ad libitum*. The animals were maintained on a 12-h light/dark cycle (lights on at 7 AM). Environmental conditions were set to maintain a temperature of 23.0 ± 3 °C and a relative humidity of 35–75%.

2.4. Breeding

Following acclimation, 12-13-week-old males and 12-week-old nulliparous females were used for mating. Mating was conducted by housing females one-to-one with males. Successful copulation was confirmed by the presence of a vaginal plug or sperm in vaginal smears noted on the following morning. The day on which copulation was confirmed was defined as “Gestation Day (GD 0)."

2.5. Treatment

Rapeseed oil, ALA-TAG oil, and ALA-DAG oil were administered orally by gavage once daily during GD 6 to GD 19 at doses of Rapeseed oil/0.0 mL/kg ALA-DAG oil (the Rapeseed oil group), ALA-TAG oil (the ALA-TAG oil group), 3.75 mL/kg rapeseed oil/1.25 mL/kg ALA-DAG oil (the 1.25 mL/kg ALA-DAG oil group), 2.5 mL/kg rapeseed oil/2.5 mL/kg ALA-DAG oil (the 2.5 mL/kg ALA-DAG oil group), and 0.0 mL/kg rapeseed oil/5.0 mL/kg ALA-DAG oil (the 5.0 mL/kg ALA-DAG oil group). Thus, the dose of ALA-DAG oil in this study was 0, 1.25, 2.5, and 5.0 mL/kg. The maximum dose of ALA-DAG oil (5.0 mL/kg) was determined based on a practical oral gavage volume, and previous safety data of DAG oil (Morita and Soni, 2009). This fat level is similar to or greater than the mean human fat consumption in the United States (Wright et al., 2004). Each group consisted of 24 bred females. Individual dosages were based on the most recently recorded body weight to provide the correct mL/kg/day dose. All animals were dosed daily in the morning.

2.6. Observations

Animals were observed twice daily throughout the study period for mortality and morbidity. Individual clinical observations were recorded daily prior to dose administration and approximately 1–3 h following dosing. Individual body weights were recorded on GD 0, 3, 6, 9, 12, 15, 18, and 20. Feed consumption was measured on GD 1, 3, 6, 9, 12, 15, 18, and 20.

2.7. Abdominal hysterectomy

On GD 20, euthanasia was performed on all females by exsanguination from the lateral iliac artery under anesthesia by tail vein injection of 30 mg/kg pentobarbital sodium. The thoracic and