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Food and Chemical Toxicology 44 (2006) 1530–1538
www.elsevier.com/locate/foodchemtox

Safety evaluation of a medium- and long-chain triacylglycerol oil produced from medium-chain triacylglycerols and edible vegetable oil

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Received 18 October 2005; accepted 16 April 2006

Abstract

To reduce the incorporation of dietary lipids into adipose tissue, modified fats and oils have been developed, such as medium-chain triacylglycerols (MCT). Typical dietary lipids from vegetable oils, termed long-chain triacylglycerols (LCT), are degraded by salivary, intestinal and pancreatic lipases into two fatty acids and a monoacyl glycerol; whereas, MCT are degraded by the same enzymes into three fatty acids and the simple glycerol backbone. Medium-chain fatty acids (MCFA) are readily absorbed from the small intestine directly into the bloodstream and transported to the liver for hepatic metabolism, while long-chain fatty acids (LCFA) are incorporated into chylomicrons and enter the lymphatic system. MCFA are readily broken down to carbon dioxide and two-carbon fragments, while LCFA are re-esterified to triacylglycerols and either metabolized for energy or stored in adipose tissue. Therefore, consumption of MCT decreases the incorporation of fatty acids into adipose tissue. However, MCT have technological disadvantages precluding their use in many food applications. A possible resolution is the manufacture and use of a triacylglycerol containing both LCT and MCT, termed medium- and long-chain triacylglycerol (MLCT).

This manuscript describes studies performed for the safety evaluation of a MLCT oil enzymatically produced from MCT and edible vegetable oil (containing LCT), by a transesterification process. The approximate fatty acid composition of this MLCT consists of caprylic acid (9.7%), capric acid (3.3%), palmitic acid (3.8%), stearic acid (1.7%), oleic acid (51.2%), linoleic acid (18.4%), linolenic acid (9.0%), and other fatty acids (2.9%). The approximate percentages of long (L) and medium (M) fatty acids in the triacylglyerols are as follows: L, L, L (55.1%), L, L, M (35.2%), L, M, M (9.1%), and M, M, M (0.6%). The studies included: (1) acute study in rats ($LD_{50} > 5000$ mg/kg); (2) 6 week repeat-dose safety study via dietary administration to rats (NOAEL of 3500 mg/kg/day), (3) in vitro genotoxicity studies using *Salmonella typhimurium* and *Escherichia coli* (negative at 5000 mg/plate), and (4) a four-week, placebo-controlled, double blind, human clinical trial utilizing 20 test subjects (no effects at 42 g MLCT/day). These data are corroborated by other studies published in the peer-reviewed literature on analogous MLCTs.

Keywords: Lipase; LD_{50}; Oral; Subchronic; Toxicity; Mutagenicity; Rat; Triacylglycerol; Clinical trial

Abbreviations: 2AA, 2-aminoanthracene; AF-2, 2-(2-furyl)-2-(5-nitro-2-furyl)acrylamide; B(a)P, benzo(a)pyrene; BMI, body mass index; DMSO, dimethylsulfoxide; HDL, high-density lipoproteins; ICR-191, 2-methoxy-6-chloro-9-[3-(20chloroethyl)aminopropylamino]-acridine 2HCl; LCFA, long-chain fatty acids; LCT, long-chain triacylglycerols; LDL, low-density lipoproteins; MCFA, medium-chain fatty acids; MCT, medium-chain triacylglycerols; MLCT, medium- and long-chain triacylglycerol; NaN_3, sodium azide; NOAEL, no observed adverse effect level; SMLCT, structured medium- and long-chain triacylglycerol; VLDL, very low-density lipoproteins.

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doi:10.1016/j.fct.2006.04.004

1. Introduction

Plant and vegetable oils contain triacylglycerols, composed of a glycerol backbone and three fatty acids (Fig. 1). Long-chain triacylglycerols (LCT) may have both saturated and unsaturated fatty acids of a chain length greater than 12 carbons (St-Onge and Jones, 2002; Stein, 1999). Medium-chain triacylglycerols (MCT) are defined as triglyceride esters of only saturated fatty acids with chain lengths of eight to ten carbons (Sucher, 1986). Recent research efforts have shown that MCT may reduce the incorporation and storage of dietary fats and oils into adipose tissue (Tsuiji et al., 2001; Nosaka et al., 2003).

Absorption and metabolism of LCT differ from MCT. Following ingestion, LCT are first acted upon by buccal, gastric, pancreatic, and intestinal lipases to form two free long-chain fatty acids (LCFA) and an sn-2 monoacylglycerol. Absorption of LCFA and sn-2 monoacylglycerols into the intestinal mucosal cells then occurs throughout the small intestine. In the mucosal cells, the sn-2 monoacylglycerol serves as a template for triacylglyceride formation, and the LCFA are converted into acyl-CoAs in the presence of acyl-CoA synthetase, followed by reesterification back onto the sn-2 monoacylglycerol to reform triacylglycerols. Triacylglycerols are then packaged into chylomicrons, which are secreted into the lymphatic system and eventually enter the systemic circulation. In the cell, carnitine is required for transport of the LCFA across the mitochondrial membrane (a rate-limiting step) to be oxidized for energy at the target tissue, or for storage as a triacylglycerol (Bell et al., 1997; Bach and Babayan, 1982).

MCT are processed differently from LCT. After hydrolysis to free fatty acids and glycerol in the small intestine, medium-chain fatty acids (MCFA) do not require micelle-containing bile salts or chylomicron formation, but are directly absorbed into the liver via the portal vein, rather than through the thoracic duct lymphatic system. Reesterification does not occur in the intestinal mucosal cell, and transport of MCFA into the hepatocyte mitochondria occurs via a carnitine-independent mechanism, with the MCFA predominantly oxidized to CO_2, acetate and ketones (Wiley and Leveille, 1973; Schwab et al., 1964; Birkhahn and Border, 1981).

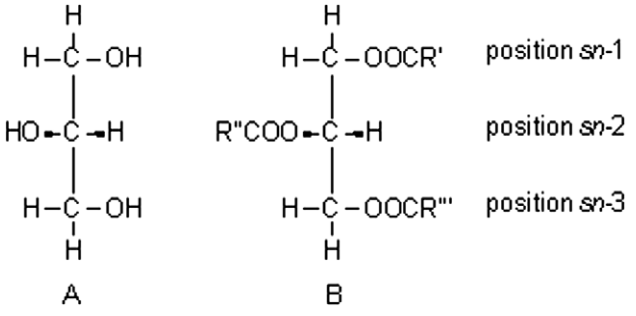

Fig. 1. (A) Glycerol. (B) Triacylglycerol with fatty acids (OOCR) and positions sn-1, sn-2, and sn-3 (Christie, 2005).

In animal studies, rats fed MCT do not gain weight as quickly as rats fed an isoenergetic amount of LCT (Matsuo and Takeuchi, 2004). A possible hypothesis is that MCFA are oxidized predominantly in the liver mitochondria to two-carbon fragments, while LCFA is preferentially incorporated into hepatic lipids for transport to tissues for storage. Very little of the original MCT is stored in adipose tissue (Greenberger and Skillman, 1969). Additional studies indicate that consumption of MCT increases thermogenesis (Mascioli et al., 1991; Seaton et al., 1986). Thus, the resting metabolic disposal of MCT is increased, while fat deposition is diminished, compared to LCT.

A new type of cooking oil has been developed that is composed of both medium- and long-chain triacylglycerols (MLCT), as it is difficult to completely substitute MCT for LCT in food production. MCT's use as a cooking oil is limited by a lower temperature threshold for producing smoke, called the smoke point, than that found for LCT (Matsuo et al., 2001a,b; Kasai et al., 2003). MLCT are structured triacylglycerols that contain MCFA and LCFA in the same triacylglycerol molecule, obtained through lipase or sodium methoxide transesterification of the starting materials, MCT and edible vegetable oil (Kasai et al., 2003; Takeuchi et al., 2002). The fatty acid composition and triacylglycerol molecule composition are presented in Tables 1 and 2, respectively. This transesterification increases the usefulness of MLCT in cooking applications, by raising the smoke point to above 200 °C. The smoke points for MCT, LCT (rapeseed oil), and MLCT are 143, 230, and 210 °C, respectively.

The present studies were conducted to determine the safety of MLCT in pre-clinical studies and in a human clinical trial. The subchronic and clinical studies presented here were previously published individually in Japanese (Noguchi et al., 2002; Nosaka et al., 2002).

Table 1
Fatty acid composition of test oils (Nosaka et al., 2002)

Fatty acid chain length	LCT (g/100 g fatty acids)	MLCT (g/100 g fatty acids)
8:0	ND	9.7
10:0	ND	3.3
16:0	6.2	3.8
16:1	0.2	0.2
18:0	2.5	1.7
18:1	48.8	51.2
18:2	30.2	18.4
18:3	9.4	9.0
20:0	0.6	0.6
20:1	1.1	1.2
22:0	0.4	0.3
22:1	0.2	0.3
24:0	0.2	0.1
24:1	0.2	0.2
Total	100	100

LCT = Long-chain triacylglycerols; MLCT = medium- and long-chain triacylglycerols; ND = not detected.