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A 24-month dietary carcinogenicity study of DAG in mice ☆

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Abstract

This study evaluated the possible carcinogenic effects of DAG (diacylglycerol) oil when given in the diet at levels up to 6.0% for 24 months to mice. Dietary fat was provided by DAG and/or the control article, TG (triacylglycerol oil). Dietary concentrations (% DAG/% TG) were 0%/6.0% (TG control), 1.5%/4.5%, 3.0%/3.0%, and 6.0%/0%. An additional control group received the standard rodent diet (fat content 4.5%).

The clinical condition of the animals, ophthalmic findings, palpable mass occurrence, body weights and gross and histopathologic findings were unaffected by DAG in comparison to TG. The findings in DAG-treated groups were no different than those observed in the TG control group. The standard basal diet had 4.5% fat content. Both TG and/or DAG, when presented separately or together in the diet at a total fat level of 6.0%, resulted in some differences relative to the basal diet control (lower survival, higher body weights, lower food consumption, and higher incidences of macroscopic and microscopic findings), presumably related to the higher dietary fat content and/or the semi-purified diet. However, these parameters were similar in groups fed a diet with 6.0% dietary fat that was either DAG or TG. Thus, DAG at dietary concentrations up to 6.0% for 24 months produced no signs of systemic toxicity and had no effect on the incidence of neoplastic findings.

Keywords: Diacylglycerol; Dietary; Mouse; Systemic toxicity; Carcinogenicity

1. Introduction

An estimated 55% of adults in the United States are overweight or obese (Flegal et al., 1998). Obesity has been associated with a large number of health risks such as heart disease, diabetes mellitus, hypertension, gallbladder disease and some types of cancer [National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 2000; Carey et al., 1997; Lamon-Fava et al., 1996; Rimm et al., 1995]. It has been reported that the proportion of obese persons in a population increases as the total fat content of the culture's diet increases [World Health Organization (WHO), 1990]. Efforts to prevent or treat obesity usually include energy (diet) restriction and increased physical activity. However, the success of these efforts is frequently limited because of poor compliance with energy restriction requirements. It is difficult to control fat energy intake for a prolonged period, because fats and oils are essential nutrients and also important elements influencing the taste and texture of food.

Another approach to limiting body fat accumulation has evaluated the use of cooking oils which are

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metabolized differently than the "standard" cooking oils, composed primarily of triglycerides (TG). Studies have shown that oils composed primarily of diacylglycerol (DAG) are metabolized differently than TG oils and may produce beneficial effects with regard to the prevention and management of obesity (Watanabe et al., 1998; Katsuragi et al., 1999; Yasukawa and Yasunaga, 2001; Matsuo and Tokimitsu, 2001; Yamamoto et al., 2001; Takei et al., 2001; Maki et al., 2002). DAG is a natural component (2–10%) of edible fats and oils from various sources that have been consumed for many years (Abdel-Nabey et al., 1992; D'alanzo et al., 1982). A cooking oil product containing 80% or greater (w/w) of DAG was approved by the authorities in Japan as a "Food for Specified Health Use" in 1999. Studies have shown that a food oil containing mainly DAG prevents or reduces the accumulation of body fat (Katsuragi et al., 1999; Maki et al., 2002; Nagao et al., 2000), but the mechanism for this effect is not known. The USFDA did not have any questions in regard to the submitted GRAS notification for DAG in the United States (United States FDA GRN 00115, 2002).

The chronic effect of DAG consumption in the mouse has not been assessed. The purpose for the present study was to evaluate the potential toxicologic and carcinogenic effects of DAG when administered orally as a macronutrient in the diet for 24 months to mice. This study was based on FDA Redbook guidelines (with regard to the numbers of animals and parameters assessed) and was conducted under Good Laboratory Practices. In this study, two control groups were employed. The first (Group 1) was given standard PMI 5002 rodent diet (which contains 4.5% dietary fat) to provide a link to historical control data in our laboratory. The second control group (Group 2) was given a triglyceride oil (6.0%) at the same fatty acid content as the test article, DAG. In some instances, the TG/DAG treated groups differed from Group 1, but not from each other. The findings were largely non-descript, but as these data may be used in the future for risk assessment purposes, data for all parameters assessed are presented.

2. Materials and methods

2.1. Experimental design overview

After a 15-day acclimation period, DAG and/or the control article, TG, were administered in the diet daily, seven days per week, for up to 104 weeks (the first week of administration was week 0) to the mice. Animal allocation to dosage groups is summarized in Table 1. Clinical observations were recorded daily and detailed physical examinations and palpable masses were recorded weekly. Body weights and food consumption were recorded weekly through week 13 and biweekly thereafter. There were no interim scheduled necropsies. If and when survival in a group fell to 15 mice/sex/group, the sex-group was terminated. If a group was terminated prior to the week 103/104 scheduled necropsy, an effort was made to collect all of the data required by protocol in conjunction with a terminal necropsy. Thus, clinical pathology parameters (hematology and leukocyte counts) were evaluated at week 51 (all groups) and at weeks 92, 96, 98, 103 and 104, but only for the sex-groups terminated during those weeks. Ophthalmic examinations were conducted prior to DAG administration (week −1) and during weeks 51, 92, 98 and 103. Necropsies were performed at study weeks 92 (Group 4 females), 96 (Group 5 males) and 98 (Group 5 females) and on all remaining surviving animals at study week 103/104. Selected organs were weighed from 10 animals/sex/group at the scheduled necropsies. Selected tissues were examined microscopically from all animals.

Table 1
Experimental design of a 24-month dietary carcinogenicity study of DAG in mice

Group	Treatment	Dietary fat/oil level			Number of animals	
		Standard (%)	TG (%)	DAG (%)	Males	Females
1	Control-1[a]	4.5	0	0	50	50
2	Control-2[b]	0	6.0	0	50	50
3	DAG-low[b]	0	4.5	1.5	50	50
4	DAG-mid[b]	0	3.0	3.0	50	50
5	DAG-high[b]	0	0	6.0	50	50

[a] Diet was standard basal diet (PMI Certified Rodent Diet 5002) with 4.5% fat content.
[b] Diet was WIL PMI Certified Rodent Diet 7247/1004740 with 6.0% fat content.

2.2. Test and control articles

The test article, DAG (diacylglycerol oil), and the control article, TG (triacylglycerol oil), were received from Kao Corporation, who confirmed the purity and stability. For dose calculation purposes, DAG and TG were considered to be 100% pure. The fatty acid composition of the oils is shown in Table 2. DAG and TG were stored at room temperature, under nitrogen, protected from light.

2.3. Preparation of test diets

The two feed types used on study are described in Table 3. The test/control article diet admixes were prepared weekly as follows. The appropriate amounts of the test materials were weighed so that the total amount of DAG and/or TG accounted for 6.0% of the total weight for each diet preparation. The appropriate