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Thirteen-week oral dose toxicity study of Oligonol containing oligomerized polyphenols extracted from lychee and green tea

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ABSTRACT

Oligonol is a functional food containing catechin-type monomers and proanthocyanidin oligomer converted from polymer forms *via* a novel manufacturing process. The catechin component of green tea extract has been associated with nasal toxicity in rats following subchronic exposure. To assess the potential for Oligonol to induce nasal toxicity a 13-week repeated oral dose toxicity study was conducted in rats using doses of 100, 300, and 1000 mg/kg/d. Clinical signs and mortality were not affected by Oligonol treatment. Compound-colored stools and an increase in food consumption were observed in some treated groups; however, there were no treatment-related differences in terminal body weights or with respect to the results of the gross postmortem examinations. Histopathological evaluation of the nasal cavity tissues revealed no treatment-related lesions. The results from this toxicity study indicate that Oligonol does not induce nasal toxicity and further supports the results of previous studies demonstrating the safety of Oligonol for human consumption.

1. Introduction

Polyphenols are abundantly found in many plant-derived foods. They are classified based on differences in phenolic acid structure (e.g., flavonoids, stilbenes, and lignans) (Harborne, 1988; Soobrattee et al., 2005). The polyphenols with low molecular weights (oligomers and monomers) function to defend plants from ultraviolet rays (Grace and Logan, 2000) or pathogens (Duzan et al., 2005). Thus, the extracts from plants, and purified polyphenolic products, have been extensively investigated as multifunctional ingredients having antioxidant, antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, anti-thrombotic, antiviral, anti-carcinogenic, vasodilatory, and neuroprotective properties (Middleton et al., 2000; Scalbert et al., 2005; Soobrattee et al., 2005, 2006). The utility of polyphenols for human consumption has been limited by the fact that as plants grow and ripen the concentration of low molecular weight polyphenols decreases due to polymerization into forms that have low bioavailability. While these forms of polyphenols appear to have important prebiotic effects (Lee et al., 2006; Davis and Milner, 2009; Laparra and Sanz, 2010; Fardet, 2010; Vendrame et al., 2011; Landete, 2012) and can be bioavailable following metabolism by gut microflora (Lee et al., 2006; Laparra and Sanz, 2010; Landete, 2012), their beneficial properties could be enhanced through consumption of polyphenols in forms that can be directly absorbed from the gastrointestinal tract (Hackman et al., 2008). Oligonol is a phenolic product derived from lychee fruit (*Litchi chinensis Sonn.*) extract and green tea (*Camellia sinensis*) extract. The polyphenols in Oligonol are mono- or oligomerized through a novel manufacturing process (Tanaka et al., 2007). For instance, proanthocyanidins, which are typical polyphenol polymers contained in various fruits and plants, are altered to oligomeric proanthocyanidins in Oligonol. In addition, Oligonol includes 29% by weight of polyphenol monomers and dimers of catechin, epicatecin, epicatecin gallate, epigallocatechin gallate (EGCG), procyanidin A1, procyanidin A2, procyanidin B1, and procyanidin B2 as reflected by the polyphenol profiles of lychee fruit and green tea (Sarni-Manchado et al., 2000; Ogasawara et al., 2009). As the result of the mono- and oligomerization process, Oligonol is readily absorbed and available for effective utilization.

The beneficial effects, as well as the safety of Oligonol, can be established in human clinical trials. Prior to conduct of such trials, it is necessary to establish the safety of dosing in man through the conduct of subchronic toxicity studies in animals. To this end, an

Abbreviations: ANOVA, one-way analysis of variance; bw, body weight; EGCG, epigallocatechin gallate; GTE, green tea extract; H&E, Hematoxylin & Eosin; IACUC, Institutional Animal Care and Use Committees; LD_{50}, median lethal dose; LFP, lychee fruit extract; LOAEL, lowest-observed-adverse-effect level; MOS, margin-of-safety; NOAEL, no-observed-adverse-effect level; UV, ultraviolet; v/v, volume to volume.

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acute toxicity study in rats and subchronic toxicity studies in rats and mice were conducted with Oligonol. In addition, genetic toxicity studies were performed, including a bacterial reverse mutation assay, and *in vitro* and *in vivo* micronucleus assays (Fujii et al., 2008). There were no adverse effects in each of the acute [median lethal dose ($LD_{50} \geqslant 2000$ mg/kg/d in rats] and subchronic studies [90 day, no-observed-adverse-effect levels (NOAELs) of 1000 mg/kg/d and 200 mg/kg/d in rats and mice, respectively]. Likewise, the results of the genetic toxicity testing demonstrated that Oligonol was without significant genotoxic activity. In addition to the animal data available that support the safety of Oligonol (Fujii et al., 2008), Nishihira et al. (2009) demonstrated that Oligonol intake (10 weeks, 200 mg/d) in a human clinical trial designed to investigate effects on obesity had no adverse effects on serum clinical chemistry parameters including those related to lipid metabolism and liver and renal function. These data indicate that Oligonol produced from lychee fruit and green tea is a safe material.

Hepatotoxicity of the green tea polyphenol EGCG, has been reported to occur in mice exposed to a single bolus dose of 1500 mg/kg body weight (bw)/d or to two consecutive daily doses of 750 mg/kg bw (Lambert et al., 2010), while lower doses of EGCG (500 mg/kg bw/d in the diet to rats and 500 mg/kg bw/d in pre-fed dogs provided as 2 divided doses in capsule form) showed no evidence of toxicity (Isbrucker et al., 2006). Based on the results of the subchronic toxicity studies conducted with Oligonol, there is no indication that treatment is associated with hepatotoxicity, at least up to daily doses of 1000 mg/kg bw/d in rats and 200 mg/kg bw/d in mice.

Recently, Chan et al. (2010) reported that green tea extract administered *via* daily oral gavage to groups of F344/N Tac rats and B6C3F1 mice caused nasal toxicity in both species, with a reported NOAEL of 62.5 mg/kg bw/d in male rats, with no NOAEL [i.e., a lowest-observed-adverse-effect level (LOAEL) of 62.5 mg/kg bw/d] identified in female rats and in mice of both sexes (Chan et al., 2010). This report indicates that the nasal cavity is the most sensitive tissue with respect to the toxicity of green tea catechins. Although the study by Fujii et al. (2008) clearly shows that Oligonol is without hepatotoxicity, which is a known adverse effect of certain green tea polyphenols, given the finding of Chan et al. (2010), it was considered prudent to more closely evaluate the theoretical possibility that Oligonol could cause toxic effects in the nasal cavity since the adverse effect level for green tea extract (LOAEL of 62.5 mg/kg bw/d) occurred at a low dose and given that Oligonol contains approximately 15% green tea derived polyphenols (Ogasawara et al., 2009).

Of note, the components of green tea extract responsible for the reported toxicity to the tissues of the nasal cavity of the rat are unknown. Thus, it is not known if the green tea components contained within Oligonol are those associated with such toxicity, and moreover, it cannot be ascertained whether such compounds may or may not be concentrated within the green tea extract used in the preparation of Oligonol. As a result, since nasal lesions have been reported to occur in rats with other polyphenol-containing extracts such as ginko biloba (NTP, 2013), and given the possible regulatory concerns (Abdel-Rahman et al., 2011), a subchronic toxicity designed to specifically assess the effects of Oligonol on tissues of the nasal cavity in rats was conducted. The results of this study are reported herein.

2. Materials and methods

2.1. Materials

2.1.1. Test article

Oligonol (>95% purity) was used as the test substance. It was produced from lychee fruit (*Litchi chinensis Sonn.*) extract and green tea (*Camellia sinensis*) extract using a patented technology process (international patent WO 2004/103988 AI) at Amino Up Chemical Co., Ltd. (Sapporo, Japan) (Nonaka et al., 2004). Lychee fruit extract (LFP) and green tea extract (GTE) for the production process were provided by Guilin Layn Natural Ingredients Corp. (Guilin, China).

Briefly, dried lychee fruits, one of the richest sources of polyphenols, were extracted with 50% [volume to volume (v/v)] ethanol. The filtrate was evaporated and passed through a DIAION HP-20 column, and eluted with ethanol. The eluate was then evaporated to dryness yielding a dark brown powder which contained a mixture of proanthocyanidins. The lychee extract was mixed with green tea extract, which was then extracted with 50% (v/v) ethanol. Lychee extract and green tea extract comprised about 84% and 16%, respectively, of the Oligonol preparation.

The reaction mixture was heated at 60 °C for 16 h, filtered through a DIAION HP-20 column, washed with water and eluted with 40% (v/v) ethanol. Evaporation of the eluate yielded a reddish brown powder that contained the monomeric and oligomeric proanthocyanidin mixture, Oligonol. The characteristics of the Oligonol preparation used in this study (Batch No. OLF1202S) are presented in Table 1. This material was identical to that which had been previously tested for subchronic toxicity and genotoxic activity (Fujii et al., 2008). The material used in Fujii et al. (2008) was produced by the exact same production process by the same manufacturer. Oligonol tested by Fujii et al. (2008) met the same batch specifications as Oligonol tested in this study (data not shown), and would be comprised of components as described in Table 1.

2.1.2. Animals

Five-week-old male and females Crl:CD (SD) rats were purchased from Charles River Laboratories Japan Inc. (Atsugi, Japan). The study was conducted according to the following good laboratory practices and regulatory guideline; "Good Laboratory Practice Regulation for Nonclinical Laboratory Studies" Notification No. 2009-183, Korea Food and Drug Administration (December 22, 2009); "OECD Principles of Good Laboratory Practice" Organization for Economic Co-operation and Development, ENV/MC/CHEM (98) 17 (as revised in 1997); "OECD Guideline for the Testing of Chemical 408, Repeated Dose 90-day Oral Toxicity Study in Rodents" Organization for Economic Co-operation and Development (Adopted: 21st September 1998). The study was reviewed and approved by the Institutional Animal Care and Use Committees (IACUC) of Biotoxtech. Co., Ltd. based on Animal Protection Act (Enactment May 31, 1991, No. 4379, Revision August 4, 2011, No. 10995) (Approval No.: 120508).

2.2. Animals and housing

Crl:CD (SD) rats (48 males and 48 females) were quarantined and acclimatized for seven days before the study, and general condition and clinical signs were observed daily. On the last day of the acclimation period, the body weight of animals was measured and 40 males and 40 females were selected (males; 190.6–219.2 g, females; 141.7–163.7 g) and assigned to four groups (10 animals/sex/group) in an attempt to equalize mean group body weights. Rats were individually housed in stainless wire mesh cages, and provided public tap water [sterilized by filtration and ultraviolet (UV) irradiation] and pelleted rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet 2918C, Harlan Laboratories Inc., Indianapolis, USA) *ad libitum*. During the study, animals were housed in a facility designed to maintain appropriate environmental conditions (21.0–22.8 °C, 12-h light/dark cycle, 40.0–62.6% humidity, ventilation frequency of 10–15 times/h).