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Effects of green tea catechin on embryo/fetal development in rats

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ABSTRACT

Evidence suggests that the health benefits associated with green tea consumption are related to tea catechins. The objective of this study was to evaluate potential maternal and fetal effects of standardized heat-sterilized green tea catechins (GTC-H). GTC-H was gavage administered to mated female rats from gestation day 6 through 17, at doses of 0, 200, 600, and 2000 mg/kg/day. There were no GTC-H-related deaths or macroscopic findings. During the entire gestation period in the high-dose (2000 mg/kg/day)-treated group and during days 6–9 and 6–18 in the 600 mg/kg/day group, mean body weight gain was lower. Mean feed consumption was lower during gestation days 6–9 in the 600 mg/kg/day group and during gestation days 6–9 and 9–12 in the 2000 mg/kg/day group. Compared to the control group, mean body weights in the 600 and 2000 mg/kg/day groups were up to 5.1% and 7.7% lower during gestation days 9–20. GTC-H administration did not affect mean gravid uterine weights or intrauterine growth and survival. There were no GTC-H-related fetal malformations or developmental variations. Based on the results of this study, the no-observed-adverse-effect level (NOAEL) for GTC-H was 200 mg/kg/day for maternal toxicity, and 2000 mg/kg/day for embryo/fetal development.

1. Introduction

Green tea, made from the dried leaves of *Camellia sinensis*, is one of the most popular beverages consumed around the world. Epidemiological studies suggest that polyphenolic compounds present in tea reduce the risk of a variety of diseases (Setiawan et al., 2001; Zhang et al., 2002; Wu et al., 2003; Jian et al., 2004). The main compounds in green tea are a group of catechins that include catechin, epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epicatechin gallate (ECG), and catechin gallate (CG). Multiple *in vitro* studies on catechins report mechanisms consistent with protection against degenerative diseases (Crespy and Williamson, 2004). Green tea catechins (GTC) have been reported to possess multiple properties such as prevention from cancer (Katiyar and Mukhtar, 1996), hypotensive effects (Henry and Stephen-Larson, 1984), antiviral properties (Nakayama et al., 1993), antioxidative properties (Yoshino et al., 1994), the inhibition of plaque formation (Hattori et al., 1990), antiallergic potential (Kakegawa et al., 1985), and blood glucose-lowering effects (Matsumoto et al., 1993). Additionally, tea catechins have been shown to affect lipid metabolism by reducing triglycerides and total cholesterol (Chan et al., 1999), inhibiting liver and body fat accumulation (Chaudhari and Hatwalne, 1977; Ishigaki et al., 1991; Nagao et al., 2001), stimulating lipid catabolism in the liver (Murase et al., 2002), and enhancing energy consumption (Dulloo et al., 2000; Osaki et al., 2001). Available evidence indicates that long-term consumption of tea catechins may be beneficial for the suppression of high-fat diet-induced obesity by modulating lipid metabolism, could have a beneficial effect against lipid and glucose metabolism disorders implicated in type 2 diabetes, and could also reduce the risk of coronary disease (Crespy and Williamson, 2004). In Japan, green tea with high levels of tea catechin has been marketed under "Foods for Specified Health Use" regulation, for people who are concerned about their body fat.

Tea catechins are water-soluble, colorless substances that impart the bitter and astringent taste characteristic of green teas. As the high polyphenolic and catechin content of tea extracts have inherent bitterness, several investigators have attempted to develop processes to prepare green tea beverages that are acceptable. Additionally, the qualitative and quantitative composition of desirable catechin isomers in beverages depends on heat-sterilization conditions that lead to epimerization of catechins (Seto et al., 1997). During heat-sterilization, approximately half of the tea catechins in green tea beverages are epimerized to catechin, gallocatechin, catechin gallate, and gallocatechin gallate (Chen et al., 2001; Seto et al., 1997; Kim et al., 2007; Ikeda, 2008). Heat-sterilization conditions

Abbreviations: FDA, food and drug administration; GD, gestation day; GRAS, generally recognized as safe; GTC, green tea catechin; GTC-H, heat-sterilized green tea catechin; ICH, International Conference on Harmonization; MHLW, Ministry of Health Labour and Welfare; NOAEL, no-observed-adverse-effect level; OECD, Organization for Economic Co-operation and Development.

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are dependent on features of the beverage, including pH and packaging, such that significant epimerization of catechins occurs in certain types of green tea beverages and not in others (i.e., sports drinks; because of their acidic pH, these types of beverages do not require severe sterilization). Products containing green tea extracts, particularly with high levels of catechins produced using heat-sterilization, are commonly marketed for health benefits. The antioxidant activity of heat-epimerized catechins have been reported to be greater than parent tea catechins (Murakami et al., 2006).

Because of its potential health benefits and increased use, the safety of green tea catechins has been investigated and is supported by several studies, including short-term (Chengelis et al., 2008) and long-term studies in rats (Morita et al., submitted for publication; Takami et al., 2008). In a human study, daily oral administration of standardized and defined green tea polyphenols for 28-days was reported to be safe (Chow et al., 2003). However, some studies raise concerns regarding adverse effects of tea consumption during early pregnancy on the fetus. Wang et al. (2007) reported that *in vitro* exposure of developing rat embryos to EGCG during early organogenesis (gestation day 9.5 through 11.5) was "mildly embryotoxic." In another study, Strick et al. (2000) reported that bioflavonoids, genistein and quercetin (basic ring structure planar similar to catechin), can cause DNA cleavage and chromosome translocation that may lead to infant leukemia (believed to occur *in utero*). In another study, Correa et al. (2000) reported an association between high periconceptional tea consumption and developmental neural tube defects. Inhibition of dihydrofolate reductase activity by tea catechins has been claimed as a link to the increased incidence of spina bifida (Navarro-Peran et al., 2005). Very few studies have systematically explored the developmental toxicity potential of green tea preparations that contain high levels of catechins or their epimers. The objectives of this study were to investigate potential adverse effects of GTC-H on embryo-fetal development following maternal exposure during the critical period of major organogenesis, to characterize maternal toxicity at the exposure levels tested, and to determine a no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity.

2. Materials and methods

2.1. Experimental overview

Green tea catechin preparation GTC-H in deionized water was administered orally by gavage to bred female Crl:CD(SD) rats (25/group) once daily from gestation days 6 through 17. Dosage levels were 0 (control), 200, 600, and 2000 mg/kg/day administered at a dosage volume of 10 mL/kg. All rats were observed twice daily for mortality and morbidity. Clinical observations, body weights, and feed consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations, and developmental variations.

2.2. Study compliance

The study was performed according to a well-designed protocol and in compliance with the United States FDA Good Laboratory Practice Regulations (21 CFR Part 58), October 5, 1987 and the Japanese Good Laboratory Practice Standards, Ordinance No.21 of the Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare (MHLW), March 26, 1997. The protocol was designed to be in general accordance with the International Conference on Harmonization (ICH) Tripartite Guideline on Detection of Toxicity to Reproduction for Medicinal Products, Federal Register, September 22, 1994, Section 4.1.3.

2.3. Test article

The test article (GTC-H) used in the present study was provided by Kao Corporation with confirmation of their purity and stability. GTC-H was prepared from aqueous GTC extract via heat treatment (at 249.8°F (121 °C) under aqueous conditions for 90 min), and a subsequent vacuum freeze-drying procedure. The composition of GTC-H was similar to commercially available green tea beverages currently marketed in Japan under "Food for Specified Health Use" for health benefits. The compositional analysis of catechins from GTC-H is presented in Table 1. The GTC-H preparation used in the present study was similar to our previous 28-day study and a detailed compositional analysis of GTC-H is summarized in our recent publication (Chengelis et al., 2008).

2.4. Preparation of dosing formulations

The test article formulations were (weight/volume) mixtures prepared in deionized water (vehicle) at concentrations of 20, 60, and 200 mg/mL for the 200, 600 and 2000 mg/kg/day groups, respectively. The test article formulations were prepared approximately weekly as single formulations for each dosage level, divided into aliquots for daily dispensation, and stored refrigerated in the dark. Homogeneity and 10-day stability following refrigerated storage of the formulations was demonstrated using an HPLC/UV absorbance detection method.

2.5. Animals and husbandry

One hundred and fifty sexually mature Sprague-Dawley Crl:CD®(SD) female rats, approximately 70 day old at receipt (mean body weight 218 ± 7.8 g), were purchased from Charles River Laboratories, Inc. (Raleigh, NC). The animals were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996). Feed (PMI Nutrition International, Inc., Certified Rodent LabDiet® 5002 in meal form) and water (reverse-osmosis-treated municipal water) were provided *ad libitum*. The animals were maintained on a 12-h light/dark cycle (lights on at 6:00 AM) in an environmentally controlled room set to maintain a temperature of 22 ± 3° C and a relative humidity of 50 ± 20%. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

2.6. Breeding

After a 14-day acclimation period, females were mated with resident males from the same strain and source. The resident males (n = 125) used were approximately 28 weeks old at mating and the mean body weight was 681 ± 70.6 g. The females were approximately 12 weeks old when paired for breeding (mean body weight 246 ± 12.3 g). Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm in a vaginal lavage. Each mating pair was examined daily. The day on which evidence of mating was identified was termed gestation day zero and the animals were separated.

2.7. Treatment

The vehicle and GTC-H formulations were administered orally by gastric intubation once daily during gestation days 6–17. All animals were dosed at approximately the same time (between 11 and 12 AM) each day. The dosage levels of GTC-H were 0 (control), 200, 600, and 2000 mg/kg/day and the dosage volume for all groups was 10 mL/kg. Individual dosages were based on the most recently recorded body weights to provide the correct mg/kg/day dose. Dosage levels were selected based on the results of a preliminary dose range-finding study in which mated female rats were administered GTC-H orally via gavage at 1000, 1500 and 2000 mg/kg/day once daily during gestation days 6–17. Mean maternal body weight losses were noted in all test article-treated groups, however, not in a dose-related manner. These losses resulted in lower mean body weights, net body

Table 1
Composition of green tea catechin preparation used in the embryo/fetal developmental toxicity study in rats.

Composition	Percent (W/W)[a]
Total catechin (Sum of below mentioned 8 catechins)	30.5
Gallocatechin (GC)	6.1
Epigallocatechin (EGC)	4.7
Catechin (C)	1.8
Epicatechin (EC)	1.9
Epigallocatechin gallate (EGCG)	5.9
Gallocatechin gallate (GCG)	6.5
Epicatechin gallate (ECG)	2.2
Catechin gallate (CG)	1.6
Epimerization ratio [Sum of EGC, EC, EGCG and ECG]/Total catechin	0.52
Caffeine	3.5
Polyphenol	46.7

[a] The specifications for total catechin and polyphenol were ">28.0" and ">37.0", respectively. The specifications for the other items listed were "Not defined".