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SCIENCE DIRECT
Toxicology 225 (2006) 48–54
TOXICOLOGY
www.elsevier.com/locate/toxicol

Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis

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Received 3 December 2005; received in revised form 3 May 2006; accepted 4 May 2006
Available online 12 May 2006

Abstract

Fullerenes characterized as an antioxidant are believed to reduce various reactive chemical species, such as free radicals, and their characteristic features have been disclosed to furnish many useful medical technologies. Despite the numerous applications for the biological efficacy of fullerenes, less is known about the toxicity of fullerenes in mammals. Hence, the protocol was designed to determine the acute oral median lethal dose and evaluate the acute toxicity of fullerenes when administrated as a single dose to Sprague–Dawley rats. In an acute toxicity test, fullerenes were administered once orally to a single group of male and female at a dose level of 2000 mg/kg. No deaths were observed and the body weights in both sexes of 2000 mg/kg group increased in a similar pattern to the control group. Genotoxicity of fullerenes was also assessed in a bacterial reverse mutation assay (Ames test) and the chromosomal aberration test in cultured Chinese hamster lung (CHL/IU) cells. Although structural chromosomal aberrations were induced at up to 5000 μg/mL, there was no significant increase in the frequency of chromosomal aberrations at any dose level regardless of presence of S9. Fullerenes did not cause genetic damage in *Salmonella typhimurium* TA100, TA1535, TA98 and TA1537 and *Escherichia coli* WP2*uvrA*/pKM101. These results indicate that fullerenes are not of high toxicological significance.

Keywords: Fullerenes; Acute toxicity; Genotoxicity; Ames test; Chromosomal aberrations; Genetic damage

1. Introduction

Despite a wide range of attempts on the biological potency of fullerenes, less is known about the toxicity in mammals. Fullerene arranging the 60 carbon atoms with 30 carbon–carbon (C–C) double bonds and 60 C–C single bonds as shown in Fig. 1 was discovered in 1985 (Kroto et al., 1985). Intense interest has been focused on this unique molecule having spherical symmetry in the last two decades for the characterization with chemical and biological properties of fullerene (Krusic et al., 1991; Arbogaast et al., 1991; McEwen et al., 1992). Various water-soluble fullerenes were prepared by the use of chemical modification of hydrophobic fullerene to widely explore biological activities. It is reported that several water-soluble fullerenes indicated low cytotoxicity while displaying their biological efficacy both *in vitro* (Tsuchiya et al., 1995; Baierl et al., 1996; Dugan et al., 1997) and *in vivo* (Yamago et al., 1995; Dugan et al.,

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doi:10.1016/j.tox.2006.05.001

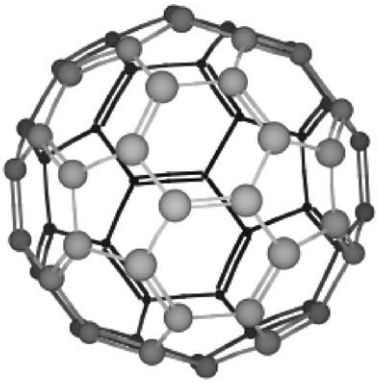

Fig. 1. The structure of fullerene, C_{60}.

1996; Satoh et al., 1997). Fullerene was recognized as a free radical scavenger and water-soluble fullerenes were proved to reduce the level of intracellular peroxidation (Xiao et al., 2005). Attempts for pharmaceutical technologies with a series of water-soluble fullerenes have been explored for their potential as anti-HIV (Schinazi et al., 1993; Toniolo et al., 1994; Rajagopalan et al., 1996) and anticancer (Chiang et al., 1995; Miyata and Yamakoshi, 1997) agents. Quite recently, Moussa have reported that pristine C_{60} is a powerful liver-protective agent against free-radical-mediated liver injury (Gharbi et al., 2005).

For the promising pharmacotherapeutic application, sufficient data regarding *in vitro* and *in vivo* toxicological studies of fullerenes should be accumulated. Thus, we focused our attention on the mixture of C_{60} and C_{70}, a more practical industrial composition of fullerenes (or fullerite), and we have designed a protocol to determine the acute oral median lethal dose and to investigate the acute toxicity of fullerenes.

2. Materials and methods

2.1. A single dose oral toxicity study of fullerenes in rats

Fullerenes (the mixture of C_{60} and C_{70}, fullerite), sublimed technical grade, purity: 99.5%, were supplied from Vitamin C60 BioResearch Corp. (Tokyo, Japan).

2.1.1. Animals

Male and female Sprague–Dawley rats (Charles River Japan Inc., Shinyokohama, Japan) at 6 weeks old were used in this study. The Sprague–Dawley rat is generally recognized as appropriate for acute oral toxicity studies and considerable historical data are available. They were housed in a controlled atmosphere with a temperature of 22.3–24.4 °C and mean relative humidity of 43.7–65.6% with a 12-h light–dark cycle (lights on at 7:00 a.m.). Sterilized animal chow (Oriental Yeast Co. Ltd., Tokyo, Japan) and water were provided *ad libitum*. After being quarantined and acclimated to the environmental conditions for 7 days, animals found to be in good health were randomly allocated to groups on a body weight basis. This study was conducted in compliance with the guiding principles for the care and use of laboratory animals in the Japanese Pharmacological Society and the guidelines of animal care in our laboratories, as approved by the Tokyo Women's Medical University Committee on animal care and use.

2.1.2. Administration of fullerenes

Fullerenes were administered once orally at a dose level of 2000 mg/kg to male and female rats. The control group that received an only vehicle was also arranged. The rats fasted for approximately 18 h prior to the initiation of treatment were administered using a disposable syringe with a gastric tube for rats, and were restrained from feed intake for 3 h after administration. Polyalkylsulfonated C_{60} derived from the test substance, unmodified fullerene, is known to indicate low toxicity (Chen et al., 1998). Therefore, a single dose toxicity of the test substance was anticipated to be similarly low toxicity. Thus, the dose was set at one level of 2000 mg/kg, which was the upper limit dose specified by the applied test guideline; for toxicity studies of drugs: Notification no. 24 of the First Evaluation and Registration Division, MHW's PAB (Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, Tokyo, Japan) dated September 11, 1989. Revisions of guidelines for single and repeated dose toxicity studies: Notification no. 88 of the Pharmaceuticals and Cosmetics Division, MHW's PAB dated August 10, 1993. Fullerenes were suspended by a dropwise addition of a small amount of the vehicle (0.5% (w/v) CMC–Na aqueous solution including 0.1% (w/v) Tween 80) in a volume 10 mL/kg. All animals were observed at 10 and 30 min, and 1, 3, and 6 h post-dose on day 0, and once daily thereafter for 14 days.

2.2. Statistical analysis

In the statistical analysis of the body weight data, the variance among groups was first determined by F test at a significance level of 5%. When no difference in variance was found, data was analyzed using Student's t test and P value was set at significance levels of 1 and 5%. If the variance was present, Aspen–Welch's t test was applied at two significance levels of 1 and 5%.

2.3. Ames test

Mutagenicity of the test substance, fullerenes, were assessed in a bacterial reverse mutation assay using *Salmonella typhimurium* TA100, TA1535, TA98 and TA1537 and *Escherichia coli* WP2*uvrA*/pKM101. The test was conducted by the pre-incubation method in the presence and absence of S9 mix. A dose-finding test was conducted at doses of 1.5, 5, 15, 50, 150, 500, 1500 and 5000 μg/plate. Based on the