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Acute toxicity, twenty-eight days repeated dose toxicity and genotoxicity of vanadyl trehalose in kunming mice

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ABSTRACT

A new trend has been developed using vanadium and organic ligands to form novel compounds in order to improve the beneficial actions and reduce the toxicity of vanadium compounds. In present study, vanadyl trehalose was explored the oral acute toxicity, 28 days repeated dose toxicity and genotoxicity in Kunming mice. The Median Lethal Dose (LD_{50}) of vanadyl trehalose was revealed to be 1000 mg/kg body weight in fasted Kunming mice. Stomach and intestine were demonstrated to be the main target organs of vanadyl trehalose through 28 days repeated dose toxicity study. And vanadyl trehalose also showed particular genotoxicity through mouse bone marrow micronucleus and mouse sperm malformation assay. In brief, vanadyl trehalose presented certain, but finite toxicity, which may provide experimental basis for the clinical application.

1. Introduction

Vanadium acts as a transition element which is distributed on earth and is present in animals and higher plants. Vanadium may be a micro nutrient and contained in plenty of foods, such as soybean, sesame, olive oil, spinach, and shellfish (Roy et al., 2015). It has been demonstrated that vanadium shows interesting biological and pharmacological properties. Vanadium has been used in clinical practice as promising antitumor drugs to treat and protect against cancer (Bishayee et al., 2010; Suwalsky et al., 2013; Wu et al., 2014). Vanadium lowers serum cholesterol, triglycerides, and glucose. It also contracts blood vessels, and enhances oxygen affinity of hemoglobin and myoglobin (Mukherjee et al., 2004). In particular, vanadium presents insulin-mimetic activity both *in vivo* and *in vitro* studies (Heyliger et al., 1985; Meyerovitch et al., 1991; Shechter, 1990). Many researchers have focused on the investigation of the mechanism involved in vanadium insulin-mimetic activity. It has been reported that vanadium enhances the use efficiency of glucose by promoting the expression and translocation of glucose transporter (Clausen et al., 1981; Paquet et al., 1992; Tsiani et al., 1998), stimulates glycolysis by improving the activity of hexokinase (Tolman et al., 1979), restrains the process of gluconeogenesis by inhibiting the glucose-6-phosphatase (G-6-pase) and phosphoenolpyruvate carboxykinase (PEPCK) (Brichard et al., 1993), activates the insulin signaling pathway by suppressing the protein tyrosine phosphatase1B (PTP1B) (Meyerovitch et al., 1991; Tracey, 2000; Winter et al., 2005), and directly regulates the insulin signal transduction by activating the protein tyrosine kinase (PTK) (Sekar et al., 1996).

However, studies have revealed that some obvious toxic effects including nose bleeding, decreased body weight gain and gastrointestinal side effects like diarrhea and dehydration appeared in animals treated with vanadium (al-Bayati et al., 1989; Meyerovitch et al., 1987; Domingo et al., 1995). In addition, it has been well documented that vanadium and its compounds could induce organ and tissue injury, blood toxicity, oxidative stress or damage,

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mitochondrial dysfunction or injuries, reproductive toxicity (embryotoxicity and teratogenicity), genotoxicity, cytotoxicity, neurobehavioral injury or neurotoxicity and inflammatory responses (including pharyngitis, rhinitis, chronic productive cough, bronchopneumonia and tracheobronchitis) *in vivo* and *in vitro* of both human and animal (Imura et al., 2013; Ousterhout and Berg, 1981; Paternain et al., 1990; Cortizo et al., 2000; Li et al., 2013; Hosseini et al., 2013; Villani et al., 2007; Afeseh Ngwa et al., 2009; Kurt et al., 2011; Cuesta et al., 2011; Dinoeva, 1982; Cui et al., 2011; Ray et al., 2006; Kleinsasser et al., 2003; Leopardi et al., 2005). Although toxic effects have largely been reported from inorganic forms, organic vanadium compounds have been associated with therapeutic effects. This may be due to the increased lipophilicity and gastrointestinal absorption (Wang et al., 2001). Thus, organic vanadium compounds have become a highlight.

Vanadyl trehalose, a new organic vanadium compound, was synthesized by Barrio et al. who also demonstrated its insulin-mimetic activities in osteoblast-like cells in culture (Barrio et al., 2003). Recently, we also synthesized vanadyl trehalose in similar ways and have shown that vanadyl trehalose could regulate the blood glucose level and relieve the diabetic symptoms of polydipsia, polyphagia, polyuria, and weight loss in Kunming (KM) mice (Jiang et al., 2016).

In the current investigation, we aimed to evaluate the toxicity of vanadyl trehalose by establishing the acute oral toxicity study, performing mouse bone marrow micronucleus assay and mouse sperm malformation assay, and carrying out a 28-day repeated oral dose toxicity study. The results might provide an experimental basis for the application of vanadyl trehalose in clinical therapy.

2. Materials and methods

2.1. Materials

All chemical reagents used were of analytical grade. Vanadyl sulfate (purity: 99.9%) was purchased from Alfa Aesar (Tianjin); trehalose was acquired from Ximake Biotech (Tianjin); ethanol was purchased from Tianjin chemical reagent factory.

2.2. Animals

Kunming (KM) mice were provided by the Laboratory Animal Center of the Academy of Military Medical Sciences of China (animal license number SCXK-2007-005). Mice were housed in a room with a 12:12 h artificial light cycle, a temperature of 20 °C ± 2 °C, and a humidity of 50 ± 5%. All mice were fed with a standard diet and raised an adaptive training for a week. All procedures were conducted in accordance with the guidelines of the Chinese Council on Animal Care as well as the University of Nankai Animal Care Committee.

2.3. The synthesis of vanadyl trehalose

The synthesis of vanadyl trehalose was conducted according to the method of Barrio et al. (2003). The aqueous solution of vanadyl sulfate (2 mmol) was dropped to a solution of trehalose (4 mmol, 10 mL). The pH value of the liquor was adjusted to 13 by an addition of NaOH (2 M). The brown miscible liquid was sealed for 12—24 h at room temperature. A microcrystalline powder was formed as the addition of absolute ethanol. The solid was filtered and washed with absolute ethanol. Then, the hygroscopic sodium salt was dried and stored in a desiccator.

IR spectra was recorded in KBr (potassium bromide) pellets with a Fourier transform infrared (JASCO FTIR-4100) spectrophotometer.

2.4. Acute toxicity study

Acute toxicity study of vanadyl trehalose was performed following the guidelines of the Organization for Economic Co-operation and Development (OECD) for testing of chemicals, TG 423 (adopted-December 2001) with minor modifications. We took

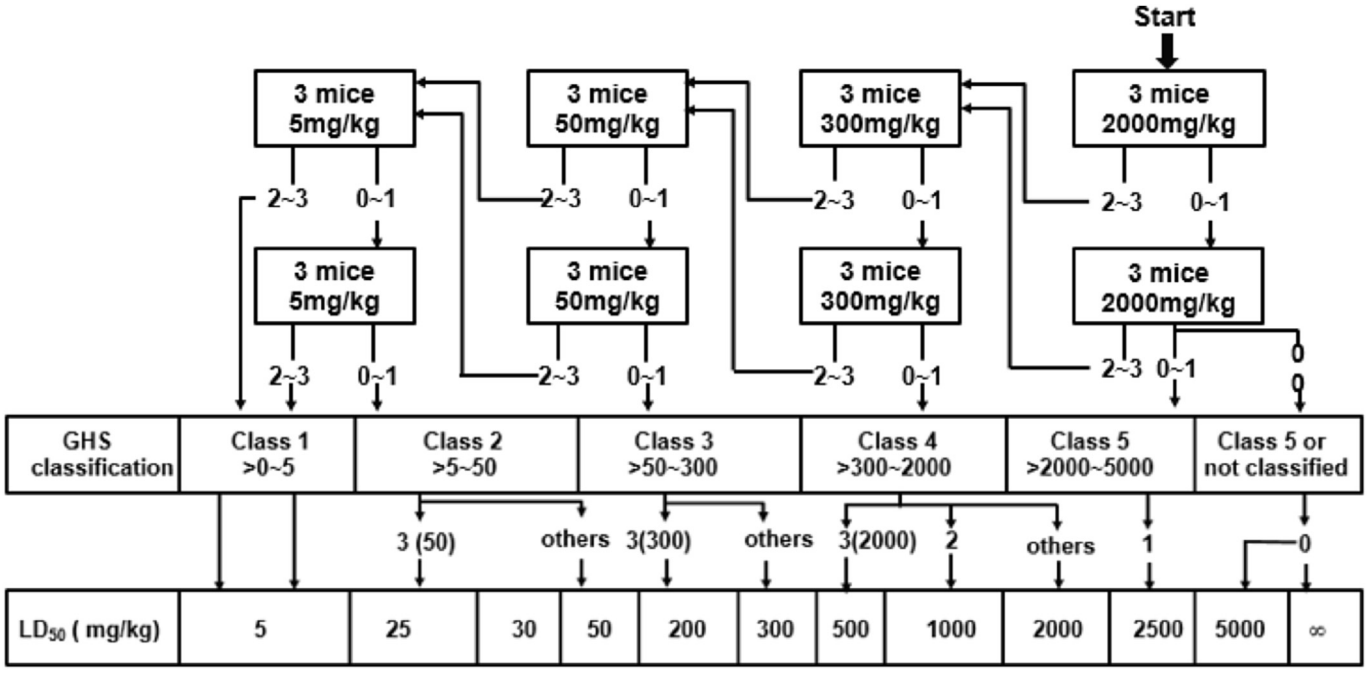

Fig. 1. The process of acute toxicity study. Number 0, 1, 2, 3, mortality of mice at each step. GHS, Globally Harmonized System of Classification and Labelling of Chemicals (mg/kg BW).