Available online at www.sciencedirect.com
ScienceDirect
Journal of Trace Elements in Medicine and Biology 22 (2008) 59–65
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

TOXICOLOGY

Acute effects on the lung and the liver of oral administration of cerium chloride on adult, neonatal and fetal mice

Masami Kawagoe[a], Kiyoshi Ishikawa[a,b], Shou-Cun Wang[a,b], Kiwamu Yoshikawa[a,b], Szilvia Arany[a,b], Xiao-Ping Zhou[a,b], Jing-Shu Wang[a], Yasuharu Ueno[a], Yukio Koizumi[a], Takashi Kameda[a], Souichi Koyota[a], Toshihiro Sugiyama[a,*]

[a]*Department of Biochemistry, Akita University School of Medicine, Akita 010-8543, Japan*
[b]*Akita University Venture Business Laboratory, Akita 010-8502, Japan*

Received 12 June 2006; accepted 24 August 2007

Abstract

We evaluated tissue changes associated with cerium chloride administration via gavage to adult mice, via milk to neonatal mice and transplacentally to fetal mice. Change in adults consisted of extensive pulmonary hemorrhage, pulmonary venous congestion, thickened alveolar septae, hepatic necrosis and neutrophil infiltrations. Those in fetal mice consisted of pulmonary and hepatic congestion. These results indicate that gavage cerium administration elicited subtle tissue changes, though oral toxicity is rather low. These changes were less severe in neonatal and fetal mice. When cerium was injected into adult mice through the tail vein, cerium was distributed mainly to the liver, spleen and lung dose-dependently with the cerium concentration gradually decreasing after 3 days. A study of cerium anticoagulation in mouse plasma showed that clotting time was significantly prolonged when cerium was added to plasma. These results suggest that cerium may disturb blood coagulation and cause pulmonary and hepatic vascular congestion.

Keywords: Cerium; Hemorrhage; Fetus; Neonate; Clotting time

Introduction

Rare-earth elements such as cerium, a lanthanide, have been used for ceramics, fluorescent materials, abrasives, magnets, etc. in high-tech industries [1]. The role of cerium in medicine has been as an antiseptic agent in burn treatment [2], nanoparticles doping for hyperthermia [3] and large-scale affinity separation of proteins [4]. The use of rare-earth elements has attracted interest in their metabolism and toxicity, triggering studies associating these agents with low toxicity following intraperitoneal [5], intravenous [6,7] and oral administration [8–10] to many experimental animal species. These studies involved tissue absorption, accumulation and distribution [11–13].

One such study showed that cerium crossed the placental barrier in late gestation and was transferred through lactation [14,15]. None of these studies, however, microscopically evaluated of potential-related tissue change.

As an environmental pollutant cerium is assumed to be absorbed by the human body orally or percutaneously

*Corresponding author. Tel.: +81 18 884 6074; fax: +81 18 884 6443.
E-mail address: sugiyama@med.akita-u.ac.jp (T. Sugiyama).

0946-672X/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jtemb.2007.08.003

with initial changes occurring in the lungs and liver. Exposure to dust containing rare metals such as cerium for 18 months was reported to cause human pneumoconiosis [16]. Biochemical analysis is especially difficult with embryonic organs. Microscopic observation of growth from an embryo to an adult most effectively measures changes to organs following cerium administration.

Using light microscopy, we evaluated representative tissues for potential changes associated with single-dose gavage cerium administration to adult mice and the effects of such treatment in fetal and neonatal mice.

We measured cerium distribution in tissue after tail vein injection with different doses of cerium. We report the effects of cerium on blood anticoagulation causing pulmonary and hepatic vascular congestion.

Materials and methods

Materials

Cerium chloride ($CeCl_3 \cdot 7H_2O$) was purchased from Wako Chemical Industries Ltd. (Osaka, Japan).

Animals

ICR mice purchased from Japan SLC (Shizuoka, Japan) were treated based on guidelines set by the Animal Committee of the Akita University School of Medicine, which approved protocols for animal experimentation. Feed and water were provided *ad libitum*. Mice were mated at night, and finding of a vaginal plug at noon on the next day was considered 0.5 days of gestation. At least three animals were analyzed at each stage.

Cerium administration by gavage

Cerium chloride in physiologic saline was administered by gavage to adult (6–10 weeks) male and female mice in a single dosage of 200 or 500 mg/kg body weight. Five mice in the control group were administered saline solution. Mice were sacrificed using cervical dislocation. Adult mice (three per group) were necropsied on days 1, 3 or 7 after gavage administration. This was also done in neonatal mice involving a single gavage dose of 200 mg/kg body weight to dams immediately after parturition. Fetal mice were sacrificed on day 14.5 of gestation 3 days after dams were given a single gavage dose.

Injection through tail vein

Adult (6–10 weeks) male mice were used similarly for intravenous injection. Control mice were injected with 5% glucose. Cerium solutions prepared with glucose were injected at 10 and 25 mg/kg body weight through the tail vein. The liver, spleen and lung were then removed and parts placed in neutral buffered formalin for histopathological study. The remainder was used to analyze cerium concentration.

Histology

Tissues were fixed overnight in a cold mixture of 95% ethanol and acetic acid (99:1 v/v), dehydrated in graded ethanol, cleaned in xylene and embedded in paraffin and 5 μm sections cut, dewaxed and stained with hematoxylin and eosin.

Analysis of cerium concentration

Portions of excised organs (0.05–0.1 g) were digested with nitric acid using a hot plate. The digest sample was diluted with 1 N HNO_3 solution. Content of cerium and cadmium were calculated using a calibration curve obtained with standard solutions prepared by $Ce(NO_3)_3$ in 1 mol/L HNO_3 and $Cd(NO_3)_2$ in 0.1 mol/L HNO_3 (Wako Pure Chemical Industries Ltd., Osaka, Japan). Content of cerium and cadmium in the 1 N HNO_3 solution was measured by ICP–AES (SPS3000, Seiko Epson Co. Ltd., Nagano, Japan).

Prothrombin time (PT)

Mouse blood was collected using a 3.8% sodium citrate solution (SCS) and centrifuged at 3000 rpm for 10 min into plasma and clots. Adding thromboplastin and calcium ions to plasma triggered a coagulation cascade. PT depends on the comprehensive activity of extrinsic pathway factors IV, VII, X, V, II and I, i.e., total coagulation cascade activity by extrinsic pathways.

Statistics

Data were assessed by analysis of variance (ANOVA). If significance was found in ANOVA, group means were compared using Student's *t*-test. The value of p for statistical significance was 0.05. Each measurement is expressed as mean $\pm$ SEM.

Results

Effects of cerium on adult mice

Among mice administered the cerium solution at 500 mg/kg body weight, 60% died within 48 h of oral administration. A second group of 12 mice administered