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Letter

Localization of the trichloroethylene-related compound *S*-(1, 2-dichlorovinyl)-*L*-cysteine in mouse cartilage

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ABSTRACT — *S*-(1, 2-Dichlorovinyl)-*L*-cysteine (DCVC) is derived from trichloroethylene (TCE) and known to cause renal cell injury after metabolic activation by cysteine conjugate *β*-lyase. We examined the *in vivo* disposition of [^{35}S] DCVC in mice after intraperitoneal administration at a dose of 30 mg/kg (2.42 MBq/6 mg/mL). DCVC and its related-substances were absorbed rapidly and distributed highly in the kidneys. Whole-body autoradiography analyses revealed high accumulation of DCVC and its related-substances in hyaline cartilage of growth plates of the femoral epiphysis, vertebral endplates of the spinal column, costal cartilage, and tracheal cartilage, in addition to the kidneys. These findings suggest that TCE and DCVC are likely to be a cause of damaged cartilage.

Key words: DCVC, Trichloroethylene, Cartilage, Autoradiography, Mouse

INTRODUCTION

Trichloroethylene (TCE) is a synthetic material generally used as an alternative fluorocarbon or metal-degreasing agent. It is a Class II Specified Chemical Substance and therefore controlled under environment conservation guidelines. Several studies revealed that TCE is toxic to mice, rats and humans (Cichocki *et al.*, 2016; Maltoni *et al.*, 1988). Furthermore, animal studies demonstrated that TCE is a carcinogen for the liver and kidney in animals (Maltoni *et al.*, 1988).

TCE is metabolized *via* several pathways, including glutathione-dependent metabolism, through which it is converted to *S*-(1, 2-Dichlorovinyl)-*L*-cysteine (DCVC) (Cummings and Lash, 2000). DCVC is considered to cause renal cell injury after metabolic activation by cysteine conjugate *β*-lyase (Cummings and Lash, 2000; Tateishi *et al.*, 1978; Tomisawa *et al.*, 1986). Current understanding of the mechanism of DCVC-induced renal injury derives primarily from studies on isolated proximal tubular cells (Cummings and Lash, 2000; Lash *et al.*, 2014; Xu *et al.*, 2008) or subcellular fractions of renal cortical homogenates from rats, rabbits, and other non-human mammals (Davis and Petry, 1994; Lash *et al.*, 2001; Wolfgang *et al.*, 1990). We also reported subchronic nephrotoxic effects of DCVC in mice (Shirai *et al.*, 2012). Furthermore, we demonstrated that DCVC adversely affects the proliferation and differentiation of cultured osteoblasts, osteoclasts, and chondrocytes (Takamoto *et al.*, 2008). However, little is known about the *in vivo* and *in vitro* effects of DCVC on hard tissues.

Cartilage, a resilient hard tissue that is smooth and elastic, covers and protects the ends of long bones at the joints and serves as a structural component of the rib cage, ear, nose, bronchial tubes, intervertebral discs, and many other body components. Cartilage is composed of chondrocytes that produce a large amount of collagenous extracellular matrix. The matrix of cartilage is composed of glycosaminoglycans, proteoglycans, collagen fibers and elastin. In general, cartilage can be classified into

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three types (hyaline, elastic, and fibrocartilage) that differ with regard to the relative amounts of collagen and proteoglycan. Hyaline cartilage is found in the trachea, parts of the joint, and the ribs. Several diseases, including chondrosarcoma, chondrodystrophy, osteoarthritis, and relapsing polychondritis, involve in hyaline cartilage tissues. In the present study, we found DCVC and its-related substances accumulate in hyaline cartilage in mice. Our findings suggest that DCVC is a significant cause of cartilage damage.

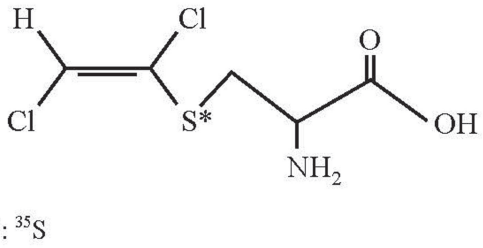

Fig. 1. Structure of DCVC.

MATERIALS AND METHODS

Materials

DCVC (see Fig. 1) was synthesized from TCE and *L*-cysteine as described previously (McKinney *et al.*, 1959), with a purity of 95% or greater. [^{35}S]DCVC was synthesized from TCE (Junsei Chemical Co., Ltd., Tokyo, Japan) and [^{35}S]*L*-cysteine (American Radiolabeled Chemicals Inc., St. Louis, MO, USA; purity > 99%) *via* Birch reduction (Hayden *et al.*, 1987) and suspended in physiologic saline at 2.42 MBq/6 mg/mL.

Pharmacokinetics of DCVC

A suspension of [^{35}S]DCVC (radiochemical purity: 98.6%) was administered intraperitoneally to three 6-week-old non-fasted male Balb/c mice (Charles River Laboratories Japan, Inc.) at a dose of 12.1 MBq/30 mg/kg. At 24 hr after administration, the mice were sacrificed by inhalation of carbon dioxide under isoflurane anesthesia, and the tissues were collected. Samples were analyzed for radioactivity by liquid scintillation counting after dissolution with SOLUENE-350 (PerkinElmer, Inc., Tokyo, Japan). The concentration of radioactivity in the tissues (μg equiv./g or mL) was then calculated.

Whole-body autoradiography

Mice were sacrificed by inhalation of carbon dioxide under isoflurane anesthesia at 24 hr after intraperitoneal administration of [^{35}S]DCVC at a dose of 12.1 MBq/30 mg/kg. Whole-body sections were prepared using a cryomicrotome (Leica Microsystems, Germany) and exposed to imaging plates (GE Healthcare, USA) for 16 hr to obtain autoradiograms using an image analyzer (GE Healthcare, USA). The distribution of radioactivity in the tissues was carefully determined from the autoradiograms. Animal protocols and procedures were approved by the Institutional Animal Care and Use Committee of Nemoto Science Co., Ltd.

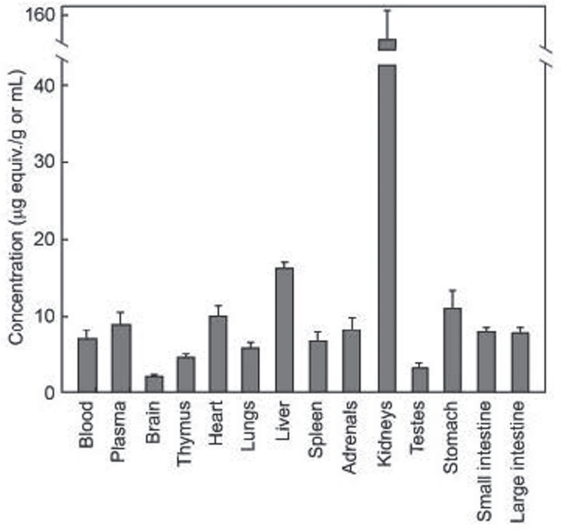

Fig. 2. Concentration of radioactivity in the tissues of male mice at 24 hr after a single intraperitoneal administration of [^{35}S]DCVC at a dose of 12.1 MBq/30 mg/kg. Each bar and vertical line represents the mean and SD for three animals.

Statistical analysis

Numerical data are expressed as the mean ± S.D. of the results from three samples. Experiments were repeated independently in triplicate, and the results were qualitatively identical in every case.

RESULTS

Distribution of radioactivity in mouse tissues

At 24 hr after intraperitoneal administration of [^{35}S]DCVC at a dose of 30 mg/kg, the concentration of radioactivity was highest in the kidneys (154.7 ± 6.0 μg equiv./g). As shown in Fig. 2, levels of radioactivity in the liver (16.2 ± 0.8 μg equiv./g), stomach (11.1 ± 2.3 μg equiv./g), and heart (10.0 ± 1.4 μg equiv./g) were low.

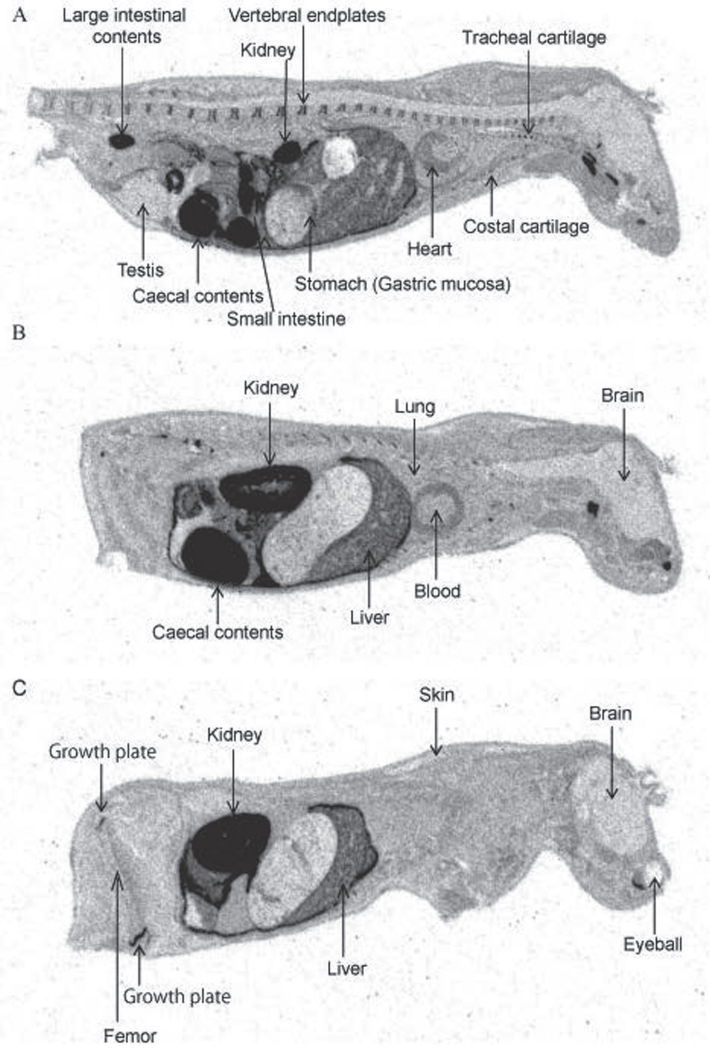

Fig. 3. Whole-body autoradiogram of a male mouse at 24 hr after a single intraperitoneal administration of [^{35}S]DCVC at a dose of 12.1 MBq/30 mg/kg. A, the medial position; B, the left paramedial position; C, left position.

The concentrations in the other tissues were less than that in plasma (8.0 μg equiv./g or mL).

Whole-body autoradiography

We performed whole-body autoradiography to determine the localization of [^{35}S]DCVC and its related-substances in mouse organs. As shown in Fig. 3A (medial position) and Fig. 3B (left paramedial position), the highest amounts of DCVC and its related-substances were found in the kidney, with moderate levels in the liver, stomach, and heart, similar to the distribution of radioactivity in the tissues (Fig. 2). Interestingly, the medial position autoradiograph (Fig. 3A) also showed high levels of DCVC and its related-substances localized at the vertebral endplates of the spinal column and in the costal cartilage and the tracheal cartilage. Furthermore, as shown in Fig. 3C (left position), DCVC and its related-substances accumulated at high levels in the growth plate of femoral epiphysis.

DISCUSSION

We found that DCVC and its related-substances localized in high amounts in the hyaline cartilage at growth

plates of the femoral epiphysis, vertebral endplates of spinal column, costal cartilage, and tracheal cartilage at 24 hr after intraperitoneal administration in mice. Sulfur-containing conjugates can be classified into three types based on mechanism of toxicity, and DCVC is classified as type II. DCVC is derived from TCE that is generally known as an environmental pollutant and metabolized to unstable 1, 2-dichlorovinyl thiol and chlorothioketene by cysteine conjugate *β*-lyase in the liver or kidneys (Chiu *et al.*, 2006; Lash *et al.*, 2000). It is unclear from our results whether DCVC or one of its related-substances localized in the hyaline cartilage. Furthermore, whether other cysteine conjugate compounds localize in hyaline cartilage similar to DCVC should be examined in future studies.

As cartilage does not contain blood vessels or nerves, it receives nutrition *via* diffusion. How and where in the hyaline cartilage DCVC and its related-substances localize is also a topic of future research. If DCVC was non-cytotoxic, it could potentially be used as the tool for delivering drugs to cartilage. However, TCE and DCVC are reportedly strong cytotoxicity (Cummings and Lash, 2000; Davis and Petry, 1994; Lash *et al.*, 2014; Lash *et al.*, 2001; Maltoni *et al.*, 1988; Shirai *et al.*, 2012; Wolfgang *et al.*, 1990; Xu *et al.*, 2008). Our previous study (Takamoto *et al.*, 2008) also showed that the TCE metabolite DCVC significantly reduced the viability of chondrogenic cells ATDC5 at a concentration of 10 μM. The accumulation of DCVC in cartilage could lead to cancer that causes the cartilage to collapse. Our findings also provide insights regarding potential causes of damaged cartilage, although it is not yet clear why DCVC and its related-substances located in hyaline cartilage.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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