Effects of Trichloroethylene Metabolite DCVC against Bone-Related Cell Lines

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Abstract

S-(1,2-dichlorovinyl)-*L*-cysteine (DCVC), one of the metabolite of trichloroethylene (TCE), is known to exert renal toxicity. We studied the toxicity of DCVC on bone-related cell lines such as chondrogenic cells (ATDC5 cells) and osteoblasts (MC3T3-E1 cells) and the formation of osteoclasts. DCVC at a concentration of 10^{-5} M significantly reduced cell viability of ATDC5 cells and MC3T3-E1 cells, and strongly inhibited formation of osteoclast-like cells. The results indicated that DCVC possessed potent toxicity against the proliferation and differentiation of the bone-related cell lines.

Introduction

Trichloroethylene (TCE) is known as an environmental contaminant that has been industrially used as synthetic material for alternative fluorocarbon, metal-degreasing and other industrial processes. While several studies reported that TCE causes hepatocarcinogenesis in mice and renal toxicity in rats. TCE also causes renal, reproductive and developmental toxicity in humans. TCE is mainly metabolized *via* at least two separate pathways, P-450 oxidative metabolism and glutathione (GSH)-dependent metabolism once taken into the body (Cummings and Lash, 2000). In the metabolic process, TCE is partially metabolized by GSH conjugation to form *S*-(1,2-dichlorovinyl) glutathione, and then metabolized by γ-glutamyl-transferase and dipeptitase to form *S*-(1,2-dichlorovinyl)-*L*-cysteine (DCVC). DCVC can then be metabolized by either cysteine *S*-conjugate *N*-acetyl-*S*-transferase to form *N*-acetyl-DCVC, or by the cysteine conjugate ß-lyase (ß-lyase) to form a reactive thiol intermediate (Cummings and Lash, 2000). Recently, DCVC has been implicated as a main route for the renal toxicity *via* the bio-activation by ß-lyase in the study with human proximal tubular cells (Cummings and Lash, 2000).

The bone is based on the complex actions of osteoclasts and osteoblasts, and the exposure to chemical compounds leads to the metabolic disorder such as osteoporosis

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by way of the imbalance of the actions of the bone cells. Although several studies demonstrated that DCVC worked injury on bone marrow and kidney *via* ß-lyase bio-activation (Lock, 1996), little is known about its effect on bone metabolism. In this study, we investigated the toxicity of DCVC on chondrogenic cells, osteoclasts and osteoblasts.

Materials and Methods

Chemicals

DCVC was synthesized according to the procedure as described previously (McKinney et al. 1959). Appropriate amount of sodium and dry *L*-cysteine (Sigma) (2 mmol) were added in portions to dry liquid ammonia, and then TCE (2 mmol) was added slowly under stirring. After the reaction for more than 30 minutes, slightly colored residue was obtained and was dissolved in 7.5 ml of water. An equal volume of ethanol was added and cooled overnight in a refrigerator. The resulting precipitate was isolated by filtration. As a result, 304 mg of crude DCVC was obtained. The product identity was determined by mass spectroscopy.

Culture of cells

ATDC5 cells and MC3T3-E1 cells were supplied by the RIKEN Cell Bank (Tsukuba, Japan). ATDC5 cells were maintained in 55-cm^2 dishes in DMEM/Ham's F12, supplemented with 5% fetal bovine serum, 10 μg/ml human transferring, 3×10^{-8} M sodium selenite, 100 units/ml penicillin, and 100 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2 in air at 37℃. MC3T3-E1 cells were maintained in 55-cm^2 dishes in α -MEM, supplemented with 10% fetal bovine serum, 50 units/ml penicillin and 50 μg/ml streptomycin, in a humidified atmosphere of 5% CO_2 in air at 37℃. After cells had reached 70% confluence, cells were detached by treatment with 0.05% trypsin, replated in 96-well plates.

MTT assay

We used 3-(4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2H tetrazolium bromide (MTT; Dojindo, Kumamoto, Japan) to examine the viability of chondrocytes and osteoblasts. Cells (1×10^3 cells/well; 96-well plates), exposed to DCVC at various concentrations, were subcultured for indicated times. After cells were washed twice with serum-free RPMI 1640 medium, cells were treated with MTT (50 μg/well) for 4 h and then absorbance at 570 nm was measured.

Formation of osteoclast-like cells in vitro

Spleen cells were collected from splenic tissues of the six-week-old male ddY mice (Sankyo Laboservice, Tokyo, Japan). Erythrocytes contaminating the spleen cell fraction were eliminated by adding 0.83% ammonium chloride in 10 mM Tris-HCl (Ph 7.4) to the cell pellet. Spleen cells (2.4×10^5 cells/well) in 96-well plates (0.32 cm^2/well) were cultured with 50ng/ml human sRANKL and 30 ng/ml M-CSF for 7 days. Fresh medium and compounds were supplied at 3-day intervals.

The Institutional Animal Care and Use Committee of Toin University of Yokohama approved the animal protocols and procedures.

Localization of tartrate-resistant acid phosphatase (TRAP)

After culture for 7 days, adherent cells were fixed in 3.6% formaldehyde for 5 min and then in a mixture of ethanol

and acetone (1:1, v/v) for 1 min. Then they were stained for TRAP activity, as previously described (Yamagishi et al., 2001; Notoya et al., 2004). TRAP has been extensively used as a cell specific marker for osteoclasts, although its function has remained obscure. TRAP-positive mononuclear cells and TRAP-positive multinucleated cells (with three or more nuclei) were counted under a microscope (IX70; Olympus).

To confirm the toxicity of DCVC against bone-related cell lines, chondrogenic cell line ATDC5 cells and mouse calvarial clonal preosteoblastic cells (MC3T3-E1) were employed in the determination of cell viability by MTT assay. Although the addition of DCVC at a concentration of 10^{-7} M exerted slight elevation of cell viability after 94 hours incubation in ATDC5 cells, DCVC at 10^{-5} M significantly reduced cell viability after 74 hours and 94 hours incubation (Figure 1).

Results and Discussion

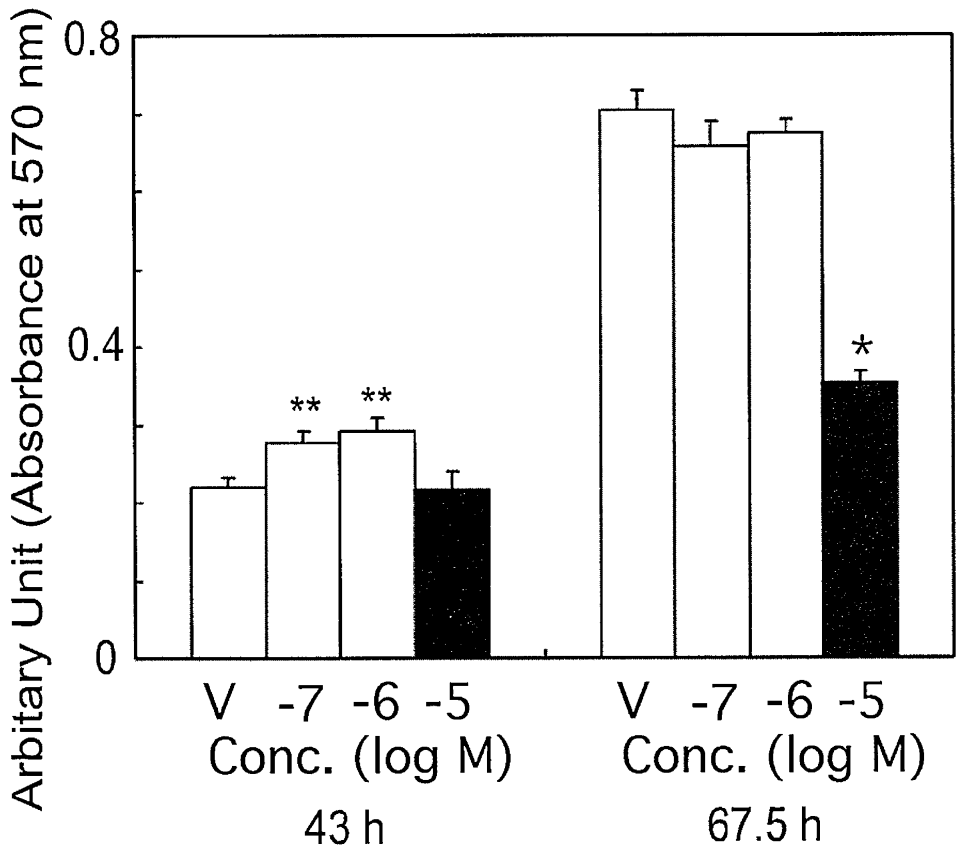

Figure 1. The effects of DCVC on chondrocyte viability. We used MTT to examine the viability of ATDC5 cells. Cells (1×10^3 cells/well; 96-well plates), exposed to DCVC at various concentrations, were subcultured for indicated times. V; vehicle. Columns and bars show the means ± S.D. of results from four wells. Data are representative of the results of three separate experiments. *P<0.01; *vs* vehicle, **P<0.05; *vs* vehicle.

The addition of DCVC at 10^{-5} M significantly reduced cell viability after 67.5 hours incubation in MC3T3-E1 cells (Figure 2). There was no inhibition after 43 hours *incubation.*

Furthermore, multinucleated osteoclast-like cells derived from mouse spleen were incubated in the presence of various concentrations of DCVC for 7 days, and stained with TRAP reagent. As shown in Figure 3, DCVC at 10^{-5} M obviously inhibited the formation of osteoclasts .

These results indicate that the addition of DCVC at a concentration of 10^{-5} M exerted potent toxicity against proliferation and differentiation of bone-related cell lines, while there was no evidence for the involvement of bio-activation of ß-lyase in these cell lines as reported in bone marrow injury (Lock, 1996).

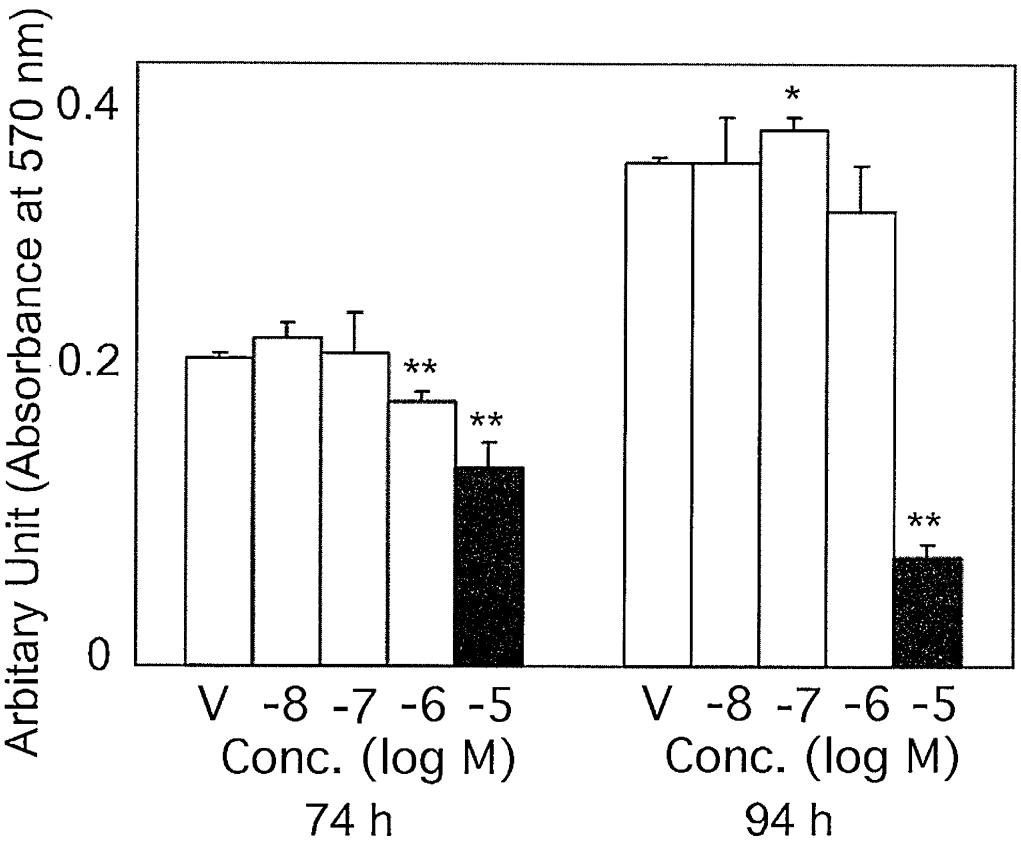

Figure 2. The effects of DCVC on osteoblast viability. We used MTT to examine the viability of MC3T3-E1 cells. Cells (1×10^3 cells/well; 96-well plates) , exposed to DCVC at various concentrations, were subcultured for indicated times. V; vehicle. Columns and bars show the means ± S.D. of results from four wells. Data are representative of the results of three separate experiments. *P<0.0001; *vs* vehicle, **P<0.01; *vs* vehicle.

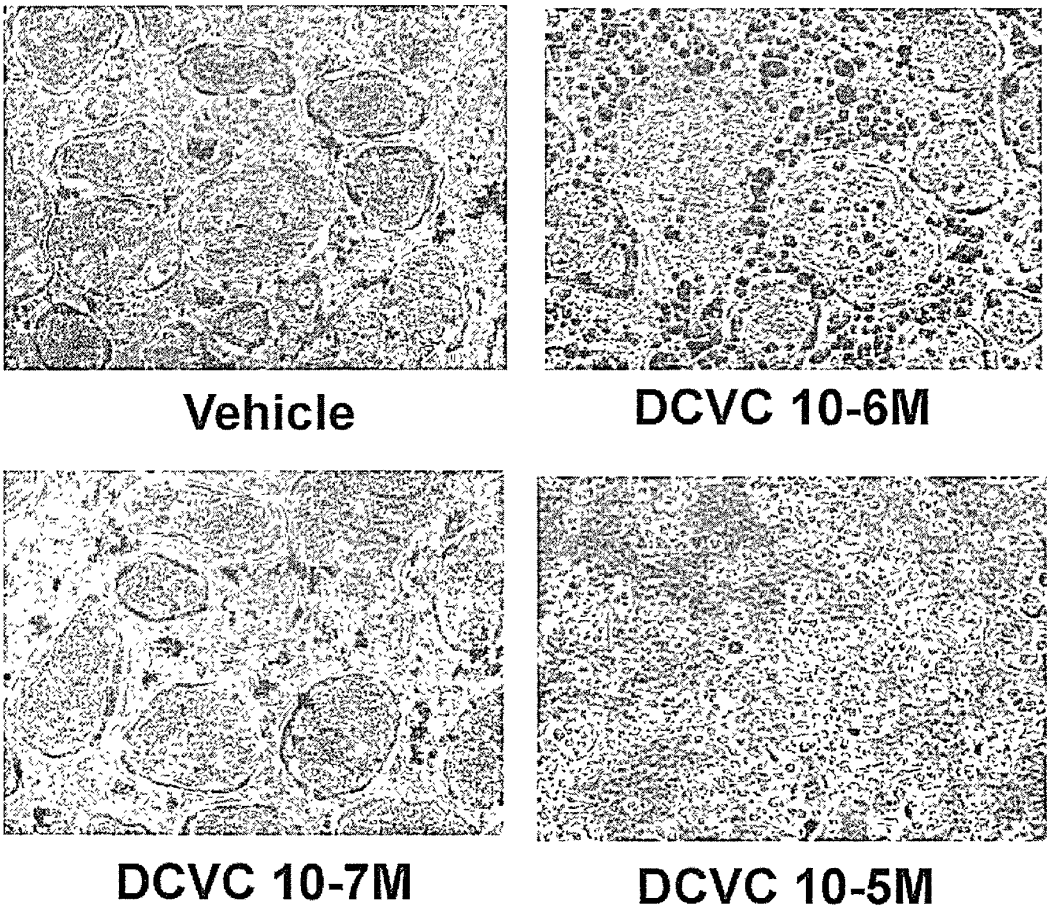

Figure 3 The effects of DCVC on the formation of multinucleated osteoclasts Osteoclasts formed from mouse spleen cells in the presence of 50 ng/ml sRANKL and 30 ng/ml M-CSF. DCVC at the indicated concentrations was added to a culture system The cultured cells were then stained for TRAP activity at day 7, and TRAP-positive multinucleated cells were counted under a microscope Typical results of staining for the detection of TRAP activity in cells treated with DCVC Bar = 50 mm.

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