In vivo Effects of Trichloroethylene Metabolite DCVC against Bone

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Abstract

DCVC is considered to cause renal-cellular injury after metabolic activation by cysteine conjugate β-lyase, DCVC orally administered at doses of 10 and 30 mg/kg exhibited no *in vivo* effects on the body weights, kidney weights, and femur bone density in contrast to the *in vitro* study in which DCVC at a concentration of 10^{-5} M significantly had inhibited the proliferation, differentiation, and calcification of bone-related cells, such as chondrocytes, osteoclasts, and osteoblasts.

Introduction

Trichloroethylene (TCE) is widely used as a synthetic material for alternatives of fluorocarbons or for metal-degreasing. It is designated as a Class II Specified Chemical Substance and required to be controlled under the guideline for environment conservation. Several studies revealed TCE toxicity in mice, rats, and humans. TCE is metabolized *via* several pathways including glutathione (GSH) -dependent metabolism through which TCE is metabolized into S-(1, 2-dichlorovinyl) -L-cysteine (DCVC)[1]. DCVC is considered to cause renal-cellular injury after metabolic activation by cysteine conjugate β-lyase[1]. However, little is known about DCVC on its distribution into the bone, biological effects, and mechanism of action. We previously investigated the *in vitro* effects of DCVC on bone-related cells such as chondrocytes, osteoblasts, and osteoclasts, and the results suggested that DCVC at a concentration of 10^{-5} M significantly inhibited the proliferation, differentiation, and calcification of these cells[2], In the present study, we investigated the *in vivo* effects of DCVC using ovariectomized (OVX)-mice as osteoporosis models. In order to investigate the effect of DCVC on the body weights, renal functions, and femur bone density, OVX-Balb/c mice were orally administered with DCVC at every third day for 28 days at doses of 10 and 30 mg/kg.

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Materials and Methods

Chemicals

DCVC was synthesized according to the procedure as described previously[3]. 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. 304.6 mg of crude DCVC was obtained. The crude DCVC was dissolved in 15 mL of water at 70℃ and the hot solution was filtrated. After recrystallization, 204.1 mg of DCVC was obtained.

Animals

Six-week-old female mice (BALB/c) were purchased from Charles River Japan, Inc. The mice were ovariectomized under anesthesia with sodium pentobarbital at 3 days before administration. The ovariectomized-mice were orally administered with DCVC suspended in isotonic saline every third day for 28 days at doses of 10 and 30 mg/kg. Body weight was measured periodically starting from the first day of DCVC administration. After the administration for 28 days, the kidneys were weighted and the blood urea nitrogen was measured using Blood Urea Test Wako Kit (Wako Pure Chemical Industries, Tokyo, Japan). The femur bone density was measured using a soft X-ray CT scanning apparatus Latheta (Aloka Co., Tokyo, Japan).

Results and Discussion

No significant differences were observed in the body weights and kidney weights among the groups of sham operation (n = 8), OVX (n=8), OVX+DCVC 10 mg/kg PO (n=7), and OVX+DCVC 30 mg/kg PO (n=7). However, blood urea nitrogen in the group of OVX+DCVC 30 mg/kg PO significantly decreased, although this action mechanism is unclear yet. Femur bone density showed no difference among each group. The results indicated that DCVC at doses of 10 and 30 mg/kg did not inhibit or accelerate the loss of femur bone density in OVX mice. In conclusion, DCVC orally administered at doses of 10 and 30 mg/kg exhibited no *in vivo* effects on the femur bone density in contrast to the *in vitro* study in which DCVC at a concentration of 10^{-5} M significantly had inhibited the prolitlration, differentiation, and calcification of bone-related cells.

References

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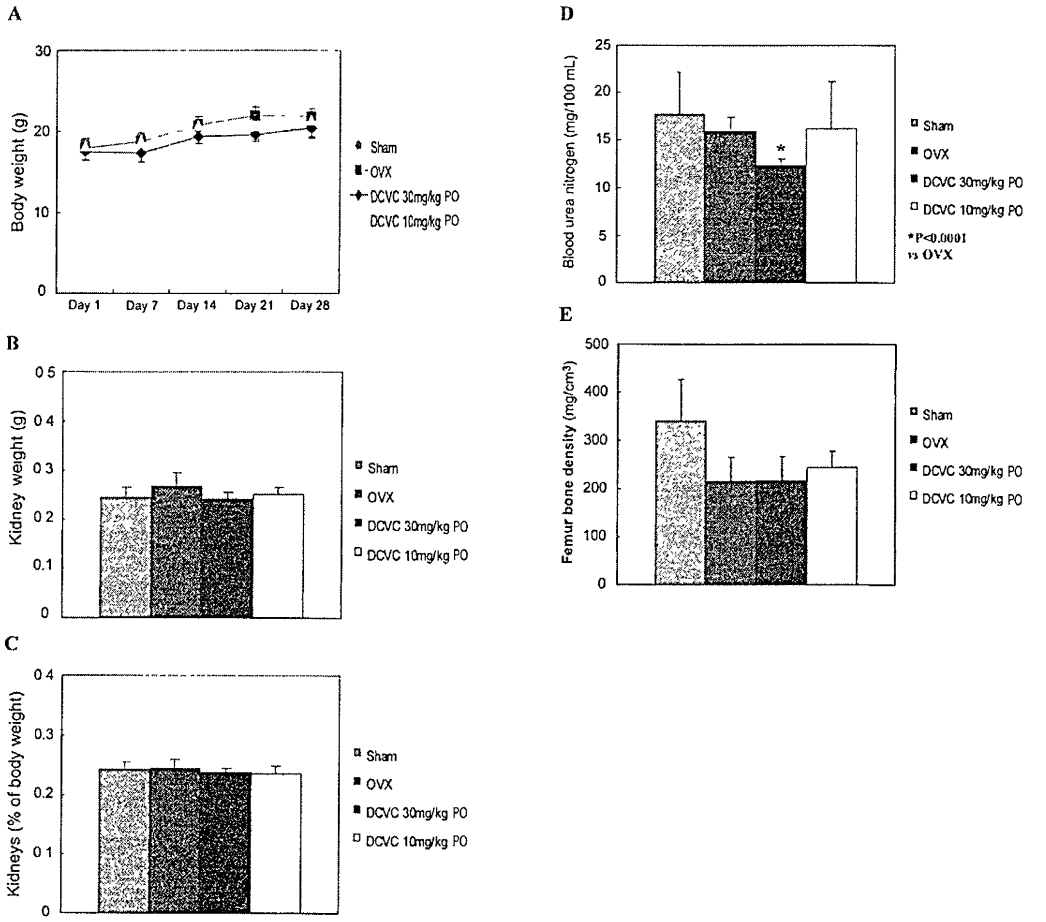

Figure 1. *In vivo* effects of DCVC on OVX-mice.

A, Body weights for each treatment group during administration were measured periodically and plotted. Points, mean (n=8 for groups of sham operation and OVX-mice, n=7 for DCVC administration groups) ; bars, SD.

B, Kidney weights for each treatment group after the last administration were measured and plotted. Columns, mean (n=8 for groups of sham operation and OVX-mice, n=7 for DCVC administration groups) ; bars, SD.

C, Kidney weight/body weight ratio for each treatment group after the last administration was measured and plotted. Column, mean (n=8 for groups of sham operation and OVX-mice, n=7 for DCVC administration groups) ; bars, SD.

D, Blood urea nitrogen for each treatment group after the last administration was measured and plotted. Statistical differences for each treatment group were analyzed by t-test.*, $P < 0.0001$, versus OVX group; Columns, mean (n=8 for groups of sham operation and OVX-mice, n=7 for DCVC administration groups) ; bars, SD.

E, Bone density of femurs for each treatment group after the last administration was measured and plotted. Columns, mean (n=8 for groups of sham operation and OVX-mice, n=7 for DCVC administration groups) ; bars, SD.