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ORIGINAL ARTICLE

# Inhibition of verotoxin (VT) 2 absorption into systemic blood from intestine by repeated administration of bovine immune colostrum antibody against VT2 in mice



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## KEYWORDS

absorption;  
bovine colostrum  
antibody;  
intestine;  
mice;  
VT2

**Background/Purpose:** Whether absorption of verotoxin (VT) 2 from the intestine in mice is inhibited by administration bovine immune colostrum antibody against VT2 was investigated.

**Methods:** Three-week-old mice were administered VT2 solution at 477.8 ng/mL or 955.6 ng/mL, and bovine immune colostrum antibody against VT2 was then administered three times. Whey without antibody against VT2 was administered to control mice. Serum levels of VT2 were measured by fluorescence enzyme immunoassay.

**Results:** Serum levels of VT2 in mice administered VT2 solution at 477.8 ng/mL and bovine immune colostrum antibody against VT2 scarcely changed. By contrast, serum levels of VT2 in control mice increased and peaked 12 hours after administration. Peak values were  $15.4 \pm 5.04$  ng/mL. Furthermore, serum levels of VT2 at 12 hours and 16 hours in control mice were significantly higher than in mice administered bovine colostrum antibody against VT2. Serum levels of VT2 in mice administered antibody at 955.6 ng/mL showed no significant differences between repeated administration of bovine immune colostrum antibody and controls. **Conclusion:** These results suggest that absorption of VT2 from the intestine was inhibited by repeated administration of bovine immune colostrum antibody against VT2 at early

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stages of *Escherichia coli* O157:H7 infection, whereas VT2 in the intestine remained at low levels.

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## Introduction

Food poisoning caused by *Escherichia coli* O157:H7 continues to occur in Japan.<sup>1,2</sup> Treatment for this type of infection generally does not involve antibiotics,<sup>3–5</sup> as verotoxin 2 (VT2) released from *E. coli* O157:H7 killed by antibiotics induces serious complications, such as hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura, and brain damage.<sup>6–8</sup> The authors have reported the neutralizing efficacy of bovine immune colostral antibody against VT2 in mice and beagle dogs.<sup>9,10</sup> We compared serum levels of VT2 between coadministration of immune colostral antibody against VT2 and saline in mice administered VT2.<sup>11</sup> Serum levels of VT2 were lower than in control mice after a single administration of immune bovine colostral antibody. In particular, serum levels of VT2 at 8 hours and 12 hours after administration of VT2 were significantly lower than in control mice.<sup>11</sup> However, the absorption of VT2 was not completely inhibited in this experiment. Thus, several administrations of bovine immune colostral antibody are necessary to inhibit the absorption of VT2 from the intestine. The aim of this study was therefore to evaluate whether absorption of VT2 from the intestine into systemic circulation is inhibited by repeated administration of immune bovine colostral antibody in mice administered VT2.

## Materials and methods

### VT2

VT2 was obtained from the supernatant of cultured *E. coli* O157:H7 isolated from humans.

### Mice

Male SPF ICR mice (age, 3 weeks) were purchased from Charles River Inc. (Yokohama, Japan). Mice were kept in cages at a temperature of  $23 \pm 2^\circ\text{C}$ , and a relative humidity of  $55 \pm 10\%$ , on a 12/12 dark (18:00 to 6:00)/light (6:00 to 18:00) cycle with the air exchanged 12 times or more/hour. Mongolian gerbils were fed MF (Oriental Yeast Co., Ltd., Tokyo, Japan), and were allowed free access to water. All experiments were approved by the Institutional Review Board of Azabu University, Kanagawa, Japan and were conducted in accordance with the Institute's Animal Experimentation Guidelines (Japanese Association for Laboratory Animal Science, JALAS, 1987).

### Animal experiments

Absorbed VT2 in mice after administration of various VT2 concentrations was first estimated. The aim of this

experiment was to determine the appropriate dose of VT2 for subsequent evaluation of inhibition by the bovine immune colostral antibody. Four VT2 concentrations (955.6 ng/mL, 477.8 ng/mL, 318.5 ng/mL, and 238.9 ng/mL) were assessed, and four mice were administered VT2 at these concentrations. Mice were sacrificed at 16 hours after administration. Serum VT2 concentrations, hemoglobin, and red blood cell counts were measured. Hemoglobin and red blood cell counts were measured by Celltac $\alpha$  (Nihon Kohden Corporation, Tokyo, Japan).

Mice were orally administered VT2 solution at 477.8 ng/mL or 955.6 ng/mL. Bovine colostral antibody against VT2 was given at 1 hour after administration, three times at 1-hour intervals (bovine immune colostral antibody group). The control group was administered whey without antibody against VT2 instead of bovine colostral antibody against VT2. Blood was collected prior to and at 4 hours, 8 hours, 12 hours, 16 hours, 24 hours, 36 hours, and 48 hours after administration. Three mice were sacrificed for blood collection at each time point. Sera were obtained by centrifugation of blood at 1610g for 10 minutes. Sera were stored at  $-80^\circ\text{C}$  until measurement.

### Measurement method for serum concentration of VT2

Serum concentrations of VT2 were measured by fluorescence enzyme immunoassay according to the procedure of Seita et al.<sup>11</sup>

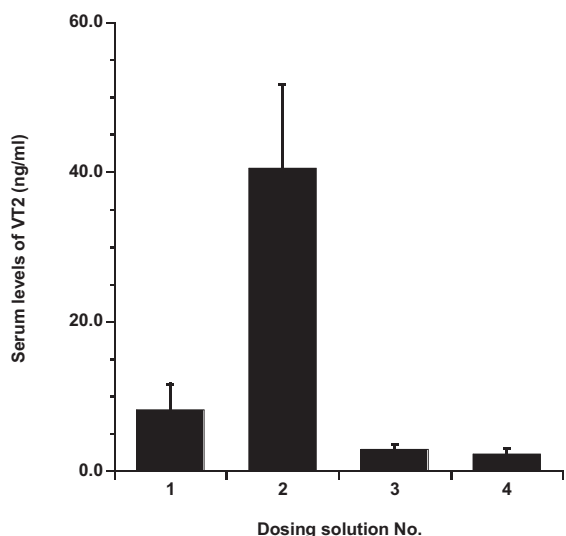
### Statistical analysis

Data are presented as mean  $\pm$  standard deviation for three mice at each time point. Statistical analysis of serum concentrations of VT2, hemoglobin, and red blood cell count were performed by unpaired Student *t* test. Differences were considered to be significant at  $p < 0.05$ .

## Results

### Determination of VT2 doses

Serum levels of VT2 were 8.2 ng/mL, 40.5 ng/mL, 2.9 ng/mL, and 2.3 ng/mL at 16 hours after administration of the various test concentrations (Fig. 1). Mean hemoglobin and red blood cell counts are shown in Table 1. Hemoglobin in mice administered VT2 solution at 955.6 ng/mL was significantly lower when compared to mice administered other VT2 concentrations. Red blood cell counts in mice administered VT2 solution at 955.6 ng/mL were also significantly lower when compared to mice administered VT2 solutions at 477.8 ng/mL or 318.5 ng/mL.



**Figure 1.** The serum levels at 16 hours after administration of different verotoxin 2 (VT2) concentrations dosing solution to mice. Dosing solutions indicated VT2 concentrations were the following: 1:955.6 ng/mL, 2:477.8 ng/mL, 3:318.5 ng/mL, and 4:238.9 ng/mL.

**Changes in VT2 levels in mice**

Serum levels of VT2 in mice administered VT2 solution at 955.6 ng/mL and 477.8 ng/mL are shown in Figs. 2 and 3, respectively. Serum levels of VT2 in mice administered VT2 solution at 955.6 ng/mL did not show significant differences between repeated administration of bovine immune colostrum antibody and controls. By contrast, serum levels of VT2 in mice administered VT2 solution at 477.8 ng/mL showed little changes in the repeated bovine immune colostrum antibody administration group. Serum levels in control mice increased after administration of VT2 and peak levels were observed at 12 hours after administration (Fig. 2). Peak levels were  $15.4 \pm 5.04$  ng/mL. Serum levels in control mice at 12 hours and 16 hours were significantly higher than those in mice repeatedly administered bovine immune colostrum antibody.

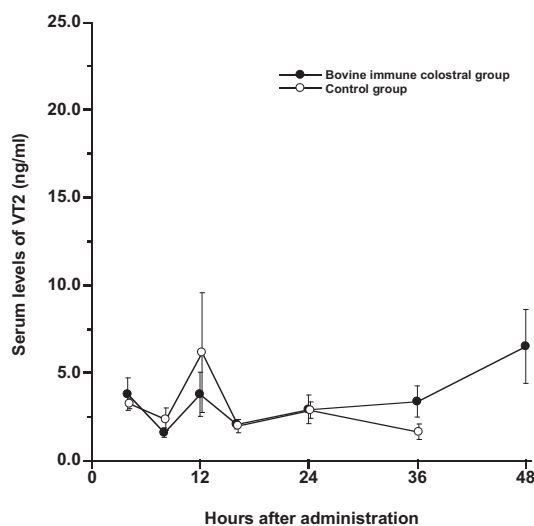
**Discussion**

VT2 derived from *E. coli* O157:H7 in the intestine is known to induce serious complications, including HUS and brain

**Table 1** Hemoglobin and red blood cell count at 16 hours after administration of different VT2 concentrations dosing solution to mice

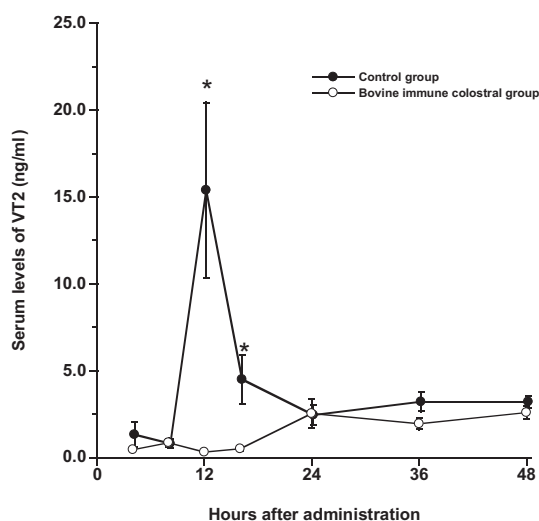
Dosing levels of VT2 (ng/mL)	Hemoglobin (g/dL)	Red blood cell count ( $\times 10^6/\mu\text{L}$ )
955.6	$7.2 \pm 1.8^a$	$386.3 \pm 102.3^a$
477.8	$10.3 \pm 2.1$	$549.8 \pm 82.7$
318.5	$11.6 \pm 2.7$	$609.3 \pm 124.6$
238.9	$9.8 \pm 1.2$	$502.3 \pm 69.1$

<sup>a</sup> Value differs significantly from mice administered 477.8 ng/mL of VT2 ( $p < 0.05$ ). Data are presented as mean  $\pm$  standard deviation ( $n = 4$ ).



**Figure 2.** The serum levels of verotoxin 2 (VT2) in mice administered VT2. Data are presented as mean  $\pm$  standard deviation ( $n = 5$ ).

damage, in patients infected with *E. coli* O157:H7.<sup>12–15</sup> In infection models, mice showing intestinal bleeding died, but those not showing intestinal bleeding did not die.<sup>9,11</sup> The cause of death was presumed toxicity of VT2 absorbed from the intestine. The authors have reported that serum levels of VT2 continue to increase for 24 hours in mice administered VT2.<sup>11</sup> Serum levels of VT2 in mice administered bovine immune colostrum antibody against VT2 were lower than those in control mice.<sup>11</sup> However, absorption of VT2 was not fully inhibited by single administration of bovine immune colostrum antibody. We presumed that serious complications were prevented by inhibiting absorption of VT2 from the intestine. Bovine immune



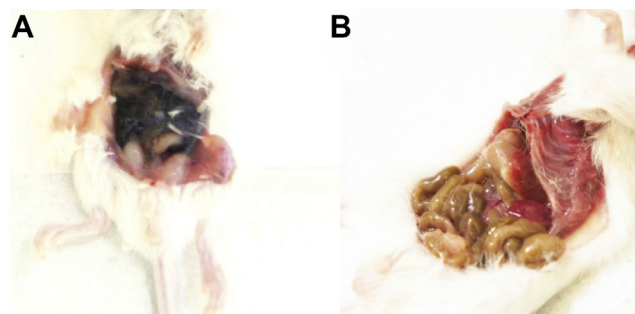
**Figure 3.** The serum levels of verotoxin 2 (VT2) in mice administered VT2. Data are presented as mean  $\pm$  standard deviation ( $n = 5$ ). \* Value differs significantly from that of 3-week-old rats ( $p < 0.05$ ).

colostral antibodies were thus administered repeatedly in this study.

Serum levels at 16 hours after administration of VT2 were highest in mice administered VT2 solution at 477.8 ng/mL (Fig. 1). By contrast, hemoglobin and red blood cell counts in mice administered the VT2 solution at 955.6 ng/mL were significantly lower than in mice administered VT2 solution at other concentrations. These results suggest that severe intestinal bleeding occurred and this interfered with intestinal function. Thus, the doses of VT2 used were 955.6 ng/mL and 477.8 ng/mL in order to evaluate the inhibition of VT2 absorption in mice.

Serum concentrations of VT2 peaked at 12 hours after administration of VT2 and decreased in control mice administered VT2 solution at 477.8 ng/mL. In particular, serum levels of VT2 at 12 hours and 16 hours in control mice were significantly higher than in mice administered bovine immune colostral antibody repeatedly. These results suggest that absorption of VT2 from the intestine was inhibited by three-time administration of bovine immune colostral antibody. However, peak serum concentrations differed from those at 16 hours after administration in the experiment to determine dosing levels. The reason for this difference is unclear. Blood was not collected from individual mice at the same time points as in rats, and the serum concentrations at each time point were recorded as the means of five mice. This difference between peak concentration after 12 hours and concentration at 16 hours in the preliminary study was thus assumed to be due to individual differences. Nonetheless, serum levels of VT2 were not significantly different between repeated administration in the bovine colostral antibody group and the control group with VT2 administered at 955.6 ng/mL. Although pathological evaluation of the intestine was not possible in this study, intestinal bleeding was observed on gross pathology and the intestine was considered to be severely damaged by VT2. It was thus assumed that VT2 was not absorbed by the intestine into systemic circulation due to severe intestinal damage (Fig. 4). The cause of death in mice with severe intestinal damage was considered to be bleeding.

Kita et al estimated the serum levels of Shiga toxin (Stx) 1 in mice after inoculation with *E. coli* O157:H7 producing Stx 1 and 2. Peak levels of  $34.8 \pm 4.6$  pg/mL were observed



**Figure 4.** The observation of intestinal bleeding and non-bleeding in mice administered various verotoxin 2 (VT2) solutions and sacrificed at 16 hours after administration. (A) Intestinal bleeding is observed; (B) intestinal bleeding is not observed.

at 4 days after inoculation.<sup>16</sup> Higher levels of VT2 were inhibited by repeated administration of bovine immune colostral antibody in this study. Furthermore, treatment of *E. coli* O157:H7 infection with fosfomycin at early stages prevents progression to serious symptoms.<sup>17,18</sup> Thus, adsorption of VT2 appeared to be inhibited by administration of bovine immune colostral antibody at early stages after infection with *E. coli* O157:H7, despite VT2 levels in the intestine increasing from disruption of *E. coli* O157:H7 by antibiotics. Furthermore, serious complications such as HUS or encephalopathy caused by VT2 were prevented.

In conclusion, absorption of VT2 was inhibited by repeated administration of bovine immune colostral antibody against VT2 in mice. Unfortunately, the mechanism of inhibition for absorption of VT2 by administration of bovine immune colostral antibody was not clarified in this study. Further studies will therefore be necessary. In the future, repeated administration of immune bovine colostral antibody against VT2 may be useful for inhibiting the absorption of VT2 released from *E. coli* O157:H7 killed by antibiotics in humans.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Acknowledgments

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