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ORIGINAL ARTICLE

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

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Background/Purpose: Whether absorption of verotoxin (VT) 2 from the intestine in mice is inhibited by administration bovine immune colostral 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 colostral 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 colostral 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 ± 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 colostral 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 colostral antibody and controls.
Conclusion: These results suggest that absorption of VT2 from the intestine was inhibited by repeated administration of bovine immune colostral 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.

Introduction

Food poisoning caused by *Escherichia coli* O157:H7 continues to occur in Japan.[1,2] Treatment for this type of infection generally does not involve antibiotics,[3–5] 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.[6–8] The authors have reported the neutralizing efficacy of bovine immune colostral antibody against VT2 in mice and beagle dogs.[9,10] We compared serum levels of VT2 between coadministration of immune colostral antibody against VT2 and saline in mice administered VT2.[11] Serum levels of VT2 were lower than in control mice after a single administration of immune bovine colostral antibody. In particular, serum levels of VT at 8 hours and 12 hours after administration of VT2 were significantly lower than in control mice.[11] 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 ± 2°C, and a relative humidity of 55 ± 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 Celltacα (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 1610*g* for 10 minutes. Sera were stored at −80°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.[11]

Statistical analysis

Data are presented as mean ± 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.