Note

In Vitro and in Vivo Evaluation of the Efficacy of Bovine Colostrum against Human Rotavirus Infection

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Received November 20, 2009; Accepted December 23, 2009; Online Publication, March 7, 2010
[doi:10.1271/bbb.90862]

Abstract

We found that skimmed and concentrated bovine late colostrum (SCBLC) obtained from normal cows at 6–7 d after parturition exhibited high potency in inhibiting replication of human rotavirus (HRV) in vitro. Furthermore, prophylactic oral administration of SCBLC once before inoculation of HRV prevented the development of diarrhea in suckling mice in vivo. SCBLC from normal cows might be useful in the prevention of HRV-induced severe gastroenteritis in immunocompromised hosts.

Key words: bovine colostrum; human rotavirus; infection; diarrhea; suckling mice

Human rotavirus (HRV) is one of the major causes of severe dehydrating gastroenteritis in infants and young children worldwide. It causes more than 527,000 deaths per year.1) Two rotavirus vaccines have been developed and approved in certain countries,1) but the development of alternative prophylactic approaches is warranted, because most patients with rotavirus diarrhea are immunocompromised, especially infants and young children.

Bovine colostrum is the early milk produced by cows during the first several days post-parturition. The importance of colostrum for the growth and health of newborn offspring is well known, as is a high concentration of immunoglobulins. Over the past two decades, it has been proposed that passive protection against rotavirus diarrhea can be achieved by using cow’s milk containing a high level of specific anti-rotavirus antibodies.2,3) These are commonly produced by hyperimmunization of pregnant cows with certain rotavirus strains,2,3) while bovine lacteal secretions in the normal state contain antibodies against rotavirus.4) There is a review regarding the clinical effects of supplementation with bovine colostrum,5) but the clinical use of bovine colostrum from hyper-immunized cows has been limited, due to difficulties in large-scale production. In addition, shipping early colostrum from cows within 5 d of parturition is illegal as a food in Japan.

To our knowledge, there has been no study on the efficacy of colostrum from normal healthy cows against HRV infection in an experimental animal model. The aim of the present study was therefore to evaluate the protective efficacy of skimmed and concentrated bovine milk from healthy lactating cows at 6–7 d after parturition, referred to as skimmed and concentrated bovine late colostrum (SCBLC), against HRV infection in vitro and in vivo. It would be of great potential if SCBLC from normal cows shows potent inhibitory activity against HRV, comparable to that exhibited by early colostrum from hyper-immunized cows.

SCBLC from normal cows was prepared at an industrial level in the facility of Kobayashi Pharmaceutical (Osaka). Briefly, pooled late colostrum from healthy cows at 6–7 d after delivery was defatted by centrifugation, pasteurized by HTST condition (heating at 73°C for 15 s), and then concentrated by ultrafiltration, followed by spraydrying.

We compared the protective efficacy against the HRV gastroenteritis between SCBLC from normal cows and the colostrum whey within the first 3 d after parturition from cows hyper-immunized with HRV (rota whey). As previously reported,2) rota whey contained high titers of neutralizing antibodies against HRV.

Replication inhibition (neutralization) assay for HRV MO strain (serotype G3P[8]) was performed using MA104 cells (African rhesus monkey kidney cell line) following a procedure described previously.6) Briefly, a suspension containing infectious virus at a titer of 10^5–10^6 FCFU/ml was treated with 10 μg/ml trypsin (Sigma-Aldrich, St. Louis, MO) for 30 min at 37°C. After appropriate dilution with Eagle’s minimum essential medium (MEM) containing 10% fetal calf serum to give a titer of approximately 4 × 10^3 FCFU per 100 μl, aliquots were mixed with an equal volume (100 μl) of one-half serially diluted samples in microtubes for 1 h at 37°C. The diluted mixtures were then used to inoculate MA104 cells (2 × 10^5 cells/ml, 200 μl), and 20-μl aliquots of each were placed into the wells of a 24-well heavy teflon (HT)-coated slide (AR Brown, Tokyo). The control gave about 100 infected foci/well without test samples. The cells were further cultured for 22 h at 37°C under an atmosphere of 5% CO2, and then

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Abbreviations: SCBLC, skimmed and concentrated bovine late colostrum; HRV, human rotavirus; HTST, high-temperature short-time method sterilization

Received November 20, 2009; Accepted December 23, 2009; Online Publication, March 7, 2010
[doi:10.1271/bbb.90862]
fixed with cold acetone for 20 min. The foci numbers of infected cells were measured by indirect immunofluorescence assay using PO-13 monoclonal antibody against HRV (rota whey, open triangles); skimmed and concentrated bovine late colostrum (SCBLC, solid squares). The inhibitory activity of each sample is expressed as percent of infected cells as compared to control cells (100%). The experiments were carried out in triplicate at least 3 times, and representative results for each sample are given as means ± SD.

In conclusion, the present study indicates promising abdominal palpation beginning at 1 d after inoculation for 3 d. Observation of muddy-mucous or liquid-mucous yellow stool yielded a judgment of diarrhea. To assess the effects of the colostrums in the prevention of HRV-induced diarrhea, test samples were given orally by gavage every 60 min before virus inoculation. Control mice were given phosphate buffered saline, pH 7.2 (PBS). The care and experimental procedures were approved by the Animal Care and Use Committee of Gifu University.

In the case of rota whey, the suckling mice were given orally a single dose of 0.25 mg in 50 µl of PBS before inoculation of the HRV MO strain. In the same experimental model, we used a single dose of 2.5 mg in 50 µl of PBS for SCBLC. This dosage was adopted based on an experimental observation in vivo (Fig. 1), showing 10-times less effective activity against the HRV MO strain than that of rota whey. As shown in Fig. 2, 2 d after inoculation of HRV, nine of the 13 mice developed diarrhea in the PBS group, and half of the mice still suffered from diarrhea even 3 d after inoculation. In contrast, all eight mice did not develop diarrhea at all in the rota whey group, and only one of the 11 mice developed diarrhea in the SCBLC group throughout the experimental period.

The effect of a single administration of a sample before inoculation was examined in this study, and a 10-times higher dose was thus required for SCBLC to be effective against HRV gastroenteritis in comparison with rota whey. A considerable reduction in the immunoglobulin level would be expected in cow’s milk as lactation progress. In SCBLC preparation, concentration through ultrafiltration was therefore applied to keep the level of antibodies high. However, for practical purposes, the smaller the quantity is ingested in a single dose the better for the immunocompromized hosts to accept it orally. Multiple administrations per d can reduce the quantity in a single dosage, as was found for a synthesized sulfated sugar compound.

In conclusion, the present study indicates promising efficacy in immunocompromized hosts of SCBLC,
skimmed and concentrated late bovine colostrum, for inhibition of infection and prevention of the development of diarrhea caused by rotaviruses. Further studies might delineate the potential of SCBLC as a prophylactic food additive against HRV infection in more detail.

Acknowledgment

We thank Dr. Osamu Nakagomi and Dr. Toyoko Nakagomi for kindly providing the HRV MO strain. This research was supported by the Japan Society for the Promotion of Science (Grant-in-Aid for JSPS Research for Young Scientists no. 09J06778 (to MI) and for Scientific Research (C) no. 1958039 (to YK)).

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