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Bovine colostrum in oral treatment of enterogenic endotoxaemia in rats

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Abstract

Introduction Under conditions of shock, bacteria and endotoxins in the intestines can traverse the mucosal barrier by translocation and enter the blood and lymphatic system. Immunoglobulins and lactoferrin have been reported to neutralize endotoxins and bacteria. We studied the essential therapeutic factors of colostrum products in an animal experiment.

Method We simulated endotoxaemia by per-oral administration of a suspension of *Escherichia coli* and antibiotics into the duodenum of anaesthetized rats after giving intraperitoneal carrageenan. At the same time, pure bovine colostrum or lactoferrin-enriched bovine colostrum was given. Therapeutic effects were studied by examining plasma endotoxin activity and bacterial contamination of mesenterial lymph nodes and peritoneal lavages. Albumin was used in a control group.

Results The most effective bovine colostrum was able to reduce the maximum plasma endotoxin value by 67% as compared with the albumin group. The combination of this colostrum with lactoferrin brought about a reduction by 80%. The reduction in bacterial contamination of lymph nodes and peritoneal lavages was also evident.

Conclusion Both gammaglobulin and lactoferrin may help to eliminate endotoxins when bovine colostrum is administered into the gut in conditions of septic shock.

Keywords colostrum, endotoxaemia, experimental septic shock

Introduction

Septic shock is a frequent cause of death in intensive care medicine. Possible translocation of bacteria and endotoxins renders the gastrointestinal tract a crucial factor in this condition [1]. Reduced perfusion of the splanchnic region because of centralization results in hypoxia and oedema of the intestinal mucosa. The protective function of the gastrointestinal tract breaks down and bacteria from the gut enter the blood and lymph system [2].

Per-oral administration of bovine milk immunoglobulin has been proved to be effective in the treatment of intestinal *Escherichia coli* infection [3]. The biological activity of bacteria and endotoxins was reduced [4,5]. The present study was conducted to determine whether those findings could be confirmed *in vivo* by using three different bovine colostrum products in animal experiments.

Materials and method

The animal experiments were approved by the Animal Care Committee (Ethikkommission) of the University Hospital of Kiel. The colostrum products were provided by the Institut für Chemie und Physik, Bundesanstalt für Milchforschung, Kiel, and were not contaminated with lipopolysaccharide.

Table 1

Composition of bovine colostrum types 1, 2 and 3, and lactoferrin

Substance	α-Lacto-albumin (μg/dl)	β-Lactoglobulin (μg/dl)	Bovine serum albumin (μg/dl)	Immunoglobulins (μg/dl)	LF (μg/dl)	Casein (μg/dl)	Iron (μg/dl)
Type 1 BC	4400	44,400	3600	79,600	1600	ND	200
Type 2 BC	4000	22,400	1600	151,600	ND	80,000	90
Type 3 BC	2800	14,000	2400	145,600	ND	80,000	200
LF	ND	ND	ND	ND	80,000	ND	125

BC, bovine colostrum; LF, lactoferrin; ND, not detected.

A total of 35 male Wistar rats (250–350 g) were anaesthetized with ketamine. In order to achieve a degree of immunosuppression (i.e. an inflammatory state [6]) in the gut and a higher initial level of plasma endotoxin activity, 80 mg of type IV carrageenan/kg (Sigma-Aldrich Corp., St Louis, MO, USA) was injected into the peritoneal cavity. The animals were randomly assigned to five groups, each comprising seven animals (Table 1): groups 1, 2 and 3 received 400 mg iron-saturated bovine colostrum/kg of different compositions (i.e. types 1–3 bovine colostra); group 4 received a combination of 400 mg type 2 bovine colostrum/kg plus 80 mg bovine iron-saturated lactoferrin/kg (Sigma); and group 5 received 400 mg human albumin/kg (control substance).

As albumin, like other proteins, has an unspecific endotoxin-binding capacity, we wanted to have comparable protein quantities as referred to a protein commonly used in intensive care medicine. At the beginning of the 5-hour period of observation, the first blood samples were taken from the exposed external jugular vein. Neomycin and bacitracin are bactericidal and stimulate release of lipopolysaccharide from *E. coli*, and their administration results in increasing plasma endotoxin levels. Therefore, a combination of 5×10^{10} colony-forming units of *E. coli*/kg (strain O:NT H16 clinical isolate; University of Kiel, Germany); 10 mg neomycin and bacitracin/kg; and the group-specific colostrum or albumin was administered through a per-oral tube (diameter 2 mm). The tube was fixed in the duodenum by laparotomy (L.N.), carefully avoiding damage to the efferent biliary tract.

Preliminary investigations without carrageenan had shown that the maximum endotoxin level in plasma (45 ± 4 EU/dl) was reached 5 hours after administration of the bacteria/neomycin–bacitracin suspension. Therefore, the plasma endotoxin activity was measured hourly for 5 hours. At the same time points, 2 ml blood was taken from the jugular vein for culture. The last assessment was followed by laparotomy (L.N.) and peritoneal lavages with 10 ml endotoxin-free 0.9% saline solution.

Furthermore, mesenteric lymph nodes from four different areas of the mesenterium (duodenum and colon) were resected and homogenized. Bacterial contamination of the lymph nodes and the hourly blood cultures were examined using smears on sheep blood agar plates incubated for 48 hours at 37°C. Each lymph node area was examined on one agar plate and the peritoneal lavage on three agar plates. Quantity and specification of the bacterial contamination were not assessed and no anaerobic cultures were performed.

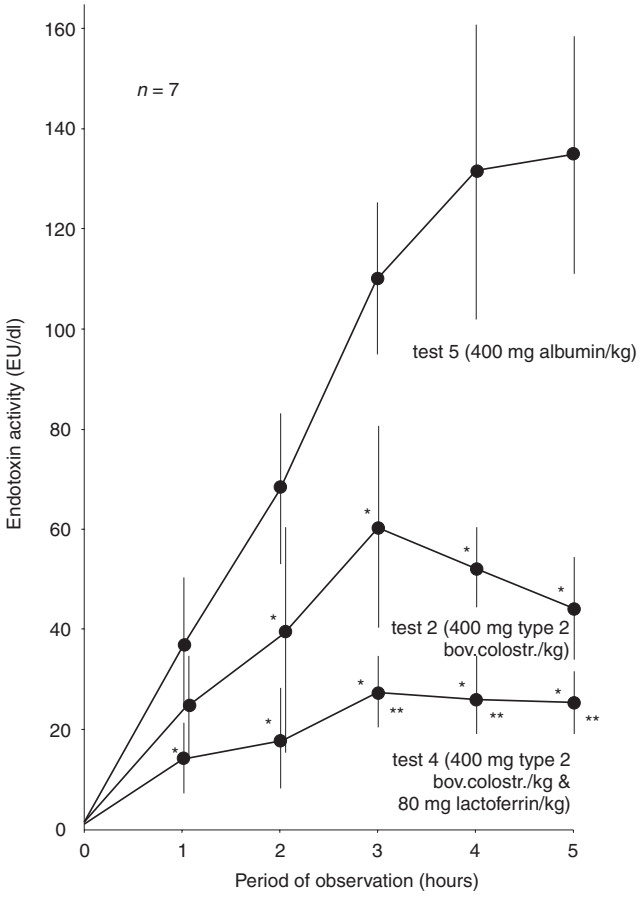
In order to measure the biological endotoxin activity in serum, we used the modified Limulus amoebocyte/lysate test with a chromogenic substrate [7]. First, 100 μl of a 1:40 diluted (0.9% NaCl) plasma sample was heated for 5 min at 80°C. Then 50 μl of Limulus lysate (Pyroquant 50, ChB: 42-109-551; Pyroquant Diagnostik GmbH, Mörfelden-Walldorf, Germany) was added and incubated for 45 min at 37°C. inally 100 μl of chromogenic substrate (S-2423; Chromogenic Company, Mölndal, Sweden) was added and incubated for 4 min at 37°C. The reaction was stopped with 50 μl acetic acid (100%) and the extinction was measured by a photometer at a wavelength of 405 nm. The lower limit of detection is 3 EU/dl. The baseline plasma endotoxin activity without application of the bacteria/neomycin–bacitracin suspension was below the lower limit of detection.

Statistical analysis of plasma endotoxin activity was based on the arithmetic means and standard deviations. Statistical significance was evaluated using the nonparametric (distribution-free method) U test (Wilcoxon and Mann–Whitney). $P < 0.05$ was considered statistically significant.

Results

Starting from a control value just above the limit of detection, there was an approximately linear rise in plasma endotoxin up to the 4-hour value of 132 ± 29 EU/dl in group 5. The 5-hour value of 135 ± 24 EU/dl was only slightly higher (Fig. 1). The bovine colostra administered in groups 1 and 3 significantly lowered biological activity from the 1-hour value onward (Table 2). The most effective suppression of biological activity was observed in group 2, in which the maximum plasma endotoxin value was 60 ± 20 EU/dl after 3 hours. At the end of the observation period the value was just 44 ± 10 EU/dl.

Figure 1



Mean values and standard deviations of plasma endotoxin activity in experimental group 2 (test 2) and 4 (test 4), and in control group 5 (test 5). *$P < 0.05$, versus control group; **$P < 0.05$, versus group 2.

Table 2

Plasma endotoxin activity

Time (h)	Albumin (EU/dl)	BC type 1	BC type 3
0	3.0 ± 1.0	2.0 ± 1.0	3.0 ± 1.1
1	36.0 ± 15.1	18 ± 2.9*	10.0 ± 4.0*
2	69.0 ± 17.2	30.0 ± 5.0*	18.0 ± 2.5*
3	110.0 ±15.7	63.0 ± 21.7*	40.1 ±5.0*
4	132.0 ± 29.3	87.3 ± 26*	62.0 ±10.7*
5	135.0 ± 24.0	75.2 ± 22.8*	52.0 ± 3.0*

Shown are mean values and standard deviations of plasma endotoxin activity for bovine colostrum (BC) types 1 and 3 (400 mg/kg each) as compared with the albumin control group (400 mg/kg). Each group contained seven rats. *$P < 0.05$, versus control group.

Table 3

Bacterial contamination after 48 hours of incubation of peritoneal lavage and lymph node specimens

Substance	Positive lavage (%)	Positive lymph nodes (%)
Albumin	62.5	62.5
Type 1 BC	27.3*	48.0*
Type 2 BC	57.0	55.3
Type 3 BC	25.0*	20.5*
Type 2 BC + 80 mg LF/kg	18.0*	17.0*

Shown are findings with bovine colostrum (BC) types 1, 2 and 3 (400 mg/kg each), and BC type 2 with 80 mg/kg lactoferrin (LF) as compared with the albumin control group (400 mg/kg). *$P < 0.05$, versus control group.

This amounts to a reduction of 67.3% (Fig. 1). In group 4 endotoxin activity was reduced to 27 ± 7 EU/dl (i.e. a maximum reduction of 80%; Fig. 1).

Bacterial contamination of the peritoneal lavages and lymph nodes was found to be lowest in experimental group 4 (Table 3). There was no bacterial contamination in any blood culture after incubation for 48 hours at 37°C.

Discussion

The gastrointestinal tract is of great importance for the development and prognosis of septicaemia [1,2]. The course of intensive care patients could be influenced favourably by selective decontamination of the intestine with local antibiotic therapy [8]. However, such bactericidal preparations can liberate lipid A fragments from the bacterial cell wall and thus increase the translocation of endotoxin [9]. It would therefore appear rational to combine antibiotic with a substance that inactivates both bacteria and endotoxins [10,11]. An oral dietetic would be of particular importance in this regard because plain parenteral nutrition lowers the concentration of secretory IgA in bile. This weakens immunological resistance and thus diminishes the barrier function of the intestinal mucosa [12]. We attempted to demonstrate that bovine colostrum is better able to inactivate lipopolysaccharide than albumin.

Administration of bovine colostrum has already proven efficacious in treating bacterial and viral enteritis in babies and infants [13]. Enteral administration of bovine colostrum with a high immunoglobulin content has been found to reduce perioperative translocation of endotoxin from the gastrointestinal tract [14]. However, it is still unclear which constituents of bovine colostrum are the crucial biological factors in this therapeutic effect. Neutralization of endotoxins and bacteria has been reported for immunoglobulins and lactoferrin [5,14].

In our animal experiments, group 2 exhibited the greatest suppression of plasma endotoxin level. This may be due to the high immunoglobulin content. The reduction in endotoxin

Key messages

- Enteral application of colostrum products is useful in septic shock
- Colostra with a high amount of lactoferrin can reduce both endotoxin activity in plasma and bacterial contamination of the peritoneal cavity
- Colostra and lactoferrin may help to improve outcomes in treatment of septic patients

activity was also significant in group 1. Because that colostrum contains only half as much immunoglobulin as the colostra in groups 2 and 3, a distinct effect of lactoferrin in group 1 is possible. The iron-saturation of the preparations rules out the possibility that the bacteriostatic effect of lactoferrin derives from removal of iron from the bacterial cell wall. Therefore, lactoferrin also confers a specific defence mechanism. Intensified elimination of the endotoxin by 'natural killer cells' is conceivable because iron-saturated lactoferrin can activate these cells. The positive therapeutic effect of combining type 2 colostrum with 80 mg lactoferrin/kg supports this.

Corresponding effects were evident in the lymph nodes and peritoneal lavages. The role of systemic and local proinflammatory cytokine levels and the significance of immunoglobulins and lactoferrin [4,15,16] in such animal models should be studied in further research. Because measurement of endotoxin in biological fluids is difficult, new techniques such as the endotoxin activity assay should be considered [17].

Competing interests

None declared.

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