Original Article

Protective effects of bovine colostrum on non-steroidal anti-inflammatory drug induced intestinal damage in rats

Jeong Wook Kim[1] MD, Woo Kyu Jeon[1] MD, Jung Won Yun[1] MD, Dong Il Park[1] MD, Yong Kyun Cho[1] MD, In Kyung Sung[1] MD, Chung Il Sohn[1] MD, Byung Ik Kim[1] MD, Joon Sup Yeom[1] MD, Hyo Soon Park[2] MD, Eun Jeong Kim[3] PhD and Myong Suk Shin[3] MSc

[1]Departments of Internal Medicine and [2]Clinical Laboratory Medicine, [3]Medical Research Institute, Kangbuk Samsung Hospital, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

The aim of this study was to examine whether bovine colostrum was able to prevent the NSAID induced small intestinal damage in animals. The animal model population of the study consisted of 4 groups: control group, diclofenac group, diclofenac with 10% low fat milk group and diclofenac with 5% colostrum group. The animals with milk or colostrum were fed with 10% low fat milk or 5% colostral solution for 5 days before the administration of diclofenac. Gut injuries were induced by administration of a single dose of diclofenac (100 mg/kg orally). Epithelial permeability values (24 hour urinary excretion of ^{51}Cr-ethylenediaminetetraacetic acid [^{51}Cr-EDTA]), enteric aerobic bacterial counts, serum biochemical profiles and pathologic findings of distal ileum were measured. Diclofenac caused a marked increase in the intestinal permeability, enteric bacterial numbers and intestinal villous damage, and enteric protein and albumin loss. Combined administration of bovine colostrum reduced the increase in intestinal permeability, enteric bacterial overgrowth, protein losing enteropathy and mucosal villous damage of the small intestine induced by diclofenac. Bovine colostrum may have a beneficial effect in prevention of NSAID induced small intestinal injuries.

Key Words: anti-inflammatory agents, non-steroidal, adverse effects, small intestine, intestinal damage, colostrum.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are usually applied in the management of patients with chronic arthropathy, in treatment of pain, as well as for prevention of myocardial infarction and stroke. Although the drugs have demonstrated a doubtless therapeutic efficacy in many conditions, the expediency of the clinical application of NSAIDs can be described as a compromise between the therapeutic efficacy and acceptable side or adverse effects. The most important NSAIDs induced side effects involve gastrointestinal disturbances, including peptic ulceration and injuries of the small and large intestine, with blood and protein loss and stricture formation.[1,2] The pathogenesis of NSAID-associated enteropathy is still uncertain. The increased intestinal permeability is believed to be an important triggering factor in the development of inflammation, facilitating the invasion of bacteria or action of other aggressive agents, such as bile acids or certain food components.[3,4]

Various approaches have been taken to the NSAIDs induced GI damage, including the development of prodrugs, enteric coated and modified-release formulations and pharmacological approaches, considering antisecretory agents and prostaglandin analogues.[5] However, none of these approaches has solved the problem of the NSAIDs induced small intestinal damage. In recent years, new NSAIDs are being developed (i.e selective COX2 inhibitors, NO-NSAIDs),[4] and definitive human trials involving these drugs are awaited. Novel therapeutic approaches to deal with these problems are therefore still required.

Colostrum is the first milk produced after delivery, and is particularly rich in growth factors, such as insulin-like growth factor (IGF), immunoglobuins and antimicrobial peptides.[6] In recent studies, addition of defatted bovine colostrum has been demonstrated to prevent villous shortening in the mouse model of indomethacin induced small intestinal injuries[7] and to reduce the intestinal permeability in human volunteers taking NSAID.[8] In this study, the potential value of a defatted bovine colostrum to reduce NSAIDs induced small intestinal injuries in rats, by measuring changes in the intestinal permeability, luminal bacteria, enteric protein loss and pathologic findings, has been examined.

Correspondence address:. Woo Kyu Jeon, M.D., Department of Internal Medicine, Kangbuk Samsung Hospital,
108, Pyung-Dong, Jongro-Ku, Seoul 110-746, Korea
Tel: + 61 (7) 3365 5404; Fax: + 61 (7) 3365 5599
Email: jeonwk@samsung.co.kr
Accepted 23 July 2004

Materials and methods

Animals

Male Sprague-Dawley rats, with body weight approximately 130-150 gm (6 week old), were purchased from Orient Co., Ltd. (Seoul, Korea). The rats were individually housed in metabolic cages in a room kept at 22 ± 1°C, with a 12 hours light and 12 hours dark cycle (lighting from 08:00 to 20:00 h). The rats were allowed free access to the diet and drinking water and their average body weight increased to approximately 160-180g during the period of the trial. The Research Committee of the hospital approved all the protocols of the experiment.

Experiment protocol

The animals were divided into four groups: group A (N =12) as the control, group B (N = 12) as the diclofenac group, group C (N = 12) as the diclofenac wtih 10% low fat milk group and group D (N =12) as the diclofenac with 5% colostrum group. All the animals were fed with standard laboratory rat chow (Basal diet 5755, PMI Nutrition International, Inc., Richimond, CA, USA). In group A and B, the drinking water was supplemented with free water, in group C with 10% solution of low fat milk, and in group D with 5% solution of bovine colostrum (Percoba liquid, Immuno Dynamics, Ltd., Perry, IA, USA) for 7 days (Table 1). Animals in group B, C and D received diclofenac sodium (100mg/kg, Ildong Pharmaceutical Co., Ltd., Seoul, Korea) orally on day 5 of the study (Fig. 1).

Table 1. Compositions of bovine colostrums, low fat milk and chow

	Bovine colostrum	Low fat milk	Chow
Protein	6.0 g/dL	3.0 g/dL	19.5 g/100 g
Albumin	2.0 g/dL		
Carbohydrate	10 g/dL	5 g/dL	60.6 g/100 g
Fat	< 0.1 g/dL	1.0 g/dL	10 g/100 g
Calorie	64 kcal/dL	40 kcal/dL	4.08 kcal/100g

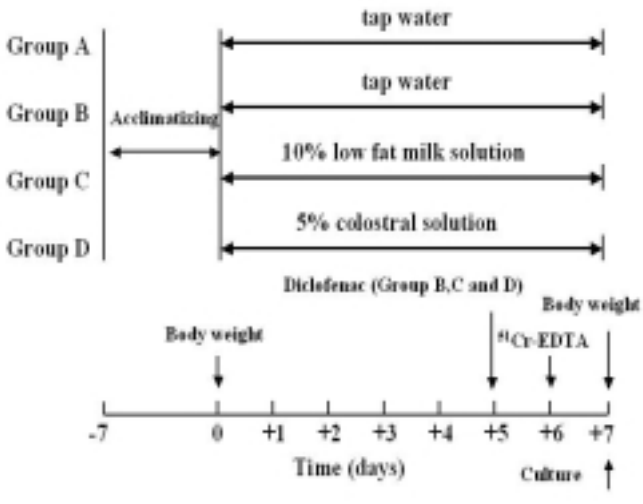

Figure 1. Study design

Measurement of intestinal permeability

Intestinal permeability was assessed by measuring the urinary excretion of ^{51}Cr-EDTA (ethylene diamine-tetraacetic acid) after oral diclofenac administration. 24 hours after the administration of diclofenac, all the animals were given 10 μCi ^{51}Cr-EDTA (in 0.5 mL distilled water) by orogastic gavage and were then placed into metabolic cages. Urine was collected for the following 24 hour period. Animals were allowed access to tap water and food during the collection period. The level of radioactivity in the urine samples was then determined by counting on a gamma spectrometer (Cobra Auto-gamma counter, Packerd Instrument Comp., Meriden, CT, USA). ^{51}Cr-EDTA excretion was quantified by running a blank sample with 10% (50 μL) of the ^{51}Cr-EDTA/H_2O used for gavage in each experiment. The data are expressed as fractional excretion of the radioactive marker because the total amount of the radioactive marker administered orally was known.

Surgical procedures

48 hours after the administration of diclofenac, the rats were anesthetized by ether. The abdomen was opened by a midline incision, and blood was aspirated from the heart and serum was obtained. Serum was frozen at 80°C until the analysis. The animals were killed by exsanguinations. The ileum was cut at the point 20cm proximal to the ileocecal junction and exposed by cutting along the anti-mesenteric border. Three cm lengths of ileum (15-20cm proximal to the ileocecal valve) were placed in 10% buffered formalin for histologic examination. The content was obtained from the ileum (same region as used for the histologic examination) and put into an empty sterile tube to measure the amount of enteric bacteria.

Determination of the amount of enteric bacteria

IIeal contents were weighed and diluted by sterile phosphate-buffered saline. Diluted ileal contents were plated on the sheep's blood and MacConkey's agar culture plates. All culture plates were incubated at 37°C in standard anaerobic chamber and examined for growth at 48 hour. Colony counts were expressed as colony-forming units per gram of ileal contents (CFU/g).

Histologic examination

The samples were embedded in paraffin, cut at 3-5 μm, and stained with hematoxylin and eosin. The pathologist, unaware of the treatment groups, evaluated the severity of the diclofenac induced gut damage in accordance with the following scoring systems: 0, normal; 1, minimal changes in crypt architecture; 2, epithelial disruption; and 3, erosion extending to the muscularis mucosae; 4, ulcers and/or necrosis (all item x 2 if disseminated rather than focal).

Serum biochemical assays

Serum level of total protein, albumin, alanine amino-transferase, alkaline phosphatase and bilirubin were determined with Advia 1650 automated serum analyzer (Bayer Healthcare Co., Ltd, Tarrytown, NY, USA).

Statistical analysis

Each measurement was expressed as mean ± SD. Mann-Whitney *U* test was used to compare the mean values in two groups. A *P* value of <0.05 was considered to be statistically significant.

Results

Body weight and amount of food and water intake

No significant difference was noted in body weight changes among each group. Groups with diclofenac showed decrease in the amount of food, water, protein and calorie intake compared to controls. No significant difference was noted in food, protein and calorie intake among these groups. However, the amount of water intake was increased in the group with diclofeanc and low fat milk compared to the group with diclofenac only and diclofenac with colostrum (Table 2).

Intestinal permeability

The fractional excretion of orally administered ^{51}Cr-EDTA in the control group averaged approximately 4%. Administration of diclofenac resulted in a significantly increased epithelial permeability (approximately 6~7-fold of levels found in the control rats). Simultaneous administration of bovine colostrum reduced the increase in the small intestinal permeability caused by diclofenac (approximately 4-fold of level found in the control rats), but not low fat milk (Fig. 2).

Amount of enteric bacteria

In controls, the number of total and gram negative aerobic bacteria within the lumen varied from ~10^4 to ~10^7 CFU/g in the distal ileum. After the administration of diclofenac, the total and gram negative aerobic bacterial counts in the ileum increased approximately from 100-fold to 10000-fold, whereas with supplements of bovine colotrum an increase of approximately from 20-fold to 500-fold was observed. However, the number of bactera in the ileum did not differ between the group with diclofenac only and the group with diclofenac and low fat milk (Table 3).

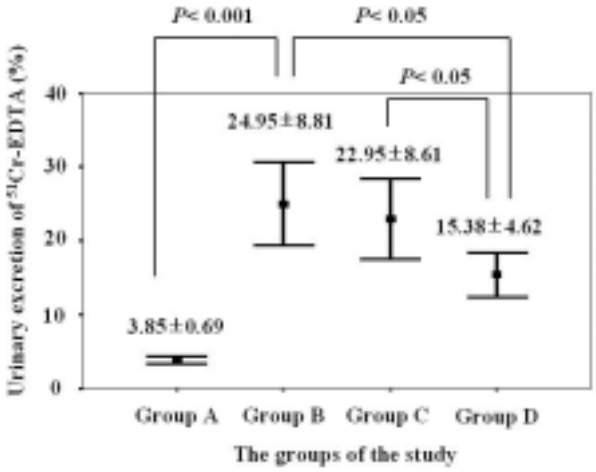

Figure 2. The changes of intestinal permeability measured at 24 hour urinary excretion of ^{51}Cr-EDTA

Serum biochemical parameters

Both total protein and albumin levels declined in animals receiving diclofenac. Simultaneous administration of bovine colostrum reduced the decline of serum total protein and albumin levels. But, supplements with low fat milk had no effect on the changes of serum total protein and albumin levels by diclofenac. Neither serum alanine transaminase nor bilirubin levels increased after the diclofenac administration. These findings indicate that these data is not confounded by hepatic dysfunction (Table 4).

Table 2. Body weight changes and amounts of food and water intakes in the animals

	Trial population			
	Group A	Group B	Group C	Group D
Body weight change (% /7 days)	133.8 ± 3.3	131.7 ± 5.1	133.3 ± 5.6	133.1 ± 4.5
Chow intakes (g/100 g BW /7 days)	131.6 ± 6.3	126.2 ± 4.8	124.6 ± 8.0	126.1 ± 7.1
Water intakes (mL/100 g BW /7 days)	237.5 ± 9.9	223.8 ± 14.8[a]	406.3 ± 22.6[ac]	242.6 ± 10.9[de]
Protein intakes (g/100 g BW /7 days)	15.52 ± 0.78	14.70 ± 0.66[a]	15.19 ± 0.79	15.24 ± 0.91
Calorie intakes (g/100 g BW /7 days)	324.7 ± 16.3	307.6 ± 13.7[a]	312.3 ± 16.4	314.4 ± 19.1

Body weight change was expressed in the percent weight change based on the body weight at operation; BW, body weight; [a]*P* <0.01 compared with group A; [b]*P* <0.01 compared with group A; [c]*P* <0.001 compared with group B; [d]*P* <0.01 compared with group B; [e]*P* <0.001 compared with group C

Table 3. Changes of the number of enteric bacterial numbers in the small intestine

	Trial Population			
	Group A	Group B	Group C	Group D
Total aerobes (log CFU/g)	6.008 ± 0.804	8.752 ± 0.668[a]	8.589 ± 0.606[a]	7.676 ± 0.070[abc]
Gram negative aerobes (log CFU/g)	5.271 ± 0.885	8.373 ± 0.681[a]	8.253 ± 0.705[a]	6.797 ± 1.159[abc]

[a]*P* <0.001 compared with group A; [b]*P* <0.01 compared with group B; [c]*P* <0.01 compared with group C

Histologic findings

The morphology of microdissected villi was determined throughout the distal ileum. Rats receiving diclofenac had markedly shortened and damaged villi in the small intestine. For example, the control rats did not receive diclofenac or colostrum and had slightly tapered villi; rats receiving diclofenac alone had markedly shortened villi with destructive changes; rats receiving diclofenac and colostrum showed much less marked changes of the villi. Supplementation with colostrum resulted in improvement of NSAID induced villous damage of the small intestine. No changes of pathologic findings was noted in the group with low fat milk compared to the group receiving only difclofenac (Table 4).

Discussion

A number of factors have been suggested in the pathogenesis of NSAID enteropathy, including impaired epithelial barrier function, luminal bacteria, bile, and others. Increased intestinal permeability may allow dietary macromolecules, bacteria, bile acids, pancreatic juices, and other intraluminal toxins access to the intestinal epithelium.[4]

Colostrum is the first milk produced after the delivery and is particularly rich in growth factors, immunoglobulins, antimicrobial peptides, and other bioactive molecules.[6,9] Major peptide growth factor constituents of bovine colostrum includes EGF, IGF-I and II, TGF (transforming growth factor)-alpha, TGF-beta family, lactoferrin and others. Among these factors, IGF-I and TGF-beta familiy are present in high concentrations in bovine colostrum compared with bovine milk and human colostrum. IGF-I was used to improve impaired intestinal barrier function induced by sepsis[10] and burn injuries,[11] and to increase the uptake and utilization of glutamine by the bowel.[12] TGF-beta has been shown to reduce NSAID-induced gastric injuries.[7] Bovine colostrum contains several antimicrobial factors, such as immunoglublins, lactoferrin, lactoperoxidase and lysozyme, which produce both specific and non-specific bacteriostatic and bactericidal effects on many pathologic microorganisms, including bacteria, viruses and fungi.[9,13]

In our study, diclofenac has been found to induce the gut barrier damage and marked atrophy of the intestinal villi with destructive changes, and to decrease the average damage score of the distal small intestine. Bovine colostrum supplements have been found to reduce the gut barrier dysfunction and intestinal villous damage with reduction of the damage score. NSAID induced declines in the serum total protein and albumin level were regarded as indices of the intestinal vascular leakage, and previous studies reported the serum albumin to be a readily measured endogenous index of acute intestinal leakage in NSAID induced enteropathy.[14] Diclofenac caused decrease in serum protein and albumin levels, bovine colostrum prevented hypoproteinemia and hypoalbuminemia without increasing the value of the protein and calorie intake, and serum biochemical profiles of the liver function. These findings have shown that bovine colostrum ameliorates NSAID induced protein-losing enteropathy. This protective effect of bovine colostrum may be associated with the beneficial proliferative effect of colostrum being rich in growth factors. A study reported that after diclofenac administration, the number of gram negative aerobic bacteria increased significantly in the small intestine, but the number of anaerobic bacteria within the small intestine was not significantly affected.[15]

In our study, bovine colostrum containing abundant antimicrobial components has been shown to reduce the diclofenac induced bacterial overgrowth of aerobic enteric bacteria, especially of gram negative bacteria. The protective effects of bovine colostrum in respect of the gut barrier damage and enteric bacterial overgrowth may be used to reduce bacterial translocation by NSAID. Bacterial translocation is a term to describe the passage of both viable and non-viable microbes and microbial products from the intestinal lumen to extra-intestinal sites. Factors that promote bacterial translocation include overgrowth with gram-negative enteric bacilli, impaired host immune defenses, and loss of the mucosal barrier function.[16] Bacterial translocation caused by NSAID has been proposed as an triggering mechanism of immune stimulation and alleviation of the disease in decompensated chronic heart failure.[17]

In summary, combined administration of bovine colostrum has been demonstrated to reduce the gut barrier damamge, overgrowth of enteric aerobic bacteria, protein lost in the intestine and the mucosal villous damage of the small intestine induced by NSAID. The present results suggest that bovine colostrum may be used as a prophylactic agent against NSAID induced small intestinal injuries and bacterial translocation. Certainly, further studies

Table 4. Values of the results of serum biochemistry and pathologic examinations 2 days after administration of diclofenac

	Trial population			
	Group A	Group B	Group C	Group D
Total protein (g/dL)	5.40 ± 0.18	4.40 ± 0.40[a]	4.43 ± 0.53[a]	5.08 ± 0.35[bce]
Albumin (g/dL)	3.60 ± 0.16	2.87 ± 0.24[a]	2.83 ± 0.38[a]	3.21 ± 0.20[ace]
ALT (IU/L)	28.6 ± 5.8	27.1 ± 5.8	26.2 ± 4.6	27.5 ± 3.7
ALP (IU/L)	194.8 ± 28.9	118.8 ± 29.6[a]	118.3 ± 51.1[a]	113.5 ± 19.9[a]
Total bilirubin (mg/dL)	0.14 ± 0.09	0.19 ± 0.08[a]	0.21 ± 0.08	0.17 ± 0.09
Pathologic score (point)	0.3 ± 0.5	6.0 ± 1.9[a]	5.5 ± 1.7[a]	4.3 ± 1.7[ad]

ALT, alanine aminotransferase; ALP, alkaline phosphatase; [a]P <0.001 compared with group A; [b]P <0.05 compared with group A; [c]P <0.01 compared with group B; [d]P <0.05 compared with group B; [e]P <0.05 compared with group C

studies are needed to determine the effect of bovine colostrum in patients undergoing long term NSAIDs therapy.

Acknowledgement
We are grateful to Dr. Richard Cockrum (Immuno Dynamics, Ltd., Perry, IA, USA) for his helpful discussions.

References

1. MC Allison, AG Howatson, CJ Torrance, FD Lee, and RI Russell. Gastrointestinal damage associated with the use of non-steroidal anti-inflammatory drugs. N Engl J Med 1992; 327: 749-754.
2. Bjarnason I, Zanelli G, Smith T, Prouse P, Williams P, Smethurst P, Delacey G, Gumpel MJ, Levi AJ. Non-steroidal anti-inflammatory drug-induced intestinal inflammation in humans. Gastroenterology 1987; 93: 480-489.
3. Hond ED, Peeters M, Hiele M, Bulteel V, Ghoos Y, Rutgeerts P. Effect of glutamine on the intestinal permeability changes induced by indomethacin in humans. Aliment Pharmacol Ther 1999; 13: 679-685.
4. Davies NM, Saleh JY, Skjodt NM. Detection and prevention of NSAID-induced enteropathy. J Pharm Pharm Sci 2000; 3: 137-155.
5. Davies NM. Review article: non-steroidal anti-inflammatory drug-induced gastrointestinal permeability. Aliment Pharmacol Ther 1998; 12: 303-320.
6. Playford RJ, Macdonald CE, Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. Am J Clin Nutr 2000; 72: 5-14.
7. Playford RJ, Floyd DN, Macdonald CE, Calnan DP, Adenekan RO, Johnson W, Goodlad RA, Marchbank T. Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. Gut 1999; 44: 653-658.
8. Playford RJ, MacDonald CE, Calnan DP, Floyd DN, Podas T, Johnson W, Wicks AC, Bashir O, Marchbank T. Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability. Clin Sci (Lond) 2001; 100: 627-633.
9. van Hooijdonk AC, Kussendrager KD, Steijns JM. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. Br J Nutr 2000; 84 Suppl 1: S127-134.
10. Chen K, Okuma T, Okamura K, Tabira Y, Kaneko H, Miyauchi Y. Insulin-like growth factor-I prevents gut atrophy and maintains intestinal integrity in septic rats. JPEN J Parenter Enteral Nutr 1995; 19: 119-124.
11. Shimoda N, Tashiro T, Yamamori H, Takagi K, Nakajima N, Ito I. Effects of growth hormone and insulin-like growth factor-1 on protein metabolism, gut morphology, and cell-mediated immunity in burned rats. Nutrition 1997; 13: 540-546.
12. Balteskard L, Unneberg K, Mjaaland M, Jenssen TG, Revhaug A. Growth hormone and insulinlike growth factor 1 promote intestinal uptake and hepatic release of glutamine in sepsis. Ann Surg 1998; 228: 131-139.
13. Korhonen H, Marnila P, Gill HS. Milk immunoglobulins and complement factors. Br J Nutr 2000; 84 Suppl 1: S75-80.
14. Atchison CR, West AB, Balakumaran A, Hargus SJ, Pohl LR, Daiker DH, Aronson JF, Hoffmann WE, Shipp BK, Treinen-Moslen M. Drug enterocyte adducts: possible causal factor for diclofenac enteropathy in rats. Gastroenterology 2000; 119: 1537-1547.
15. Reuter BK, Davies NM, Wallace JL. Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohepatic circulation. Gastroenterology 1997; 112: 109-117.
16. Berg RD. Bacterial translocation from the gastrointestinal tract. Adv Exp Med Biol 1999; 473: 11-30.
17. Rauchhaus M, Sharma R, Bolger A. NSAIDs, intestinal cell integrity, and bacterial translocation in chronic heart failure. Arch Intern Med 2000; 160: 3004-3005.