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Diet Supplemented with Whey Protects Balb/c Mice from Indomethacin-Induced Gut Injury

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The discovery of whey as a functional food exerting health promoting activity elevated it from a waste-by- to a co-product in the manufacturing of cheese. Many of its bioactive components are being investigated for their anti-inflammatory, immunomodulatoty and prohealing effects. Nonsteroidal anti-inflammatory drugs are undoubtedly effective in the treatment of rheumatic diseases, but cause gastrointestinal injury. We examined the efficacy of whey on indomethacin-induced gut damage, using a mouse model of small intestinal injury. The villus morphology of jejunum and ileum in experimental and control animals was studied by scanning electron microscopy, light microscopy and morphometric measurements. Our results indicated that diet supplemented with whey prevented distortion, epithelial lesions and shortening of the villi in indomethacin treated mice. We suggest that the beneficial effect of whey on gut mucosa is mainly due to the abundant growth factors and antimicrobial peptides.

Key words: bioactive peptides, indomethacin, small intestinal damage, villus morphology.

Introduction

Millions prescriptions dispensed each year play an important role in managing diseases. However, increasing incidence of drug-induced complications are associated with medications' adverse effects. The gastrointestinal tract is commonly the site of these iatrogenic adverse effects.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are some of the most commonly prescribed medicines worldwide. They are undoubtedly effective in the treatment of rheumatic diseases. The main concern with these drugs is the frequency of their digestive side effects including bleeding and perforation of the stomach and intestine.

Emerging studies are recently defining potential therapeutic roles for specific nutrients and their compounds in gut mucosal turnover, repair and barrier function. Results of these investigations define new nutritional methods for trophic and cytoprotective impact on the intestine in different gastrointestinal conditions such as NSAIDs-induced enteropathy.

Milk is the first functional food available to mammals. The high nutritional value and multiple functional and biological properties of milk components are widely recognized. It contains two primary sources of protein, the caseins and whey. After processing, the

caseins are the proteins responsible for making curds, while whey remains in an aqueous environment, having a protein concentration of about 65%. Whey has long been considered by the dairy industry to be a waste-by product and thus, a disposal problem. Its discovery as a functional food with nutritional applications elevated the whey to a co-product in the manufacturing of cheese. Today many of its bioactive components (α-lactalbumin, β-lactoglobulin, lactoferrin, lactoperoxydase, immunoglobulins, glycomacropeptide and a variety of growth factors) are being investigated for their anti-inflammatory, immuno-modulatory and prohealing effects.

Mucosal integrity is maintained when the damaging effects of aggressive factors are counter-balanced by mucosal defense mechanisms. These include a high rate of cellular turnover, an efficient mucosal blood flow, a continuous adherent mucous layer and a presence of growth factors that can directly stimulate repair and also influence all of the other protective factors. The increasing number of growth factors with intestinotrophic activity raises the possibility that one or more of these peptides may be useful for prevention and regeneration of intestinal epithelium in diseases characterized by intestinal injury.

Major peptide growth factor constituents of bovine colostrum, milk and whey are: transforming growth factors α and β (TGF-α and TGF-β), insulin-like growth factors I and II (IGF I and IGF II), epidermal growth factor (EGF), platelet —derived growth factor (PDGF) and several other peptides which structure and function are less clearly defined [5].

Orally introduced growth factors stimulate the gut repair process at the sites of injury [13]. H o w a r t h et al. [5] found that growth factors from cheese whey ameliorated chemotherapy-induced mucositis in rats. P o r t e r et al. [12] also observed reduced colonic inflammation in experimental colitis in rats after administration of whey-growth factors.

The aim of our study was to examine the potential value of whey in preventing and/or treating small intestinal damage using a mouse model of indomethacin-induced gut injury.

Material and Methods

Fresh cheese whey was obtained from dairy Purshevitsa in Vratsa region, pasteurized (72°C for 15 s) before microfiltration through a 0.2-μm pore membrane to remove fat; then it was frozen and stored at –20°C. The whey solution was used as a substitute for drinking water.

Mouse small intestinal injury model

BALB/c mice, aged 3-4 months and weighing 25-30 g were randomized into 2 experimental groups of 6 animals and fed on a standard chow diet. The drinking water of group 1 was replaced by whey for 7 days before and an additional 1 day concomitant with indomethacin application. Our pilot studies showed that the total volume drunk was the same in the two experimental groups (mean 5 ml/mouse/day). Indomethacin (China Taicang Pharmaceutical Factory) was suspended in saline with a trace of Tween 80 to facilitate dispersion. According to the method of E t t a r h and C a r r [3] small intestinal injury was induced to the 2 experimental groups by two subcutaneous injections of indomethacin (85mg/kg) separated by 20 hours. However, using the same protocol in our pilot studies, high mortality rate due to severe gastrointestinal bleeding was obtained. We therefore administered a single dose of indomethacin to the two experimental groups. This reduced dosage caused a marked decrease in villus height, epithelial lesions and altered villus architecture in indomethacin treated mice. Two control groups (n=6) were included in our experiments: the first group

received whey without indomethacin (K1) and the second group received neither whey, nor indomethacin (K2). All mice were sacrificed 20 hours after indomethacin application, as it was shown that this was the time of maximal gut damage [10]. Animal procedures were done after the rules of animal ethics committee.

Scanning electron microscopy (SEM)

Small intestinal fragments from jejunum and ileum, defined by their percentage length [10] were prepared for SEM according to the method of Potter and Collins [11] with some modifications. Jejunal and ileal segments were thoroughly rinsed with cold PBS, fixed with 2% glutaraldehyde in 0.05M phosphate buffer, pH 7.4, washed with 0.1M phosphate buffer, pH 7.4 with 0.15M sucrose added, post-fixed with 1% Osmium tetraoxide in 0.05M phosphate buffer, dehydrated through an ascending ethanol series, critical point dried, and finally coated with gold. The examination was performed on a JEOL JSM-35 scanning electron microscope under different magnifications: from ×100 to ×860. The mucosal surface, predominant villus pattern and shape in jejunum and ileum of animals from the experimental and control groups were studied. The most characteristic features, typical for the group, were shown and analyzed.

Light microscopy and morphometry

The contents of the small intestine were flushed thoroughly with saline, and 2 cm segments from jejunum and ileum were cut, fixed in 10% buffered formalin and embedded in paraffin, using standard techniques. Cross-sections from jejunum and ileum of each mouse were cut and stained with hematoxylin and eosin. Light microscopy and morphometric measurements were performed under light microscope (Karl Zeiss Jena) equipped with a micrometer at a magnification of 10. Only complete in continuity longitudinal sections of villi were evaluated from the villus tip to the intervillus region. Morphometric measurements were performed as ten well oriented villi were measured for villus height and the mean values were determined for each animal both at jejunal and ileal sites. The mean values ± standard error of the mean were then computed for each group. The statistical significance of differences observed between groups was assessed using Student's t-test.

Results and Discussion

SEM: The examination of mucosal surface of jejunum and ileum showed that animals from control groups had leaf-like upright villi, usually uniform in height (Fig. 1 and Fig. 2). In the same parts of the small intestine of indomethacin-treated mice we found that most of the villi lost their leaf-like pattern and uprightness, some were bent or tilted (Fig. 3). In higher magnification epithelial lesions were apparent (Fig. 4). The drug treated and whey supplemented mice had, however, markedly reduced morphological changes in mucosal surface. In Fig. 5 almost normal appearance of villus morphology could be seen. In some of the specimens irregular pattern of the villi was observed–high and thin villi were alternated with small and thick ones.

Light microscopy and morphometry:

The shortening of the small intestinal villi is one of the typical features of early NSAID damage. We found that whey supplementation had no effect on villus height in animals that did not receive indomethacin. All mice from control groups (K1 and K2) had long,

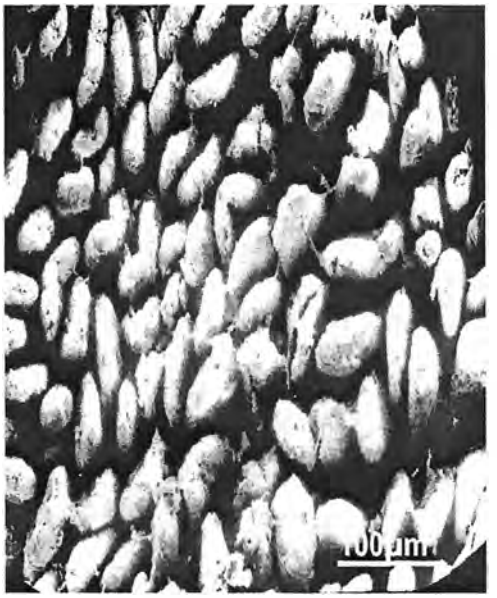

Fig. 1. Representative scanning photomicrograph of ileum, control group, bar =100 μm

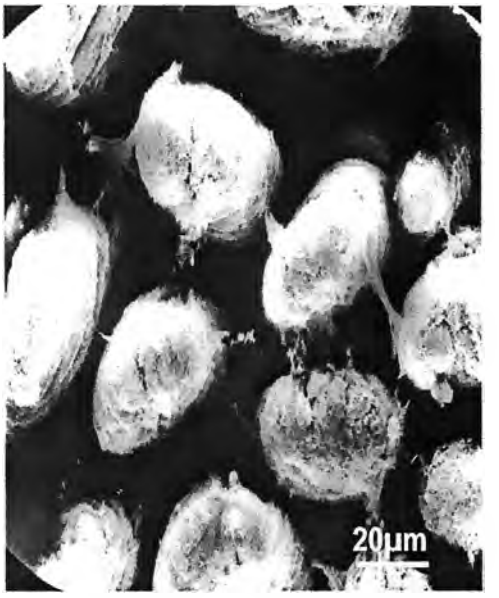

Fig. 2. Representative scanning photomicrograph of ileum, control group, bar=20 μm

Fig. 3. Representative scanning photomicrograph of ileum, indomethacin-treated group, bar=20 μm

Fig. 4. Representative scanning photomicrograph of ileum, indomethacin-treated group, bar=10 μm

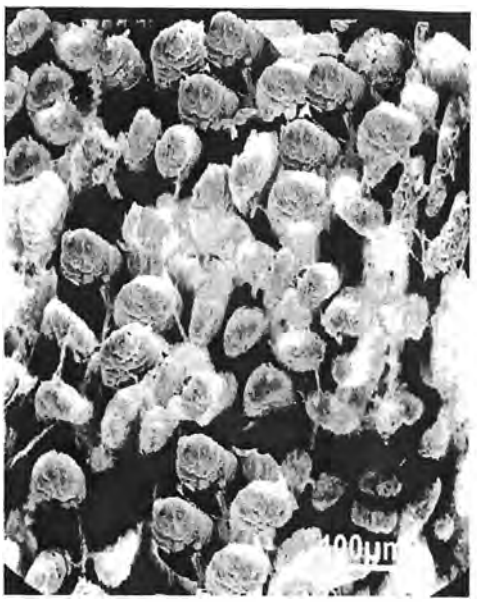

Fig. 5. Representative scanning photomicrograph of ileum, indomethacin-treated and whey supplemented group, bar=100 μm

Fig. 6. Representative photomicrograph of hematoxylin-eosin stained transverse section from jejunum of control mice. × 40

slightly tapering villi, almost uniform in height (Fig. 6). Indomethacin treated animals had however markedly shortened and buckled villi with flat tips and irregular pattern (Fig. 7). In mice received whey supplementation indomethacin caused much less marked changes to the villi — the shortening was hardly discerned and the villus tips looked more rounded (Fig. 8). The data from morphometric measurements showed statistically significant dif-

Fig. 7. Representative photomicrograph of hemato-xylin-eosin stained transverse section from jejunum of indomethacin-treated mice. × 40

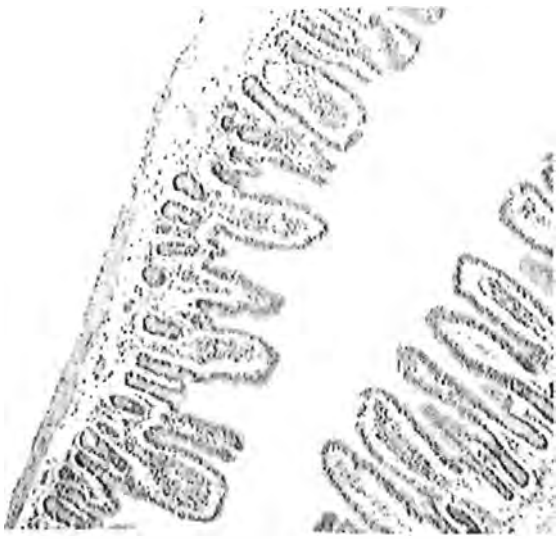

Fig. 8. Representative photomicrograph of hemato-xylin-eosin stained transverse section from jejunum of indomethacin-treated and whey supplemented mice. × 40

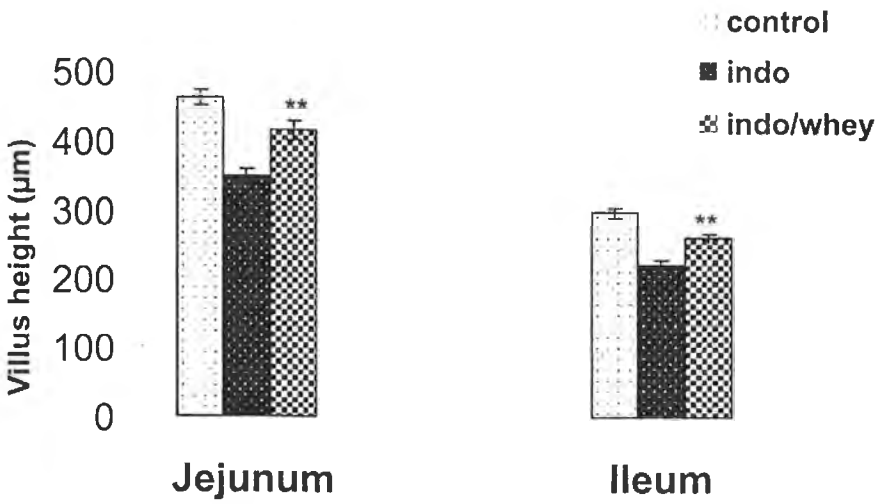

Fig. 9. Villus height in jejunum and ileum of experimental and control mice. The significant difference between whey supplemented and indomethacin-treated and only indomethacin treated groups of mice is denoted (**p<0.01; t-test)

ferences in jejunal villus height among experimental groups (Fig. 9) The villi of indomethacin treated and whey supplemented mice were significantly higher (414±11.32μm), than those of animals received indomethacin alone (348±10.59μm)(p<0.01). In both treatment groups indomethacin caused decrease in villus height, compared to controls (460±14.00μm).

A similar tendency showed the data obtained from ileal samples. Indomethacin treated and whey supplemented mice had significantly greater villus height (260±7.71μm) than animals, which received indomethacin alone (222±5.30μm) (p<0.01). In both treatment groups villus height was decreased in comparison with controls (296±12.50μm).

At both the jejunal and ileal sites indomethacin caused about 25% reduction in the villus heights, compared with control mice. Animals that received whey, however, had only about a 10% reduction in villus height for jejunum and 12% for ileum (Fig. 10).

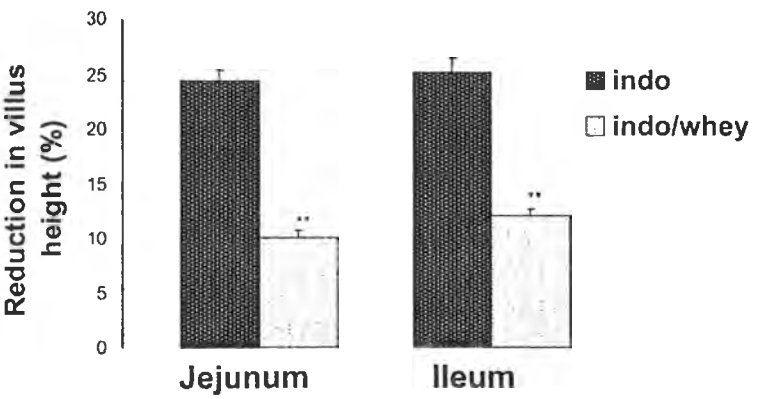

Fig. 10. Reduction in villus height (%) in jejunum and ileum of experimental and control mice The significant difference between whey supplemented and indomethacin-treated and only indomethacin treated groups of mice is denoted (**p<0.01; t-test)

The balance between aggressive factors and mucosal defense mechanisms supports gastrointestinal mucosal integrity. After acute mucosal injury, the rapid recover is vital to prevent extension of the damage due to luminal acid and proteolytic enzymes, bacteria and their products. One of the earliest healing responses is the stimulation of cell migration over the denuded area to reestablish epithelial continuity. It begins within the first hour following injury and is termed restitution. The maintenance of this process is complex and depends mainly on local and systemically derived growth factors, prostaglandins, endogenous gut flora and mucins.

NSAIDs such as indomethacin, contribute to multiple disruptions in homeostatic mechanisms that control the integrity of the mucosal epithelium. They cause damage to the gastrointestinal tract by reduction of protective mucosal prostaglandin levels, reduction of mucosal blood flow, stimulating neutrophil activation and possibly, stimulating apoptosis [7].

The detailed mechanism by which whey reduces the degree of damage in small intestines is unclear, although the findings that growth factors stimulate epithelial cell migration at wound sites and promote restitution, provide some possible explanation. TGF-alpha and TGF-beta are reported to stimulate epithelial cell migration at wound sites [6], but some studies suggest that the major physiologic role of TGF-alpha is to act as a mucosal-integrity peptide maintaining normal epithelial function in the non-damaged mucosa [9]. B e r l a n g a et al. [1] demonstrated that EGF reduced intestinal damage in vivo. Data from the medical literature suggest that more than one peptide is likely to be involved as synergistic activity between various factors has been observed [4]. It would therefore seem reasonable to hypothesize that the application of mixtures of growth factors may be more effective than the addition of a single factor. Bovine whey is a rich source of several classes of growth factors and it contains the bulk of their activity present in milk. However it is impossible to rule out effects on indomethacin-injured mucosa by components of whey that are not growth factors.

The role of bacteria in the pathogenesis of indomethacin-induced gut injury has been demonstrated by the study of M e l a r a n g e et al. [8] in which indomethacin provokes very few macroscopic lesions in germ free animals, and by B j a r n a s o n et al. [2], who found that pretreated with antibiotics animals showed decrease in NSAID's induced enteropathy. Due to the significant participation of luminal bacteria in the pathogenesis of indomethacin —induced gut injury, the marked reduction of alterations in small intestinal villi of whey supplemented mice may be also attributed to the antimicrobial peptides. Lactoferrin, lactoperoxidase, lyzozyme (major non-specific antimicrobial components) and immunoglobulins with their specific bacteriostatic and bactericidal activity may have a direct effect on luminal bacteria.

In summary, whey supplementation significantly improved morphological features of disease activity in indomethacin-induced intestinal injury. These actions are mediated probably by protection of injury and also by increasing of epithelial repair. The present study defines a new approach for trophic and protective effects on the gut and may also be of value in other ulcerative conditions of the bowel.

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