Peptide therapy and the gastroenterologist: colostrum and milk-derived growth factors

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Abstract—Colostrum is the specialized milk produced for the first few days following parturition. It is much richer in immunoglobulins, antimicrobial peptides and growth factors than the subsequent mature milk. In this article, some of these constituents of human and bovine colostrum in comparison with mature milk are reviewed. Marked species differences exist in the constituents of both colostrum and mature milk. Recent studies suggest that colostral fractions, or individual peptides present within colostrum, might be useful for the treatment of a wide variety of gastrointestinal conditions including inflammatory bowel disease, non-steroidal anti-inflammatory drug-induced gut injury and chemotherapy-induced mucositis. The relative merits of using colostral fractions as opposed to an individual recombinant peptide for the treatment of these various conditions are discussed. © 2001 Harcourt Publishers Ltd.

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Introduction*

Colostrum is the first milk produced after birth and is particularly rich in immunoglobulins, antimicrobial peptides (e.g. lactoferrin, lactoperoxidase) and other bioactive molecules including growth factors. In combination with the milk that is subsequently produced, it is important for the nutrition, growth and development of the newborn infant, and contributes to the immunological defence of the neonate. There are continuous changes in the composition of the mammary secretions throughout the suckling period; however, for the purposes of this review colostrum is considered as the milk produced in the first 48 h after birth.

Recent studies suggest that the growth factors contained within colostrum might provide novel therapeutic opportunities to treat a variety of gastrointestinal medical conditions. Some of the bioactive constituents of human and bovine colostrum in comparison with the milk produced later post-partum and some of the specific properties of the numerous growth factors that have been identified within the colostrum and milk are discussed. In addition, the therapeutic possibilities of using whole colostrum, or individual peptides present within the colostrum, for the treatment of various gastrointestinal diseases and the relative merits of using the two approaches are explored.

Constituents of colostrum

Growth factors are so called because historically they were identified by their ability to stimulate growth of various cell lines in culture situations in vitro but, in reality, the functions of these peptide-based molecules are considerably more diverse. Various nomenclatures have been ascribed to molecular species as they have been identified. As characterization has become more sophisticated, however, it is apparent that some of these differently named species are structurally and functionally similar and may, in fact, be identical. Although there are many similarities, there are also marked species differences in the nature and concentration of growth factor constituents: for example human colostrum has much higher concentrations of epidermal growth factor (EGF) than the bovine equivalent whereas the reverse is true for insulin-like growth factors I and II. Further details of individual peptides that form the major growth factor constituents of colostrum and milk are given below with the various members of related peptides grouped together.

EGF receptor ligand family

This group of polypeptides, with the common property of binding to the EGF receptor (also known as the c-erbl receptor), includes EGF itself, transforming growth factor-α (TGF α), mammary derived growth factor II (MDGF-II) and human milk growth factor III (HMGF-III, which might be the same molecule as EGF, see later). Other related polypeptides with these binding characteristics, but which are not present in significant amounts in colostrum, are the transforming growth factor-β (TGF β), bone morphogenetic protein 2 (BMP-2) and fibroblast growth factor 2 (FGF-2).
concentrations in colostrum, include amphiregulin, betacellulin and heparin-binding EGF (for fuller review of these peptides see 1).

Epidermal growth factor (EGF)
EGF is a 53 amino-acid peptide produced by the adult salivary glands and also the Brunners glands of the duodenum. Although EGF is present in human colostrum (200 ng/ml) and milk (30–50 ng/ml) and in many other species, it is not found in bovine secretions (2), although related molecules have been identified and characterized. In vitro experiments using gastric juice from preterm infants indicate that milk-borne EGF is not deactivated under typical gastric proteolytic conditions (3). In contrast, we have shown that adult gastric juice digests EGF₁₋₅₃ to a EGF₁₋₄₉ form which only has 25% of the biological activity of the intact EGF molecule (4). Once EGF enters the small intestine, it is susceptible to proteolytic digestion under fasting conditions but it is preserved in the co-presence of ingested food proteins (5).

There is currently much controversy over the function of EGF present within the gastrointestinal lumen under physiological conditions. Recent studies in the normal adult human gastrointestinal tract have shown that the EGF receptor is only present on basolateral membranes and is not present on the apical (luminal) surfaces (6). EGF present within the intestinal lumen will therefore not exert biological activity except at sites of injury. Evidence in favour of this role for EGF include the finding that rats which had their salivary glands removed do not get spontaneous ulcers or atrophy of the gut, but do develop increased ulceration and diminished repair if artificial ulcers are induced (7). This has led to the suggestion that EGF acts as a ‘luminal surveillance peptide’ readily available to stimulate the repair process at sites of injury (8). It is important to note, however, that luminal EGF might gain access to basolateral receptors in the immature neonate gut (9).

Transforming growth factor-alpha (TGFα)
TGFα is a 50-amino acid molecule which is present in human colostrum and milk at much lower concentrations than EGF (2.2–7.2 ng/ml), (10). In contrast to EGF, TGFα is produced within the mucosa throughout the gastrointestinal tract (11). Systemic administration of TGFα stimulates gastrointestinal growth and repair, inhibits acid secretion, stimulates mucosal restitution after injury and increases gastric mucin levels (1).

Within the small intestine and colon, TGFα expression mainly occurs in the upper (non-proliferative) zones, which suggests that its physiological role may be to influence differentiation and cell migration rather than cell proliferation. TGFα may therefore play a complementary role with TGFβ (see later) to control the balance between proliferation and differentiation in the intestinal epithelium (12). Upregulation of TGFα expression has been shown to occur in the gastrointestinal mucosa at sites of injury as well as in the liver following partial hepatectomy, supporting a role for TGFα in mucosal growth and repair (13). Further evidence for this role comes from research in mice who have had the TGFα gene ‘knocked out’ using homologous recombination. They have a relatively normal phenotype under control conditions but an increased sensitivity to colonic (14), although not small intestinal (15), injury. These findings support the role of TGFα in maintaining epithelial continuity but suggest that the relative importance of peptides such as this might vary from one region of the gut to another. Taken together, most studies suggest the major physiological role of TGFα is to act as a ‘mucosal integrity peptide’ maintaining normal epithelial function in the nondamaged mucosa (8).

Other peptides within this family
These include the bovine mammary-derived growth factor II (MDGF-II) (16), and human milk growth factor III (HMGF-III). HMGF-III has a molecular weight of approximately 6 kDa and is the major growth factor present in human milk, accounting for approximately 75% of total mitotic activity (17). There is uncertainty as to whether HMGF-III is a distinct molecule or is, in fact, EGF.

Transforming growth factor β family
This family of molecules are structurally distinct from TGFα and, in most systems, actually inhibit proliferation. There are at least five different isoforms of TGFβ and their major site of expression in the normal gastrointestinal tract is in the superficial zones where they may function to inhibit proliferation once the cells have left the crypt region (12). TGFβ has many diverse functions, which include being a potent chemo-attractant for neutrophils and stimulating epithelial cell migration at sites of wounds (18). It is therefore likely to be a key player in stimulating restitution, the early phase of the repair process where surviving cells from the edge of a wound migrate over the denuded area to re-establish epithelial continuity. TGFβ and TGFβ-like molecules are present in high concentrations in both bovine milk (1–2 mg/l) and colostrum (20–40 mg/l). These concentrations are sufficient to prevent indomethacin-induced gastric injury in rats (19), suggesting that TGFβ in colostrum may be a key component in mediating its ability to maintain gastrointestinal integrity in the suckling neonate.

A TGFβ-like milk growth factor (MGF) has been described as being associated with the casein fraction of cows’ milk; this has since been shown to be a mixture of TGFβ1 and β2, predominantly the β2-form (85%) (20).
Insulin-like growth factors (somatomedins)

IGF-I and IGF-II promote cell proliferation and differentiation (21). They are similar in structure to pro-insulin and it is possible that they also exert insulin-like effects at high concentrations. The liver is a major site of IGF synthesis (22) and IGF-I and II are both also expressed at particularly high levels in the developing human foetal stomach and small intestine with expression reaching a maximum soon after birth (23).

Bovine colostrum contains much higher concentrations of IGF-I than human colostrum (500 vs 18 ng/ml) (24, 25) with lower levels being present in mature bovine milk (10 ng/ml) (26). These growth factors are relatively stable both to heat and acid conditions and therefore survive the harsh conditions of both commercial milk processing and gastric acid to maintain their biological activity (27). IGF-I is known to promote protein accretion, i.e. it is an anabolic agent (28) and is at least partly responsible for mediating the growth-promoting activity of growth hormone. IGF-II is present in bovine milk and colostrum at much lower concentrations than IGF-I but, like IGF-I, has anabolic activity and has been shown to reduce the catabolic state in starved animals (29).

Other less well defined peptides

Bovine and human milk contain several other peptides whose structure and functions are less clearly defined. These include:

- Mammary-derived growth factor I; a 62 kDa peptide which has been shown to stimulate growth of mammary cells and enhance collagen production (30, 31).
- Human milk growth factors (HMGF) I and II; acidic polypeptides which are poorly characterized (32).
- Bovine colostral growth factor; this 35 kDa molecule accounts for the major mitogenic activity of bovine colostrum. It appears to be biochemically similar to HMGF-II and possibly also to platelet derived growth factor (32, 33).
- Other bovine mammary derived growth factors (b-MDGF-I and II) have also been described; b-MDGF-I has a molecular weight of about 30 kD and possesses EGF-like activity, while b-MDGF-II is a larger, 50–150 kD, molecule (34).

The existence of several other peptides has been reported. However, some of these have been shown subsequently to be highly homologous with known existing molecules, while for others the details of structure and function are still not elucidated.

Clinical applications for the gastroenterologist

Colostrum, milk or recombinant peptides are unlikely to be of value for the treatment of oesophagitis or peptic ulceration due to the high efficacy of acid suppressants and Helicobacter pylori eradication regimens. There are, however, a number of serious gastrointestinal pathologies where these novel therapies might prove useful.

Short bowel syndrome

Some patients have an insufficient length of bowel to digest and absorb food adequately, usually as a result of massive intestinal resection for vascular insufficiency or following repeated operations for inflammatory bowel disease. Present therapeutic options are unpleasant and associated with a high risk of morbidity or mortality; for example, to instigate long term parenteral nutrition (PN) or, in a few selected cases, to perform small bowel transplantation. Strategies to optimize the function of residual bowel and ultimately wean patients off PN would therefore be of great benefit, and there is some evidence that growth factors could be instrumental in this. Systemic administration of individual growth factors such as EGF has been shown to stimulate bowel growth in rats receiving PN (35). O'Loughlin and co-workers (36) have shown that oral EGF assists in restoring glucose transport and phlorizin binding in rabbits following jejunal resection and studies examining the effect of colostrum supplementation of piglet feeding regimens have shown a significant increase in intestinal proliferation (37). Colostrum supplementation may be of particular value in young children who have undergone intestinal resection as gut adaptation during early childhood may be more responsive than adults.

Non-steroidal anti-inflammatory drug-induced gut injury

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed and effective for the treatment of musculo-skeletal injury and chronic arthritic conditions. Nevertheless, approximately 2% of subjects taking NSAIDs for one year will suffer from gastrointestinal side effects that include bleeding, perforation and stenosis formation of the stomach and intestine (38). Acid suppressants and prostaglandin analogues have been shown to be effective in reducing gastric injury induced by NSAIDs, but are less efficacious in preventing small intestinal injury. Novel therapeutic approaches to deal with these problems, such as using recombinant peptides, are therefore still required. A recent series of in vivo and in vitro studies support this idea; EGF (4), TGFα and TGFB (39) have all been shown to reduce NSAID-induced gastric injury. The beneficial effect of recombinant growth factors on NSAID-induced small and large intestinal injury is, however, less well documented. It has recently been shown that a defatted colostrum preparation, which is rich in the growth factors discussed earlier, reduces NSAID-induced gastric injury in rats (39). This material has also been shown to be effective in reducing gastric erosions in human volunteers taking NSAIDs (J. Hunter, Addenbrooke's
Hospital, Cambridge, UK, personal communication). Further support for this approach comes from our recent findings that this defatted colostrum preparation reduced small intestinal permeability, used as a marker of intestinal damage, in human volunteers taking clinically relevant doses of the drug indomethacin (40). Clinical trials involving patients taking long-term NSAIDs are presently under way.

Chemotherapy-induced mucositis

Current regimens for the treatment of cancers require patients to take much higher doses of chemotherapeutic agents than were used previously. As a result of this escalation in dosing, toxic side-effects on the bone marrow or gastrointestinal tract can be the factor limiting the dose or duration of treatment. Strategies to protect these tissues and encourage their recovery may facilitate the use of higher dosage with greater potential for cure. Examples include the findings that EGF enhances repair of rat intestinal mucosa damaged by methotrexate (41), TGFβ ameliorates chemotherapy induced mucositis (42) and administration of a cheese whey-derived preparation reduces methotrexate-induced gut injury in mice (43). Not all studies have shown favourable results, however, as EGF had only a minor beneficial effect in reducing mouth ulceration in a phase I clinical study of patients undergoing chemotherapy (44).

If peptides with growth stimulatory or inhibitory effects are to be used, timing of administration is likely to be critical; growth arresting factors might protect bone marrow or gut from the damaging effects of chemotherapy (which tends to affect areas with the highest cell turnover) if given prior to chemotherapy. In contrast, growth-stimulating factors might 'rescue' recovery of injured areas if administered following chemotherapy. This latter approach is already being used clinically as colony stimulating growth factor is used to stimulate bone marrow recovery following chemotherapy.

Inflammatory bowel disease and necrotising enterocolitis

The aetiology of ulcerative colitis and Crohn's disease is unknown, and current treatment of these severe, incapacitating conditions therefore has to be on an empiric basis. Studies examining the effect of administration of EGF, PDGF, TGFβ or IGF-I in animal models of colitis have given encouraging results (45), and a cheese whey growth factor extract containing several of these growth factors has also shown positive results in a similar model (46). Other peptides, not present in milk or colostrum at significant concentrations, which are undergoing study as potential therapeutic agents for these conditions include keratinocyte growth factor (47) and trefoil peptides (48). These are still at very early (animal model) stage and are unlikely to be in standard clinical usage for many years.

Milk-derived products are already in clinical use for the treatment of inflammatory bowel disease. Casein-based enteral feeds are used for the treatment of Crohn's disease and their efficacy might be due, in part, to the presence of mammary-derived growth factors within the preparation which are preserved during the processing of the milk protein (see earlier). In addition, clinical trials of the use of colostrum enemas for the treatment of ulcerative colitis and resistant proctitis are underway presently and results are awaited with interest.

Necrotising enterocolitis is a severe life-threatening illness of young children which causes severe ulceration of the small and large bowel. Its aetiology is unclear and initial treatment consists of general supportive measures including fluid replacement and antibiotic therapy, although intestinal resection is often required. A recent case report indicated that a continuous infusion of EGF had a remarkable restorative effect on gut histology (49) and a larger clinical trial is ongoing.

Liver disease

The liver is capable of remarkable regeneration and can regrow to normal size despite having over 60% of its total mass removed. Regeneration is also potentially possible after an even greater degree of damage or resection, but artificial liver support (analogous to renal dialysis) would be required during the regeneration period. If such a machine were available to maintain patients for the few weeks following massive resection, it would lead to the possibiility of curative surgery for patients with colonic carcinoma and liver metastases. As part of such an approach, administration of growth factors to accelerate liver regeneration would obviously be helpful. Until now, studies have been restricted to animal models, e.g. EGF has been shown to increase hepatocyte regeneration following partial hepatectomy in rats (50).

Recombinant peptides may also be beneficial in reducing injury and stimulating repair of liver function following exposure to hepatotoxins. For example, EGF has been shown to reduce the amount of hepatotoxicity induced by the toxin carbon tetrachloride in rats (51). The mechanisms underlying this protective effect are unclear but may be related to the induction of free-radical scavengers. Further in vivo and in vitro studies are required, however, before embarking on clinical trials.

Infective diarrhoea

The vast majority of cases of infective diarrhoea resolve spontaneously, or occasionally require a short course of antibiotics. For immunocompromised subjects, such as those with human immunodeficiency virus (HIV) infection, prophylaxis against the unusual organisms to
which they are susceptible e.g. Cryptosporidium, may be beneficial. Hyperimmune milk or colostrum preparations have been shown to be of benefit in reducing infection and increasing weight gain in both clinical and veterinary practice. For example, vaccination of cows with specific viruses or bacteria, to produce ‘hyper-immune’ milk, has been shown to be beneficial in the prevention and treatment of enteropathic infections due to Escherichia coli (52) and rotavirus (53). It is also important to note that the use of colostrum for these conditions is likely to stimulate the repair process (due to the presence of the growth factors) and well as eradicate the infection due to the antimicrobial effects of the high levels of immunoglobulins and antibacterial components.

Should we use single recombinant peptides, colostrum, or milk preparations?

Advances in molecular biology techniques now allow the large-scale production of individual recombinant peptides. Some of these have already found a place in clinical practice, e.g. erythropoietin for the treatment of renal failure induced anaemia and interferon for the treatment of viral hepatitis. The use of growth factors for gastrointestinal disease is, however, at a much earlier stage of development (54).

Although the potent growth factor activity of many of these peptides appears advantageous for stimulating the repair process, there is concern over their potential risks. Systematically administered growth factors could, potentially, induce proliferation in other regions of the body which harbour pre-malignant cells. In contrast, luminally administered growth factors, given orally or via enema, could be delivered at much higher local concentrations. A further advantage of luminal administration is that a proliferative response could be specifically targeted to affect only injured areas. This could be achieved by giving a growth factor, such as EGF, whose receptors are normally restricted to basolateral membranes, as it is only at sites of injury that these receptors would be exposed. If luminal administration of growth factors are to be effective, they must be protected from proteolytic digestion in the stomach and intestine (5). Possible strategies would be to deliver the growth factors in site-specific delivery formulations, to co-administer acid suppressants to reduce proteolytic digestion within the stomach (4) or to co-administer proteins which would act as competitive substrates for the proteolytic enzymes, milk proteins such a casein being particularly beneficial in this effect (5).

Until recently, most research has focused on using a single peptide for the treatment of a particular condition. There is now increasing evidence, however, that administration of multiple peptides can result in additive or synergistic activity. Examples include the findings that growth hormone and IGF-I have additive effect on stimulating anabolism (55) and that co-administration of EGF with trefoil peptides have synergistic activity in preventing NSAID-induced gastric injury (56). Orally-administered colostrum-derived preparations therefore appear to be an attractive therapeutic option as they contain multiple growth factors in a formulation which provides inherent protection against proteolytic digestion.

Current farming methods provide the possibility of producing large amounts of bovine colostrum for clinical use. It would clearly be important that batch variations in production were kept to a minimum to ensure consistency between treatments, and that processing methods can be used or, if necessary, developed to prevent deactivation. The use of such preparations also has the advantage of being perceived as providing ‘natural’ products that might result in greater patient acceptance and compliance. There is also the scope to develop formulations specifically tailored for individual conditions, e.g. using a hyperimmune milk or colostrum formulation for the treatment of immunocompromised patients who have gut disease, thereby reducing the incidence of gut infection whilst stimulating gut repair.

In summary, research examining the potential benefits of such colostral-derived preparations for a wide variety of gastroenterological conditions is underway. Early results are encouraging and we envisage that these products will be used in standard clinical management within the next decade.

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