Is Milk-Borne Insulin-Like Growth Factor-I Essential for Neonatal Development?1,2

Douglas G. Burrin

USDA/Agricultural Research Service Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

ABSTRACT Insulin-like growth factor-I (IGF-I), a polypeptide growth factor found in milk, is hypothesized to play a functional role in the growth and development of neonates, particularly the gastrointestinal tract. Considerable evidence, based on direct tracer studies with 125I-labeled IGF-I and measurements of circulating IGF-I concentrations in neonatal animals fed a range of IGF-I doses, indicates that the intestinal absorption of IGF-I and the possible effect on metabolism and somatic growth are negligible. However, studies in neonatal animals indicate that oral administration of pharmacological doses of IGF-I increases small intestinal mucosal growth, whereas oral IGF-I provided within the physiological range may enhance the development of intestinal lactase. Therefore, clinical trials exploring the therapeutic use of oral IGF-I as an intervention for preterm neonates and those with compromised intestinal function seem warranted. However, milk-borne IGF-I may not be essential for normal healthy infants, perhaps because endogenous IGF-I provides a sufficient stimulus for maintenance of gastrointestinal structure and function. Future studies should explore the significance of endogenous IGF-I and whether milk-borne IGF-I may be important under pathological conditions in which the endogenous IGF-I production may be compromised. J. Nutr. 127: 975S–979S, 1997.

KEY WORDS: • insulin-like growth factors • neonates • pigs • intestinal • development

There has been considerable interest in the function of milk-borne growth factors since Klagsburn’s original finding (1978) that human milk stimulates the proliferation of cultured fibroblasts. This observation has led to a concerted effort to isolate and characterize the mitogenic factors present in milk. In the last 15–20 yr, numerous polypeptide growth factors have been found in milk, including insulin-like growth factor-I (IGF-I) (see reviews Grosvenor et al. 1992, Zumkeller 1992). Additional studies have found that both the growth factor content and mitogenic activity in colostrum are high, but that they decrease considerably during the course of lactation (Donovan et al. 1994, Read et al. 1984).

As this area of research has evolved, it has been repeatedly hypothesized that milk-borne growth factors have a functional role in the growth and development of neonates, but identifying the functional significance has met with limited success. The original studies of Widdowson (Widdowson and Crabb 1976, Widdowson et al. 1976) demonstrated the rapid growth of various organs and tissues of suckling neonatal pigs during the first 10 d after birth. Subsequent studies have focused on the growth of the gastrointestinal tract, because this is the first tissue exposed to ingested milk-borne growth factors (Berseth et al. 1983). Moreover, because many animal species, and perhaps preterm human infants, have an enhanced intestinal permeability to macromolecules, in part related to the absorption of colostral immunoglobulins during the early neonatal period, it has been argued that ingested milk-borne growth factors may be absorbed into the blood and thus could conceivably affect the growth and metabolism of peripheral organs. This review will focus on the most recent findings and discuss the potential significance of milk-borne IGF-I in the growth and functional development of neonates, particularly the gastrointestinal tract.

MILK-BORNE VS. ENDOGENOUS INSULIN-LIKE GROWTH FACTOR-I

The presence of immunoreactive IGF-I in milk was first demonstrated in humans (Baxter et al. 1984), and IGF-I has since been identified in the mammary secretions of several species (Donovan et al. 1991, Francis et al. 1986, Simmen et al. 1988). These and other studies (Donovan et al. 1994) demonstrated that the concentrations of both IGF-I and IGF-

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II are severalfold higher in colostrum than in mature milk and that the levels decreased precipitously in the first few days of lactation. An additional truncated form of IGF-I (des-IGF-I) that was originally isolated from bovine colostrum lacks the N-terminal tripeptide (Gly-Pro-Glu), has reduced affinity for the IGF-binding proteins (IGFBP) and hence exhibits 10- to 100-fold greater biological activity than the normal full-length peptide in in vitro bioassays (Francis et al. 1986). A consideration that is particularly relevant, given that preterm human neonates frequently receive formula rather than breast milk, is that IGF-I has not been detected in infant formulas, presumably because it is either destroyed or removed during processing (Nagashima et al. 1990).

The IGFBP also have been identified in human (Baxter et al. 1984), porcine (Donovan et al. 1994, Simmen et al. 1988), bovine (Skaar et al. 1991) and rodent (Donovan et al. 1991) milk. Four (BP-1, BP-2, BP-3, BP-4) of the six known IGFBP have been found in milk, including the 150-kDa IGFBP-3 complex, which contains the IGF-I peptide and the 85-kDa acid-labile subunit. In pigs, total IGFBP binding activity peaks on day 4 of lactation and then declines (Donovan et al. 1994).

Despite the presence of these IGFBP in milk, there is little evidence to indicate whether these IGFBP either serve to protect IGF-I from intestinal proteolytic activity or facilitate its binding to the enterocyte IGF-I receptor.

It is very important to recognize that in addition to the IGF-I ingested in milk or colostrum, the neonatal gastrointestinal tract is also exposed to endogenous IGF-I secreted in saliva (Costigan et al. 1988), biliary fluid (Kong et al. 1995) and pancreatic juice (Chaurasia et al. 1994). Furthermore, the IGF-I concentrations present in gastrointestinal secretions are either similar to or greater than that reported in milk and thus may be quantitatively and functionally significant. Analysis of fetal and adult tissues has shown that both the IGF-I mRNA and peptide are expressed in all regions of the gastrointestinal tract, although they seem to be localized to the mesenchymal cells in the submucosa and lamina propria of the stomach and small intestine (Lund et al. 1986). This observation, coupled with the finding that the abundance of the IGF-I receptor is greater in the crypt vs. villus enterocytes, suggests that IGF-I may exert a local paracrine growth stimulus for mucosal cell proliferation (Laburthe et al. 1988). However, IGF-I receptors are also present in other cell types within the intestinal mucosa, suggesting that circulating IGF-I also may affect intestinal mucosal growth.

The IGFBP are also expressed in gastrointestinal tissues, but their cellular localization and the regulation of their expression are poorly understood, especially in neonates (see review Zumkeller 1992). Some evidence suggests that IGFBP-3 is localized in the lamina propria (Winesett et al. 1995), whereas other data indicate that some intestinal IGFBP are altered by intestinal resection and differentiation. However, as is the case with milk-borne IGFBP, it remains unclear to what extent IGFBP produced within the intestinal mucosa have any functional role in modulation of IGF-I action. Although the presence of IGF-I, the type I receptor and several IGFBP has been demonstrated in various tissues of the gastrointestinal tract of neonates, their regulation by diet, endocrine status or stage of development is largely unknown and remains a critical area for future research.

**ANABOLIC EFFECTS OF INSULIN-LIKE GROWTH FACTOR-I ON GASTROINTESTINAL TISSUES**

In the last 10 y, as quantities of recombinant IGF-I sufficient for in vivo studies have become available, a number of research groups have demonstrated that intestinal growth is particularly responsive to parenteral administration of relatively pharmacological doses of IGF-I and its related analogues in normal weaned rats (Steele et al. 1994) and after intestinal resection (Lemnay et al. 1991), dexamethasone treatment (Read et al. 1992), total parenteral nutrition (Yang et al. 1994) and intestinal transplantation (Zhang et al. 1995). These and other studies indicate that parenteral IGF-I increases both crypt cell proliferation (Potten et al. 1995) and protein synthesis (Lo et al. 1996) and is associated with increased mucosal thickness and intestinal length. Despite the convincing evidence that parenteral IGF-I stimulates intestinal growth, until recently few studies have examined the potentially anabolic effect of orally or enterally administered IGF-I. Although intraluminal infusion of IGF-I has been shown to increase mucosal growth in rats (Olanrewaju et al. 1992), more recent studies with both neonatal pigs (Houle et al. 1996, Xu et al. 1994) and calves (Baumrucker et al. 1994) have shown that oral administration of physiological concentrations of IGF-I in formula results in measurable increases in crypt cell proliferation but no demonstrable increase in intestinal mucosal mass or length. Thus, in contrast to studies in weaned rats showing a marked anabolic effect of parenteral IGF-I, recent studies in neonatal animals suggest that oral administration of physiological doses of IGF-I has relatively modest effects on intestinal growth. These findings are consistent with our research on colostrum-fed neonatal pigs (Burrin et al. 1995), which suggests that the predominant factor affecting gastrointestinal protein synthesis is nutrient intake, and only a modest stimulation of intestinal protein synthesis can be attributed to non-nutritive components, which include IGF-I.

In addition to the possible physiological importance of either colostral or milk-borne IGF-I, however, there also is growing interest in the therapeutic use of recombinant IGF-I to enhance intestinal growth and maturation in preterm infants and neonates with loss of intestinal function as a result of resection, total parenteral nutrition or diarrhea. To establish the therapeutic potential of orally administered IGF-I, we investigated whether oral administration of recombinant human IGF-I (rhIGF-I) in formula would induce intestinal growth in neonatal pigs at a dose comparable to that which induces intestinal growth in weaned rats when given parenterally (Burrin et al. 1996b). The daily oral dose of rhIGF-I we used (3.5 mg/kg body wt) is severalfold greater than the maximum amount of IGF-I normally ingested daily by neonatal pigs consuming either colostrum (~100 μg/kg body wt) or mature milk (~5 μg/kg body wt). This pharmacological dose of rhIGF-I increased small intestinal mucosal growth and was associated with increased mucosal thickness secondary to a lengthening of the villus rather than an increase in crypt depth.

We also recently demonstrated the intestinal trophic effects of orally administered IGF-I ingested naturally in sucking mice (Burrin et al. 1996a). Using a recently developed transgenic mouse model (Hadess et al. 1996), which exhibits targeted overexpression of des(1-3) human IGF-I in the mammary gland, we tested whether increased concentrations of des(1-3) human IGF-I, when ingested naturally in mother’s milk, would result in differences in intestinal growth in suckling neonatal offspring. This transgenic approach should be considered pharmacological, because measurements using a human-specific immunoassay indicate that the concentration of des(1-3) human IGF-I in milk collected from the IGF-I transgenic mice is approximately 50 μg/mL and is more than 1000-fold greater than IGF-I in wild-type mouse milk (approximately 10–50 ng/mL). Consistent with our findings in pigs, measurements of small...
intestinal weight, protein synthesis and villus height in 8-d-old neonatal mice suckled on IGF-I transgenic dams were significantly greater than for mice suckling wild-type dams. The observations from these studies demonstrate that pharmacological doses of IGF-I, whether given parenterally or enterally, stimulate intestinal growth. However, the characteristic effects on intestinal morphology seem to be dependent on the route of administration. Parenteral IGF-I stimulates crypt, lamina propria and villus cell growth. However, when IGF-I is given orally, the increase in mucosal thickness is confined to an increase in the villus height, perhaps because of limited infiltration of luminal IGF-I into the lower crypt region of the mucosa. The cellular mechanism ordinarily ascribed to the cell-proliferative response to IGF-I is its mitogenic effect as a progression factor during DNA synthesis or the S-phase of the cell cycle (Baserga and Rubin 1993). Alternatively, however, it is plausible that IGF-I treatment increases enterocyte lifespan, because IGF-I has been shown to inhibit cellular apoptosis (Harrington et al. 1994). An important area for future research will be to establish to what extent the anabolic effects of local IGF-I are mediated through these two cellular mechanisms, under both pharmacological and normal physiological conditions.

EFFECT OF INSULIN-LIKE GROWTH FACTOR-I ON INTESTINAL DEVELOPMENT AND MATURATION

In contrast to the number of reports describing the effects of IGF-I on intestinal growth, few studies have characterized its effect on intestinal development and maturation. During the neonatal period there are numerous changes in intestinal function, notably the appearance of lactase during the early neonatal period and its subsequent decline associated with weaning. One of the earliest reports of the effect of IGF-I in neonatal rats demonstrated that both oral and intraperitoneal administration increased the specific activity of several intestinal brush-border enzymes, including lactase (Young et al. 1990). Consistent with this, recent studies in neonatal pigs have demonstrated that oral administration of IGF-I increases small intestinal lactase and decreases leucine aminopeptidase activity and does so in a dose-dependent manner within a range of physiological doses (Houle et al. 1996). Results from this study also indicated that the effects of orally administered IGF-I on lactase activity were greater at 14 d than at 7 d of age, and in effect, retarded the normal ontogenic decline in lactase activity. In contrast to these studies with relatively physiological doses of IGF-I (Houle et al. 1996, Young et al. 1990), our studies have not observed any effect of oral pharmacological doses of IGF-I on intestinal lactase activity in either neonatal pigs or mice (Burrin et al. 1996a and 1996b). There are many possible mechanisms that might explain the effect of an oral physiological dose of IGF-I on intestinal lactase. In suckling neonates, the maturation of the intestinal enterocyte during its migration from the crypt to the tip of the villus involves the transcription of the lactase phlorizin hydrolase gene, followed by multiple steps of post-translation processing before the mature form of the enzyme is inserted into the brush border (Danielsen et al. 1984). It has been suggested that the increased enterocyte migration rate associated with weaning contributes to the decreased expression of mature lactase and its specific activity (Tsuboi et al. 1981). Thus, if indeed oral IGF-I affects enterocyte half-life by altering either cell migration rate or apoptosis, this might influence lactase expression. However, it is conceivable that milk-borne IGF-I increases lactase activity by altering any one of these critical cellular events during maturation of the enterocyte. The finding that oral IGF-I may increase intestinal lactase activity at relatively physiological doses reveals a potentially important function of milk-borne IGF-I, and the possible mechanism for this phenomenon deserves more in-depth investigation in the future.

INTESTINAL ABSORPTION OF MILK-BORNE INSULIN-LIKE GROWTH FACTOR-I

Another fundamental question regarding the possible function of milk-borne IGF-I and whether IGF-I can be used therapeutically in infants is whether it survives luminal digestion and is absorbed into the peripheral circulation in a biologically active form. Because neonates of many species are capable of significant absorption of intact macromolecules during the perinatal period, a function critical for acquisition of maternal immunoglobulins (Lecce et al. 1964), there is a reasonable probability that ingested milk-borne IGF-I might also be absorbed intact. An additional characteristic of the gastrointestinal tract of neonates that may enhance the survival of milk-borne IGF-I is the relatively low level of gastric acidity and luminal proteolytic digestion (Britton and Koldovsky 1989).

Despite numerous reports that have characterized the extent of luminal digestion and intestinal absorption of several milk-borne peptides, remarkably few such studies focus on IGF-I (Britton and Koldovsky 1989). Studies in vitro suggest that as little as 20% of 125I-labeled IGF-I is degraded when it is incubated with intestinal luminal contents from neonatal rats and that incubation with formula significantly decreases IGF-I degradation (Rao et al. 1993). The protective action of milk protein on IGF-I degradation has been confirmed in more recent in vitro studies demonstrating that nearly 40% of the intact 125I-labeled IGF-I coincubated with casein survives even when incubated with the intestinal lumen contents from adult rats (Xian et al. 1995). These in vitro studies, coupled with the evidence of intestinal growth effects in neonatal animals, suggest that some proportion of orally administered IGF-I may well survive intestinal digestion. However, in vivo studies with both neonatal rats (Phillips et al. 1995b) and pigs (Donovan et al. 1997) indicate that, although as much as 30% of an orally administered dose of 125I-labeled IGF-I can be recovered in the intestinal mucosa, there is very limited absorption into peripheral circulation. In our studies using transgenic mice, despite the ingestion of 1000-fold higher concentrations of des(1-3) human IGF-I, no des(1-3) human IGF-I was detected in plasma of the pups suckled on transgenic dams (Burrin et al. 1996a). Furthermore, only one of the reported studies in which IGF-I was given orally showed a statistically significant increase in either the circulating IGF-I concentration or peripheral organ growth compared with control groups (Burmucker et al. 1994, Burrin et al. 1996b, Houle et al. 1996, Xu et al. 1994); the one exception is a recent study that used neonatal rats (Phillips et al. 1995a). Taken together, the available evidence suggests that intestinal absorption of orally administered rhlIGF-I, even when given in pharmacological amounts, is of limited physiological significance in neonates.

IMPLICATIONS FOR HUMAN NEONATES

Because there are no reported clinical studies, it is impossible to predict the extent to which findings in neonatal animals are directly applicable to human infants. Nevertheless, the current evidence suggests that oral administration of pharma...


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