Gastrointestinal growth factors and pancreatic islet hormones during postoperative IGF-I supplementation in man

T Leinsköld¹, T E Adrian², U Arnelo²,³, J Larsson³ and J Permer³

¹Department of Surgery, University of Linköping, Linköping, Sweden
²Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska, USA
³Department of Surgery, Karolinska Institute at Huddinge Hospital, Stockholm, Sweden

(Requests for offprints should be addressed to T Leinsköld, Department of Surgery, University of Linköping, s-581 85 Linköping, Sweden)

Abstract

Insulin-like growth factor-I (IGF-I) has been demonstrated to exert a nitrogen sparing effect, both experimentally and in patients after abdominal surgery. IGF-I is a major mediator for the anabolic effects of growth hormone (GH). Whether elevated circulating IGF-I levels are the sole mediator of the anabolic effects of GH has not been clarified. IGF-I influences glucose metabolism, both through its own specific receptor and by activating the insulin receptor, and has also been proposed to influence pancreatic islet secretion directly. In the present study, the postoperative effects of IGF-I on plasma levels of other gastrointestinal and pancreatic islet hormones and growth factors were measured in patients after abdominal surgery.

Fifteen patients who were candidates for large bowel resection were randomly divided into two groups: IGF-I-treated (n=8) and placebo-treated (n=7). The IGF-I group received daily two s.c. injections of human recombinant IGF-I (80 µg/kg body weight) for five days, beginning on the morning of the first postoperative day. The other group received placebo injections. Fasting plasma levels of gastrointestinal growth factors (epidermal growth factor, transforming growth factor-α, IGF-II), gastrointestinal hormones (gastrin, enterogluca- gon, peptide YY), and islet hormones (insulin, islet amyloid polypeptide (IAPP) and pancreatic glucagon) were determined by RIA preoperatively and after five days of treatment.

No significant effects of IGF-I on other growth factors or gastrointestinal hormones were seen. A marked increase in plasma insulin postoperatively compared with the preoperative levels (42 ± 3 vs 61 ± 5 pm, P<0·05) was seen in the placebo group, whereas the postoperative levels in the IGF-I-treated patients remained unchanged (44 ± 3 vs 45 ± 4 pm). A similar pattern was observed for IAPP and cortisol concentrations. No differences in glucagon concentrations were seen.

In conclusion, these results suggest that IGF-I does not influence production of other gastrointestinal hormones thought to be involved in alimentary growth or pancreatic glucagon. In contrast, IGF-I caused a marked reduction of insulin and IAPP secretion. The inhibition of β-cell secretion could be direct or, alternatively, could involve an improvement in postoperative insulin resistance, perhaps by reducing serum cortisol.

Journal of Endocrinology (2000) 167, 331–338

Introduction

Trauma, such as that which accompanies major surgery, is associated with increased protein catabolism and decreased glycogen synthesis. Treatment with growth hormone (GH), after surgery and trauma, has been shown to improve nitrogen balance, decrease carbohydrate oxidation and increase fat oxidation (Wilmore et al. 1974, Ward et al. 1987, Pointing et al. 1988, Mjåland et al. 1993). Supplementation with insulin-like growth factor-I (IGF-I), the proposed mediator of GH’s somatotropic actions in vivo, was recently shown to reduce nitrogen excretion after surgery and to improve nitrogen balance in healthy human volunteers (Clemmons et al. 1992, Leinsköld et al. 1995). Other studies in humans have demonstrated that IGF-I exerts insulin-like effects on glucose metabolism (Boulware et al. 1992, Turkajl et al. 1992). When administered at pharmacological doses, IGF-I reduces circulating insulin levels and exerts a hypoglycaemic effect (Guler et al. 1987, Elahi et al. 1993, Leinsköld et al. 1995). IGF-I is a polypeptide consisting of 70 amino acids that has structural and biological similarities to insulin. IGF-I is produced in the liver and several other tissues (Daughaday & Rotwein 1989). While IGF-I appears to act by binding to a specific receptor homologous to the insulin receptor in muscle, its effects on adipose tissue appear to be mediated via insulin receptors (King et al. 1980, Bolinder et al. 1987). The half-life of free
IGF-I is short, about 15 min in humans (Guler et al. 1987, Baxter & Martin 1989). The circulating half-life is prolonged, however, because IGF-I is bound to specific IGF binding proteins (IGFBP), predominantly, in adult human plasma, to the GH-dependent IGFBP-3 (Baxter & Martin 1989).

Growth hormone stimulates growth of the gastrointestinal tract. This effect is likely to be mediated by IGF-I, since an increase in IGF-I mRNA was found in the intestinal mucosa in GH-transgenic mice (Ulshen et al. 1989). Long-term administration of IGF-I has a positive effect on the growth of the gastrointestinal tract (Steeb et al. 1994). This effect is also seen after intestinal resection and transplantation (Vanderhoof et al. 1992, Zhang et al. 1995). Control of the growth of intestinal mucosa is, however, a complex process and other growth factors and hormones are involved as well as luminal factors, such as nutrients (Wirén et al. 1995). In addition, postresectional adaptation of intestinal growth and function probably involves all of these factors (Besterman et al. 1982, Sagor et al. 1982, Savage et al. 1985, Riecken et al. 1989, Rutten et al. 1991, Björk et al. 1993, Vanderhoof 1993, Wirén et al. 1995). The relationship between exogenous IGF-I and surgery on other gastrointestinal hormones and secondary effects on intestinal mucosa have not yet been clarified.

We have previously reported a nitrogen-sparing effect during IGF-I treatment in patients after major abdominal operations and most of the patients involved in this present study were subjects in this earlier presentation from our group (Leinsköld et al. 1995).

IGF-I has been demonstrated to inhibit pancreatic islet beta-cell secretion, to reduce blood glucose levels, and to stimulate growth of intestinal mucosa in healthy volunteers and in rats. Since the role of IGF-I in traumatised patients is unknown, the aim of the present study was to investigate the postoperative effect of IGF-I on plasma levels of pancreatic islet hormones, gastrointestinal hormones and growth factors in patients after abdominal surgery.

### Study Design and Patients

The study included seventeen consecutive patients who were candidates for large bowel resection because of malignancy. Patients were excluded if they had a history of an endocrine disorder, inflammatory bowel disease, or treatment with immunosuppressive agents (including corticosteroids), or signs of metastatic disease. Two of the patients originally included were withdrawn during the study because they withdrew consent. Characteristics of the 15 patients who completed the study are shown in Table 1. There were no major postoperative complications, and the degree of operative trauma, as reflected by the duration of surgery and operative blood loss, was comparable in the two groups (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>IGF-I (n=8)</th>
<th>Placebo (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men:women)</td>
<td>6:2</td>
<td>1:6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64 (5)</td>
<td>69 (5)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1·74 (0)</td>
<td>1·65 (0)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 (3)</td>
<td>66 (5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (0-5)</td>
<td>24 (1)</td>
</tr>
<tr>
<td>Duration of operation (min)</td>
<td>132 (9)</td>
<td>131 (15)</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>157 (45)</td>
<td>158 (42)</td>
</tr>
</tbody>
</table>

The study was double-blind, and the patients were studied during the first five postoperative days. On the morning of the first postoperative day the patients were randomly allocated into two groups, one to receive IGF-I (n=8) and the other to receive a placebo (n=7). IGF-I was given daily as two s.c. injections (2 × 80 μg/kg body weight) of recombinant human IGF-I (rhIGF-I, 2 mg/ml, Pharmacia, Stockholm, Sweden), starting on the morning of the first postoperative day. Patients in the placebo group received injections of the vehicle only. Starting at the same time, each patient was given a continuous infusion of glucose (3 g/kg/day) and amino acids (0-1 g/kg/day) (Vamin 9 TM; Pharmacia) which was stopped four hours before blood samples were collected. This regime continued until the study was finished.

Blood glucose concentrations were measured before operation and on each postoperative day in the morning prior to the first injection of IGF-I or placebo. On postoperative days one and five, it was also measured two and four hours after the IGF-I or placebo injection.

Serum cortisol levels were measured before operation and on postoperative days one and six.

IGF-I was assayed from blood samples taken each morning before injection of IGF-I or placebo, starting on the day of operation.

Plasma concentrations of insulin, islet amyloid polypeptide (IAPP), pancreatic glucagon, insulin-like growth factor-II (IGF-II), gastrin, epidermal growth factor (EGF), transforming growth factor-α (TGF-α), peptide YY (PYY) and enteroglucagon were measured before operation and at postoperative day six.

Clinical condition and function were monitored daily. Urea, creatinine, and serum concentration of electrolytes (sodium and potassium) were monitored daily. The study was approved by the ethics committee of Linköping University, Sweden.

### Materials and Methods

All blood samples were collected in the morning after an overnight fast. During parenteral nutritional support, the
infusions were stopped four hours before the blood samples were taken. Samples for standard laboratory analysis and for analysis of cortisol were taken on the day of operation, before the first injection of IGF-I, and on the last day of treatment. Blood for IGF-I analyses was taken in serum tubes and centrifuged within 20 min at 3000 r.p.m. The serum fraction was separated and frozen at −70 °C for subsequent assay of IGF-I. Samples for analysis of the other peptides were collected into ice-cold tubes containing aprotinin (400 KIU/ml blood) and EDTA (5 mg/ml blood). The samples were immediately separated in a refrigerated centrifuge and frozen for subsequent assay of the different peptides.

Blood glucose and other biochemical analyses

Blood glucose was measured using the glucose oxidase method (Glucostat, Beckman Instrument Corp., Fullerton, CA, USA). Routine biochemistry, electrolytes and analysis of haematological parameters were performed at the Department of Chemistry, Linköping University Hospital, by standard procedures.

Growth factor and hormone analysis

IGF-I  IGF-I was analysed in serum. Protein was extracted from the samples with acidified alcohol (0·1 M hydrochloric acid, 75% ethanol 1:4) and the IGF-I concentration was measured by radioimmunoassay. A polyclonal anti-human IGF-I antibody was used. The minimum detectable concentration was 20 ng/ml. At 200 ng/ml, the variations within and between assays were below 4 and 10% respectively. The assay had negligible cross-reactivity with proinsulin, insulin (<0·01% for both), and IGF-II (<1%).

Insulin, glucagon (total and pancreatic), IAPP, gastrin, PYY, EGF, IGF-II, TGF-α  The growth factors and hormones were analysed in plasma and were measured with specific and sensitive radioimmunoassays (RIAs) as previously described (Bloom & Long 1982, Savage et al. 1985, Permert et al. 1994, Skullman et al. 1994). Enteroglucagon was determined by subtracting values from a pancreatic glucagon specific assay from levels measured using an antibody that fully cross-reacts with pancreatic and enteric glucagon-like immunoreactivity.

Statistics

All values are presented as means (s.e.m.). The significance of differences between the two groups of patients was assessed by analysis of variance (ANOVA), using a statistics program (InStat, GraphPad, San Diego, CA, USA). Bonferroni’s correction for multiple comparisons was used. Paired or unpaired analysis was used as appropriate.

Results

Haemoglobin, and serum albumin, urea, creatinine and electrolytes (sodium and potassium) were not significantly different between the two groups either before operation or during the period of treatment (Table 2).

The day of the first sign of functional bowel movements measured as first stool, and the first day of oral intake of liquids, as well as the day of admittance were not significantly different between the two groups (data not shown).

Serum cortisol levels were significantly increased ($P<0·05$) on the first postoperative day in the placebo group, but not in the IGF-I-treated group, compared with preoperative values (Table 2).

Serum concentrations of IGF-I were similar in the two groups before initiation of treatment, and increased significantly during the treatment in the group receiving IGF-I, both compared with the initial values and with those in the placebo group ($P<0·001$) (Fig. 1). During the postoperative period there were no changes in serum concentrations of IGF-I in the placebo group compared with basal levels but during the IGF-I treatment the levels declined
by postoperative day 4 and continued to decline throughout the rest of the study period, although the fall did not reach statistical significance (Fig. 1). Before surgery, blood glucose concentrations were similar in the two groups (IGF-I group: 5·41 (0·70) vs placebo group: 5·07 (0·53) (mmol/l), Fig. 2). After operation glucose levels rose in both groups, but after the first postoperative day the mean concentration in the IGF-I-treated group declined to basal levels. In contrast, blood glucose levels in the placebo group did not reach preoperative control levels until the fifth day after surgery. This difference was significant on day 4 (P<0·05) (Fig. 2). IGF-I did not acutely affect blood glucose concentrations two and four hours after injection, on either the first or fifth days of treatment (data not shown).

Plasma insulin and IAPP concentrations were significantly increased postoperatively in the placebo group. In contrast, in the IGF-I-treated group they remained unchanged during treatment (Figs 3 and 4 and Table 3). There were no significant differences in plasma concentrations of gastrin, PYY, enteroglucagon, EGF, TGF-α or IGF-II either before operation or during IGF-I treatment (Table 3).

Discussion

The effect of exogenously administered IGF-I on plasma levels of pancreatic islet hormones, gastrointestinal hormones and growth factors in patients after abdominal surgery was investigated. Postoperative IGF-I treatment significantly decreased postoperative insulin, IAPP and cortisol as well as blood glucose levels. This dose of IGF-I given together with nutrients does not cause hypoglycaemia, which is in line with previous studies (Miell et al. 1992, Yarwood et al. 1997). No effects of IGF-I on gastrointestinal hormones or growth factors were seen.

Figure 1 Serum concentrations of IGF-I in patients given IGF-I (▲) and placebo (■) before the onset of treatment (day 1) and during treatment (days 2–6). The differences between the groups were significant on days 2–6 (*P<0·001).

Figure 2 Blood glucose concentrations in patients given IGF-I (▲) and placebo (■) before the first injection and during treatment. *P<0·05.

Figure 3 Plasma insulin concentration in patients given IGF-I (open bars) and placebo (solid bars) before the onset of treatment (day 1) and on the last day of treatment (day 6). *P<0·05.
Previous studies of IGF-I in non-traumatised animals and humans have shown that the peptide has a potent hypoglycaemic effect, despite a reducing effect on pancreatic islet cell secretion (Boulware et al. 1992, Mauras et al. 1992, Turkajl et al. 1992, Elahi et al. 1993). In the present study a reduction in postoperative hyperglycaemia was seen in catabolic patients after major operation. To examine the effects of the surgical trauma and the administration of IGF-I on pancreatic islet β-cell function, we measured insulin and IAPP. IAPP is a 37-amino acid polypeptide produced in the beta cells of the pancreatic islets secreted in parallel to insulin from the same secretory granules (Westermark et al. 1986). In the IGF-I-treated group, IAPP levels were reduced in parallel to those of insulin. This suggests that IGF-I reduced insulin secretion rather than increasing the clearance of the peptide. Insulin and IAPP were not suppressed below basal levels as a result of IGF-I treatment, as was previously reported from studies in non-operated healthy subjects (Elahi et al. 1993). The significant increase in concentrations of insulin and IAPP compared with preoperative levels observed in the placebo group did not occur in the IGF-I-treated groups.

Postoperative glucose metabolism is influenced by several factors, such as the severity of injury, body temperature, nutrients and septic complications (McManson et al. 1988, Sjölin et al. 1989, Larsson et al. 1990, Sjölin 1993). In the present study, however, no such differences were seen between the groups. As an unfortunate consequence of randomisation, there was a pronounced difference in gender in the groups. However, under normal conditions there is no sex-related difference in circulating levels of GH, IGF-I, insulin, cortisol, IGFBP-1 or IGFBP-3 (Yamamoto et al. 1991, Vahl et al. 1997a,b, Janssen et al. 1998). Also, it has recently been demonstrated that a body mass index (BMI, kg/m²) below 30 kg/m², peripheral resistance is linearly related to BMI (Elton et al. 1994). In this study, in spite of the difference in sex distribution, BMI was not significantly different between the two groups, and all patients had a BMI below 30 kg/m².

Serum cortisol levels were increased in the immediate postoperative period and this increase was abolished by IGF-I administration. A previous report revealed a similar blunting of the postoperative cortisol response by IGF-I in gastrectomized patients on the first postoperative day (Goeters et al. 1995). Since corticosteroids are associated with insulin resistance, it is possible that inhibition of this counter-regulatory response by IGF-I contributes to a reduction in insulin resistance and thereby prevents insulin levels from rising postoperatively. The mechanism for inhibition of the postoperative cortisol response to surgery is not clear since IGF-I enhances adrenocorticotrophin-stimulated but not basal cortisol secretion in vitro (Weber...
et al. 1995). Alternatively, another way to affect insulin sensitivity is via the high circulating levels of IGF-I which downregulate the release of growth hormone, thus reducing the lipolytic effects of growth hormone (Chapman et al. 1998).

IGF-I mimics insulin’s ability to stimulate glucose uptake and metabolism in both muscle and adipose tissue, although it acts only through its capacity to bind to the insulin receptor in adipocytes (King et al. 1980, Bolinder et al. 1987, Sinha et al. 1989, Elahi et al. 1993). As mentioned above, insulin levels rose significantly in the placebo group postoperatively, while levels remained similar to baseline in the IGF-I-treated group. We have previously reported the effect of major abdominal operative and IGF-I treatment on circulating IGFBP-1 and IGFBP-3 levels (Leinskold et al. 1995). The plasma concentration of IGFBP-1 is primarily influenced by insulin levels in an inverse manner (Benbassat et al. 1997, Russell-Jones et al. 1997). The serum levels of IGFBP-1 were slightly reduced in the placebo group after the trauma, which corresponds with the higher levels of insulin (Benbassat et al. 1997, Russell-Jones et al. 1997). In the IGF-I-treated group the levels of IGFBP-1 showed a tendency to increase by postoperative day 3 compared with the placebo group. The plasma concentration of IGFBP-1 remained increased during the rest of the study, reaching statistical significance at postoperative day 4 compared with the placebo group (Leinskold et al. 1995). The tendency for higher mean levels of IGFBP-1 in the IGF-I-treated group could be explained by the suppressed insulin levels. Also, the IGF-I treatment per se could explain the tendency for higher serum concentrations of IGFBP-1 (Baxter et al. 1993, Russell-Jones et al. 1997). The serum levels of the growth hormone–dependent IGFBP-3 were decreased in both groups after operation, although they were statistically significant in the IGF-I group only. After initiation of the IGF-I/placebo treatment the mean level of IGFBP-3 increased significantly in the IGF-I group compared with the values on the first day after the operation. This is rather surprising as growth hormone levels could be expected to be low in the IGF-I–treated group and, also, it has recently been shown that trauma has a negative influence on IGFBP-3 ( Cotterill et al. 1996). Blood glucose levels in the placebo group remained elevated until the fourth postoperative day. In contrast, blood glucose was not significantly different from basal in the IGF-I–treated group, except on postoperative day one. This observation is in line with previous studies in normal subjects indicating an increased rate of glucose disposal as a result of IGF-I infusion ( Jacob et al. 1989, Elahi et al. 1993). The effectiveness of IGF-I to stimulate peripheral glucose uptake is only about 4–6% of a molar equivalent of insulin, although circulating levels of IGF-I in healthy subjects are an order of magnitude higher than insulin levels (Guler et al. 1987, Elahi et al. 1993). However, the effect of IGF-I on blood glucose levels in the patients in this study may indicate that IGF-I is more potent than insulin in the stimulation of peripheral glucose uptake in the traumatised patient. This could be an effect of the trauma which increases proteolysis of IGFBP-3, thus producing a higher level of free IGF-I (Bang et al. 1998).

The gastrointestinal hormones measured in the protocol were selected because they are of importance for function or growth of the gastrointestinal tract (Yamada 1986). In this experiment we did not investigate the effect of surgery or IGF-I on the intestinal mucosa. Following resection of the small intestine or colon the administration of IGF-I can produce changes in the wall of the gut, so it was interesting to investigate the effects of major surgery and administration of IGF-I on circulating gastrointestinal hormones and growth factors (Adrian et al. 1987, Vanderhoof et al. 1992, Steeb et al. 1994, Wirén et al. 1995, Zhang et al. 1998, Inaba et al. 1997, Shimoda et al. 1997). It has been reported that both enteroglucagon and PYY increase massively following small bowel resection (Armstrong et al. 1991). Distal gut PYY appears to play an important inhibitory role in foregut secretions and motility by delaying small bowel transit, whereas enteroglucagon may have trophic effects on the small bowel mucosa (Adrian et al. 1987, Armstrong et al. 1991). However, there were no significant changes in plasma enteroglucagon or PYY in either group in response to surgery or IGF-I. Although concentrations of both enteroglucagon and PYY increased following colonic resection in the dog ( Armstrong et al. 1991), no changes were seen in a previous study in humans (Hallböök et al. 1996). None of the gastrointestinal hormones or growth factors investigated was significantly influenced by surgery or the administration of IGF-I.

Further studies are required to elucidate the mechanism of the effect of IGF-I on peripheral glucose uptake in the post-traumatic period. While the insulin–like properties of IGF-I are well established, the way in which it improves post-traumatic glycaemia has not been investigated.

Acknowledgements

This study was supported by grants from The Swedish Medical Research Council (MFR-B93–17X-10402–02A) and from the Local Research Council of Östergötland.

References


Received 3 December 1999
Revised manuscript received 27 June 2000
Accepted 18 July 2000