Effect of insulin-like growth factor I on in vivo intestinal absorption of \( \text{D-galactose} \) in cirrhotic rats

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PATIENTS WITH ADVANCED cirrhosis frequently show disordered nutritional status, a complication that negatively affects survival of the patients and outcome after liver transplantation (18). The mechanisms leading to malnutrition in cirrhotic individuals are complex and still understood (18, 21), although it has been suggested that impaired intestinal absorption of nutrients and diminished bioavailability of the anabolic hormone insulin-like growth factor I (IGF-I) participate in the genesis of this complication (3, 21). In fact, it has been recently shown (21) that administration of low doses of IGF-I to cirrhotic rats improves food efficiency and incorporation of dietary nitrogen to muscle.

Although IGF-I is produced in many tissues, the liver is the main source of the circulating hormone (8, 26). IGF-I is synthesized in response to growth hormone, and it is transported in plasma bound to different forms of IGF-I binding proteins, which play an important role in modulating the biological effects of IGF-I (11). Both diminished serum levels of IGF-I and the altered profile of IGF-I binding proteins contribute to reduced bioavailability of this hormone in liver cirrhosis (8, 21, 26).

Previously, we showed (3) that in vitro galactose transport by everted jejunal rings and brush-border membrane vesicles was reduced in rats with CCl\(_4\)-induced liver cirrhosis. However, in cirrhotic rats that received low doses of IGF-I for 2 wk, galactose transport was similar to that found in healthy control animals (3). In the present study, we have extended our work to analyze whether IGF-I treatment, given as sustained therapy for 2 wk or as a single dose administration, was able to influence in vivo intestinal sugar absorption in experimental liver cirrhosis.

MATERIALS AND METHODS

Animals and experimental design. All the experimental procedures were performed in conformity with the Guide for the Care and Use of Laboratory Animals. Rats of \(- 145–170 \text{ g body wt}\) were housed in an air-conditioned room at 22 ± 2°C with a 12:12-h light-dark cycle. Liver cirrhosis was induced by carbon tetrachloride (CCl\(_4\)) inhalation twice a week for 11 wk (1). The organic solvent was administered twice a week with a progressively increasing exposure time, ranging from 1 to 5 min, during the eleventh week of induction of cirrhosis (1). To accelerate the development of cirrhosis, we added phenobarbital (Luminal, Bayer, Leverkusen, Germany) to drinking water (400 mg/l) throughout the entire period of the induction of cirrhosis, beginning 1 wk before the first CCl\(_4\) exposure (4).

Two different study protocols were followed. In protocol 1, two groups \((n = 8 \text{ each})\) of cirrhotic rats received either saline (CI group) or IGF-I subcutaneously \((2 \mu g \cdot 100 \text{ g body wt}^{-1} \cdot \text{day}^{-1})\) (CI plus IGF-I group) for 2 wk beginning 1 wk after stopping CCl\(_4\) administration (day 0 of the experimental period). In parallel, two groups of healthy rats \((n = 8 \text{ each})\) received saline (CO group) or IGF-I (CO plus IGF-I) at the same dose as the CI plus IGF-I group during the 2 wk of the study. In protocol 2, healthy control rats and cirrhotic rats received \((\text{after basal assessment of D-galactose absorption})\) saline (CO and CI groups, respectively; \(n = 8 \text{ each}\)) or IGF-I as a bolus injection of \(1 \mu g / 100 \text{ g body wt}\) followed by continuous infusion of the same dose for 90 min (CO plus IGF-I and CI plus IGF-I groups).

On day 0 of the experimental period, blood \((6 \text{ ml})\) from the retroorbital plexus was collected. On day 15, animals fasted overnight were anesthetized with an intramuscular injection of thiopental sodium \((4 \text{ mg/100 g body wt} \text{; Merck})\) and jejunal D-galactose transport was studied in vivo.

As mentioned above, we studied intestinal absorptive function on day 15, 3 wk after stopping CCl\(_4\) administration. At that time, malondialdehyde (MDA) levels \((2 \text{ (a reflection})
of lipid peroxidation caused by CCl₄ in the liver were still significantly raised in cirrhotic rats (n = 16) compared with controls (n = 16) (142 ± 27 vs. 46 ± 4 nmol/mg tissue), while in the intestine MDA was similar in cirrhotic and normal rats (35.3 ± 4.6 vs. 28.8 ± 1.6 nmol/mg tissue; P = not significant (NS)). These data indicate that, in our study protocols, changes in intestinal function are unlikely to be due to toxic effects of CCl₄ on the intestine.

In vivo absorption of α-galactose by isolated jejunal loops. The methodology was as described previously by Ponz et al. (22). A jejunal loop of 15 cm (starting 5 cm downstream of Treitz’s ligament) was hooked between two glass cannulas, replaced in the abdomen, and connected to a perfusion system, equipped with a constant-flow electric pump (Microperpex model 2123, LKB Products, Bromma, Sweden). The loop was perfused with 50 ml of saline and then perfused through a recirculating technique for 15 min, at a flow rate of 2 ml/min, with solution containing 2.0 mM α-galactose and 0.1 µCi/10 ml of radioactive substrate ([14C]). At the end of the perfusion period, the intestine was washed with saline for 5 min. All solutions were kept at 37°C. The effluent was collected, and the amount of α-galactose absorbed was estimated as the difference between the sugar in the solution before and after perfusion plus the substrate remaining in the final washing solution. Radioactive samples were counted in a Packard Tricarb liquid scintillator in a Beckman LS 188 β-counter (model LS 1800). Absorption (A; in µmol of α-galactose·15 min⁻¹·cm jejuno-1) was calculated according to the following formula: A = [2 mM [Cᵢ – Cᵢ (Pᵢ/Pᵢ)] · I⁻¹ where 2 mM is the sugar concentration in the perfused solution, Cᵢ is the counts per minute (cpm) of the solution infused, Cᵢ is the cpm after absorption, Pᵢ and Pᵢ are the weights of the solution infused and collected at the beginning and at the end of the experimental period, respectively (these weights were used to calculate the volumes of liquids perfused and collected), and I is the length (in cm) of the jejunal loop in which sugar transport was evaluated in vivo.

In protocol 2, the right jejunal vein was catheterized and the catheter was connected to a syringe installed in a perfusion pump (Harvard Apparatus model 2681, St. Louis, MO). During the absorption experiments, animals were kept in a glass cabinet in which temperature was maintained at 35–37°C. A jejunal loop was isolated between two cannulas that were connected to the peristaltic pump at a rate of 5 ml/min, with solution containing 2.0 mM α-galactose and 1 µCi/100 ml of radioactively labeled substrate ([14C]) (basal period). Then a bolus of 0.5 ml solution containing 1 µg/100 g body wt of IGF-I was administered through the jejunal vein, followed by a continuous infusion of 0.5 ml of the same IGF-I solution (1 µg/100 g body wt) for 90 min. During this time, α-galactose absorption was assessed in six successive 10-min periods (at 30, 45, 60, 75, 90, and 105 min). Between these periods, the intestine was washed with Ringer solution for 5 min.

Once the study of α-galactose absorption was completed, blood samples were obtained and liver and jejunal specimens were collected for optical and/or electron microscopy studies. Analysis of passive permeation of inulin across the gut. In additional experiments, five control and five cirrhotic rats were used to analyze passive permeation of inulin across the gut. A jejunal loop was isolated between two cannulas that were connected to a constant-flow electric pump (Microperpex model 2123, LKB Products). The right jejunal vein was catheterized using an intravenous cannula (16 gauge × 2 in.; Becton Dickinson, Agustin de Guadalix, Madrid, Spain), and 1 ml of saline solution containing [3H]inulin (5 µCi) (American International, Buckinghamshire, United Kingdom) was infused at time 0. From time 0 to time 40 the jejunum was perfused with saline at a rate of 2 ml/min, and samples from the effluent were collected at time 20. Radioactivity readings were performed in a Beckman β-counter (model LS 1800). Analytic and histological methods. Serum levels of albumin, total proteins, glucose, cholesterol, bilirubin, alkaline phosphatase, and aminotransferases (aspartate aminotransferase and alanine aminotransferase) were determined by routine laboratory methods using a Hitachi 747 autoanalyzer (Boehringer Mannheim). Conventional histological techniques for optical and electron microscopy were carried out using a light projection microscope (Micro Promar Leitz, Wetzlar, Germany) and an electron microscope operating at 60 kV (EM10C/CR, Zeiss, Oberkochen, Germany), respectively. In jejunum, four or five fields from each animal were evaluated twice by two different observers and the length of villi and microvilli was recorded. The arithmetic mean of the two scores was taken as the final measurement.

Statistical analysis. Data are expressed as means ± SE. To assess the homogeneity among groups, we used the Kruskall-Wallis test, followed by multiple post hoc comparisons using the Mann-Whitney U test with Bonferroni adjustment. Within groups, differences between pre- and posttreatment values were assessed by means of Wilcoxon matched pairs signed rank sum test. P < 0.05 was considered to be statistically significant. Calculations were performed with the SPSSWin computer program (version 6.0; SPSS, Chicago, IL).

RESULTS

On day 0 of the study, rats that received CCl₄ showed a significant increase of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, cholesterol, and bilirubin and a significant reduction of serum albumin, total protein, and blood glucose (Table 1). Examination of liver samples at the end of the study evinced established liver cirrhosis in all animals that received CCl₄. None of the cirrhotic rats showed ascites.

At the end of the study (day 15), blood glucose was significantly reduced in CI rats (156 ± 7 mg/dl) compared with both CO (196 ± 6 mg/dl) and CI plus IGF-I animals (192 ± 11 mg/dl) (P < 0.005 for both comparisons). In CO plus IGF-I animals, blood glucose (197 ± 8 mg/dl) was similar to that found in untreated healthy controls.

Table 1. Biochemical data from healthy control rats and rats that received CCl₄ after 11-wk protocol for induction of cirrhosis

<table>
<thead>
<tr>
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<th>Healthy Control Rats</th>
<th>Rats That Received CCl₄</th>
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<tbody>
<tr>
<td>AST, IU/l</td>
<td>52 ± 5</td>
<td>289 ± 42*</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>23 ± 2</td>
<td>266 ± 39*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>220 ± 15</td>
<td>111 ± 5*</td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>3.6 ± 0.1</td>
<td>3.0 ± 0.2†</td>
</tr>
<tr>
<td>Total proteins, g/dl</td>
<td>7.1 ± 0.1</td>
<td>6.2 ± 0.2†</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>81.6 ± 3.8</td>
<td>115.5 ± 4.9*</td>
</tr>
<tr>
<td>Alkaline phosphatase, IU/l</td>
<td>308 ± 43</td>
<td>712 ± 125†</td>
</tr>
<tr>
<td>Bilirubin, mg/dl</td>
<td>0.4 ± 0.1</td>
<td>1.3 ± 0.4†</td>
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</table>

Values are means ± SE. Data were obtained on day 0 of the study from 12 control rats and 24 rats that received CCl₄. AST, aspartate aminotransferase; ALT, alanine aminotransferase. *P < 0.001, †P < 0.05 vs. control values.
As shown in Fig. 1, in vivo D-galactose absorption by isolated jejunal loops was significantly reduced in CI rats compared with CO rats (0.29 ± 0.01 and 0.42 ± 0.01 μmol D-galactose·15 min⁻¹·cm⁻², respectively; P < 0.001). Interestingly, cirrhotic rats treated with IGF-I for 2 wk (CI + IGF-I) showed values of intestinal sugar absorption (0.38 ± 0.01 μmol D-galactose·15 min⁻¹·cm⁻²) significantly higher than in CI animals (P < 0.001) although still slightly below the values observed in CO animals. However, D-galactose absorption in healthy controls treated with IGF-I (CO + IGF-I) was similar to that found in untreated normal rats.

Using light microscopy, we observed a slight, although significant, increase in the length of villi in CI animals (1.24 ± 0.02 mm) compared with CO animals (0.86 ± 0.02 mm; P < 0.001), whereas CI plus IGF-I rats showed values (0.86 ± 0.03 mm) comparable to controls. Using electron microscopy (Fig. 2), we found that microvilli from jejunum were markedly elongated in CI animals compared with CO animals. Treatment of CI animals with IGF-I for 2 wk reduced microvillus length to values similar to those found in controls (Table 2). No differences in microvillus lengths were found in CO plus IGF-I animals with respect to CO animals (2.34 ± 0.09 vs. 2.61 ± 0.17 μm, CO and CO plus IGF-I, respectively; P = NS). Electron micrographs also demonstrated a reinforcement of the anchorage of actin microfilaments at the base of microvilli in CI plus IGF-I rats compared with untreated cirrhotic animals (Fig. 2, arrows).

Protocol 2. In this protocol, we investigated if IGF-I had acute effects on intestinal sugar absorption in cirrhotic rats similar to those observed when the hormone was given for extended periods of time (14 days in protocol 1). As illustrated in Fig. 3, in basal conditions in vivo D-galactose absorption was significantly reduced in cirrhotic rats compared with normal controls (P < 0.01). Low doses of IGF-I administered as a bolus of 1 μg IGF-I/100 g body wt followed by a continuous infusion of the same amount of IGF-I for 100 min resulted in a prompt increase of intestinal galactose absorption in cirrhotic animals, reaching values similar to those found in untreated cirrhotic rats (arrows). Original magnification, ×16,000.

Table 2. Morphometric study of microvilli of jejunum from 3 experimental groups

<table>
<thead>
<tr>
<th></th>
<th>CO Rats</th>
<th>CI Rats</th>
<th>CI + IGF-I Rats</th>
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<tr>
<td>Length of jejunal microvilli, μm</td>
<td>2.34 ± 0.09</td>
<td>4.98 ± 0.39*</td>
<td>3.19 ± 0.12†</td>
</tr>
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| Values are means ± SE; n = 5 rats for each group. CO, control; CI, cirrhotic; IGF-I, insulin-like growth factor I. *P < 0.001 vs. CO rats. †P < 0.001 vs. CI rats. ‡P < 0.01 vs. CO rats.
lar to those found in normal rats by time 75. The acute stimulatory effect of IGF-I on sugar absorption was already noticeable at time 15. IGF-I treatment, however, did not produce changes in galactose absorption in healthy rats (Fig. 3).

Cirrhotic rats from protocol 2 showed similar microvillus elongation as observed in cirrhotic rats from protocol 1. Acute administration of IGF-I to cirrhotic animals caused an apparent, although nonsignificant, shortening of microvilli and an increase in the density of actin microfilaments at the base of the microvilli (Fig. 4, arrows); these changes were similar to those observed during chronic IGF-I administration to CI rats.

Absence of passive permeation of inulin to intestinal lumen in cirrhotic rats. To investigate whether increased back diffusion to the intestinal lumen could play a role in reduced galactose absorption in experimental cirrhosis, we injected labeled inulin intravenously (at time 0) and measured the radioactivity in the effluent of a perfused jejunal loop at different time intervals until time 40. No radioactivity was found in any of the samples of the effluent in either normal (n = 5) or cirrhotic rats (n = 6); radioactivity at time 40 for controls was 0.033% cpm and for cirrhotic rats was 0.035% of initial cpm inulin infusion. These data suggest that back diffusion of nutrients to intestinal lumen does not contribute significantly to impaired sugar absorption in rats with well-compensated cirrhosis.

DISCUSSION

The mechanisms responsible for malnutrition in cirrhotic patients are plural and not well understood (14, 29). Anorexia, high basal metabolic rate, increased levels of catabolic hormones, and diminished bioavailability of the anabolic hormone IGF-I have been proposed as factors contributing to poor nutritional status in advanced cirrhosis (2, 8, 14, 21, 24, 29). Although altered digestion and/or absorption of nutrients has also been considered to play a role in the malnutrition seen in cirrhosis, there are few studies analyzing the existence of specific defects in intestinal transport mechanisms in this condition. Recently, we have reported (2) impaired galactose transport by brush-border membrane vesicles and everted jejunal rings from rats with experimental liver cirrhosis. In the present study, using an in vivo assay, we have also observed reduced intestinal galactose absorption in rats with CCl4-induced cirrhosis. Taken together, our in vitro and in vivo data indicate that disturbed intestinal absorption occurs in experimental liver cirrhosis, suggesting that this alteration could contribute to malnutrition in cirrhotic patients.

Fig. 3. Jejunal transport of d-galactose in animals from protocol 2 (acute treatment). A bolus of IGF-I (1 µg/100 g body wt) or saline was administered at the beginning of assay (at time 15, arrow) after collection of the first sample of the perfusate to obtain baseline data. Then IGF-I (1 µg/100 g body wt) or saline was administered as a continuous infusion until time 90. b1, First bolus of IGF-I; b2, end of continuous IGF-I infusion. **P < 0.01, CI vs. CO. At baseline both groups of cirrhotic rats presented significant differences vs. controls. **P < 0.01, CI vs. CO. No differences were found between CO vs. CO + IGF-I or CI + IGF-I after first bolus of IGF-I. & & & P < 0.001 baseline values (time 0) vs. those at end of experimental period (time 105) in CI + IGF-I group.

Fig. 4. Electron micrographs of jejunal enterocyte microvilli in rats from protocol 2 (acute IGF-I treatment). A marked and significant elongation of microvilli was observed in untreated cirrhotic rats. Microvilli tended to shorten after acute IGF-I administration. The bundle of actin microfilaments at the base of microvilli (anchoring microfilaments) was more dense and prominent in cirrhotic rats treated with IGF-I than in untreated cirrhotic animals (arrows). Original magnification, ×16,000.
The mechanisms responsible for galactose malabsorption in cirrhosis have only been partially characterized. In a previous study (2), we found that the expression of the sodium-dependent glucose-galactose cotransporter 1 (SGLT-1) at the intestinal brush border was not diminished in cirrhotic rats compared to normal controls. On the other hand, in the present study, we did not find increased passive permeation of inulin through the gut in cirrhotic or control rats, suggesting that back leakage of nutrients to the intestinal lumen does not play a significant role in reduced sugar absorption in rats with compensated cirrhosis. However, we observed a significant elongation of microvilli of jejunal brush border in untreated cirrhotic rats. The finding that administration of IGF-I improves galactose absorption while simultaneously causing shortening of microvilli suggests that impaired galactose transport might be linked to altered structure and function of the intestinal brush border.

Although IGF-I is synthesized in many tissues, it is mainly produced in the liver on stimulation by growth hormone (11). IGF-I receptors are densely expressed in the intestinal tract (15, 20, 30, 32), and it has been shown that IGF-I stimulates intestinal function and increases nutrient absorption, particularly glucose absorption, in different experimental settings (5, 16, 23, 27, 28, 32). In the present study, we show that IGF-I significantly increases in vivo intestinal sugar transport in cirrhotic rats not only after chronic IGF-I treatment (14 days in protocol 1) but also after acute administration of IGF-I (protocol 2). In fact, sugar absorption is already enhanced at time 15 after IGF-I administration, reaching values comparable to normal controls by time 75.

As shown in Fig. 3, intestinal sugar absorption decreases slightly during the experimental period in both control and cirrhotic rats, possibly due to a slow decay in the viability of intestinal mucosa during the course of the experiment. However, in cirrhotic animals subjected to IGF-I infusion, intestinal absorption of galactose increases significantly during the same time period, reflecting the stimulatory effect of the hormone on the absorptive process in rats with cirrhosis (but not in normal controls). It is also worth noting that while acute IGF-I administration completely restores galactose absorption to normal values, chronic IGF-I treatment causes a significant increase in sugar absorption but without reaching the values found in normal rats. This discrepancy in the effects of acute vs. chronic treatment might be explained by the fact that in protocol 1 (chronic treatment) the last dose of the hormone was received ~24 h before the absorption test, whereas in protocol 2 the study was performed while the animal was receiving the treatment.

The mechanisms through which IGF-I enhances galactose transport by the gut in cirrhotic rats remain to be elucidated. IGF-I does not seem to act by increasing the expression of SGLT-1 at the brush border of enterocytes (2). As mentioned before, both acute and sustained treatment with IGF-I produces a change in the shape of microvilli in parallel with an enhancement of sugar absorption. Microvilli consist of fingerlike projections of the cell membrane with a central core formed by a bundle of actin filaments (2, 6). The parallelism between absorptive function and microvillar changes suggests that IGF-I may stimulate sugar transport by influencing cytoskeletal organization in microvilli. In agreement with this idea, we found a reinforcement of anchoring microfilaments at the base of the microvilli in IGF-I-treated cirrhotic rats.

The effects of IGF-I on cytoskeletal architecture have been described in other cell types, such as cardiomyocytes and human epidermoid cell lines (6, 12). In some cells, IGF-I has been reported to cause rapid and dramatic modifications in morphology due to microfilament reorganization (12). Similar to IGF-I, epidermal growth factor (EGF) has also been shown to cause cytoskeletal changes in various cell types (12), to stimulate intestinal sugar absorption, and to induce changes in microvilli height (7, 19). These effects also occur rapidly, being apparent 10 min after mucosal exposure to EGF (7).

However, while EGF can stimulate sugar absorption in normal animals (19), IGF-I enhances galactose absorption in cirrhotic rats but not in normal controls. Moreover, while EGF increases intestinal transport of nutrients in association with an increase in microvilli height (7), IGF-I improves galactose absorption in cirrhotic rats in parallel with shortening of microvilli. Although there is no obvious explanation for this apparent paradox, it could be hypothesized that elongation of microvilli as occurs in cirrhotic rats may alter the positioning of transporters in the brush-border membrane, while microfilament contraction and microvilli shortening due to IGF-I might correct these changes and increase the effectiveness of the transport process. In fact, in a previous study (2) we found that the galactose transporter had reduced affinity for the ligand in cirrhotic rats, this defect being corrected after IGF-I treatment. Further studies are clearly needed to clarify the mechanisms through which IGF-I stimulates nutrient absorption in cirrhosis.

Finally, we should mention that our results point to a possible therapeutic role of IGF-I in the correction of intestinal dysfunction in liver cirrhosis. Both malabsorption and deranged gut barrier (13, 14, 18, 19), leading to malnutrition and spontaneous bacterial peritonitis, are important complications of advanced cirrhosis. Because IGF-I not only stimulates absorption of nutrients but also has been reported to exert trophic effects on the intestine protecting against bacterial translocation (10), the potential of IGF-I as a therapeutic agent in cirrhotic patients deserves consideration.

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