Hormonal regulation of dipeptide transporter (PepT1) in Caco-2 cells with normal and anoxia/reoxygenation management

Bing-Wei Sun, Xiao-Chen Zhao, Guang-Ji Wang, Ning Li, Jie-Shou Li

Bing-Wei Sun, Ning Li, Jie-Shou Li, Department of General Surgery, School of Medicine, Nanjing University, Nanjing 210093, Jiangsu Province, China
Research Institute of General Hospital, Chinese PLA General Hospital of Nanjing Military Area, Nanjing 210002, Jiangsu Province, China
Xiao-Chen Zhao, Guang-Ji Wang, Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, Jiangsu Province, China
Supported by
Xiao-Chen Zhao, Guang-Ji Wang,
Research Institute of General Hospital, Chinese PLA General Hospital
Jiangsu Province, China

Abstract

AIM: To determine the regulation effects of recombinant human growth hormone (rhGH) on dipeptide transporter (PepT1) in Caco-2 cells with normal culture and anoxia/reoxygenation injury.

METHODS: A human intestinal cell monolayer (Caco-2) was used as the in vitro model of human small intestine and cephalexin as the model substrate for dipeptide transporter (PepT1). Caco-2 cells grown on Transwell membrane filters were preincubated in the presence of rhGH in the culture medium for 4 d, serum was withdrawn from monolayers for 24 h before each experiment. The transport experiments of cephalexin across apical membranes were then conducted; Caco-2 cells grown on multiple well dishes (24 pore) with normal culture or anoxia/reoxygenation injury were preincubated with rhGH as above and uptake of cephalexin was then measured.

RESULTS: The transport and uptake of cephalexin across apical membranes of Caco-2 cells after preincubation with rhGH were significantly increased compared with controls (P=0.045, 0.0223). Also, addition of rhGH at physiological concentration (34 nM) to incubation medium greatly stimulates cephalexin uptake by anoxia/reoxygenation injured Caco-2 cells (P=0.0116), while the biological functions of PepT1 in injured Caco-2 cells without rhGH were markedly downregulated. Northern blot analysis showed that the level of PepT1 mRNA of rhGH-treated injured Caco-2 cells was greatly increased compared to controls.

CONCLUSION: The present results of rhGH stimulating the uptake and transport of cephalexin indicated that rhGH greatly upregulates the physiological effects of dipeptide transporters of Caco-2 cells. The alteration in the gene expression may be a mechanism of regulation of PepT1. In addition, Caco-2 cells take up cephalexin by the Proton-dependent dipeptide transporters that closely resemble the transporters present in the intestine. Caco-2 cells represent an ideal cellular model for future studies of the dipeptide transporter.

INTRODUCTION

Transport of protein in the form of small peptides (di/tripeptides) across the small intestinal wall is a major route of dietary protein absorption. The H⁺-coupled dipeptide transporter, PepT1, is known to be located in the intestine and the kidney, and plays an important role in the absorption of di/tripeptide; in addition, it mediates the intestinal absorption of β-lactam antibiotics, angiotensin-converting enzyme inhibitors, and other peptide-like drugs[3].

To investigate the properties of the human dipeptide transporter, the human intestinal cell line Caco-2 as an in vitro model was employed. The dipeptide transporters normally found in the small intestine are present in Caco-2 cells[2-4]. Also, Caco-2 cells spontaneously differentiate in culture to polar cells possessing microvilli and enterocytic properties[5]. Confluent monolayers form tight junctions between cells[5], and exhibit dome formation[6] and electrical properties of an epithelium[7]. These cells have been evaluated in detail as a model to study both transcellular and paracellular transport of nutrients and drugs in the gut[8-12].

Previous studies have shown that some hormones metabolically regulate the expression of the intestinal dipeptide transporter[13,14]. For example, as the key hormone, insulin increases the membrane population of PepT1 by increasing its translocation from a preformed cytoplasmic pool[14]. However, little is known whether another important hormone, growth hormone (GH), also metabolically regulate the functions of PepT1. The present study was undertaken to evaluate the potential effects of recombinant human growth hormone (rhGH) for the study of the properties of the human PepT1. Especially, we observed the regulation effects of rhGH to PepT1 in Caco-2 cells which were normally cultured or anoxia/reoxygenation injured. Because dipeptide and some β-lactam antibiotics (cephalexin, cefaclor etc.) share certain structural features such as a peptide bond with an α-amino group and a terminal carboxylic acid group (as illustrated in Figure 1 for cephalexin), it is not surprising that these compounds share a common transport mechanism. Unlike dipeptides, these orally absorbed antibiotics are not hydrolyzed by intestinal peptidases. Consequently, these kinds of drugs are ideal substrates to characterize the PepT1[15-17]. In this study, the uptake and transport of cephalexin were examined.

MATERIALS AND METHODS

Materials

Cephalexin (Figure 1) was obtained from Nanjing No.2 Pharma.Co. Ltd. rhGH was purchased from Laboratoires Serono S.A. (Switzerland). All other chemicals were purchased from Sigma (St. Louis, MO), unless specified. Cell culture
reagents were obtained from Gibco (Grand Island, NY) and culture supplies from Corning (Corning, NY) and Falcon (Lincoln Park, NJ).

Figure 1 Structure of cephalixin used in this study.

Cell culture
The human adenocarcinoma cell line Caco-2 was obtained from American Tissue Culture Collection (ATCC, Rockville, MD, U.S.A). The cells were passaged as previously described[18]. Caco-2 cells were grown in Dulbecco’s Modified Eagle’s Medium supplemented with 1 % nonessential amino acids, 10 % fetal calf serum (FCS), 1 000 UL·L⁻¹ penicillin, and 1 mg·L⁻¹ streptomycin (Complete DMEM). For transport Studies, Caco-2 cells were seeded on the polycarbonate filter (0.4-µm pores, 4.71-cm² growth area) in the Transwell Cell Culture Chamber System (Costar, Cambridge, MA) at a density of 600 000 cells/filter. The cell monolayers were cultured with 1.5 and 2.6 ml of complete DMEM at the apical and the basolateral chamber, respectively. To assess the integrity of the monolayer, transepithelial electrical resistance (TEER) was monitored by measuring the transmembrane resistance. After subtracting intrinsic resistance (filter alone without cell monolayers) from the total resistance, TEER was corrected for surface area and expressed as Ω cm². Transport studies were carried out with cell monolayers that were 21-25 days old. For uptake studies, cells were seeded in the collagen-coated multiwell dishes (24 pore) with the same cell density. All the medium were replaced every day when each experiment began. Monolayers were kept at 37 °C, 5 % CO₂, and 90 % related humidity.

Transport studies
Before the transport experiments, the culture medium was replaced every day from both sides of the monolayers, 1.5 ml pH 7.0 culture medium with 34 nM rhGH were added to apical chamber of Transwell, while 2.6 ml pH 7.0 culture medium without rhGH were added to basolateral chamber. Then Caco-2 cells were cultured for 4 days. The controls were incubated with PH 7.0 culture medium both in apical side and basolateral side. All the monolayers were washed during each experiment. The apical chamber were then filled with 1.5 ml solution containing 1 mM cephalixin. Transport was examined within the time range of 0, 5, 10, 30, 60, 90, and 120 min. At the end of the incubation period, cell monolayers were washed with pH 7.4 PBS to remove any extracellular cephalixin. Accumulated concentrations of cephalixin were determined by HPLC after the cells were lysed.

Anoxia-reoxygenation cells model
After Caco-2 cells were plated, media within the wells was reduced to a uniformly thin layer to reduce the diffusion distances for atmospheric gases, while maintaining cell ability. During culture, the monolayers were normally exposed to an atmosphere consisting of 95 % air and 5 % CO₂. Anoxia was produced with an atmosphere of 95 % N₂ and 5 % CO₂ for 90 min, reoxygenation was produced by restoration of the 95 % air, 5 % CO₂ for 30 min. After the period of reoxygenation, the volume of media was restored to the initial volume of 1.0 ml[20] (Figure 2).

Figure 2 Appearance of scan electronic microscopy of Caco-2 cells. (A) Tight junction formation of normal Caco-2 cells. x×1 000; (B) Tight junction of A/R Caco-2 cell’s was destroyed partially. x×1 000.

Protein determination
Cells were lysed for 20 min or ice in 2 % Nonidet P-40, 0.2 % SDS, 1 mM dithiothreitol (DTT) in PBS, and total cellular protein was determined using DC Protein Assay Reagent (BioRad).

Concentration dependence of cephalixin transport
To examine the kinetics of cephalixin transport by rhGH-treated Caco-2 cells, the different concentrations of cephalixin range of 20 to 80 µg·ml⁻¹ were used.

Northern blotting
Total RNAs were isolated from the Caco-2 cell fractions by extraction with acid guanidine thiocyanate-phenol-chloroform. Total RNAs were denatured by heating at 70 °C in 10 mmol·L⁻¹ 3-(N-morpholino) propanesulfonic acid (pH 7.0) containing 5 mmol·L⁻¹ sodium acetate, 1 mmol·L⁻¹ ethylenediaminetetraacetic acid (EDTA), 2.2 mol·L⁻¹ formaldehyde, and 50 % (vol/vol) formamide for 5 minutes and subjected to electrophoresis in 1.2 % agarose gel containing 2.2 mol·L⁻¹ formaldehyde. Resolved RNA was transferred to hybridization
and was performed in a solution that contains 50% formamide, 5x sodium chloride-sodium phosphate-EDTA, 2x Denhardt’s solution, and 1% sodium dodecyl sulfate (SDS). The membranes were exposed and analyzed by Fuji BAS-2000 system. The cDNA probe was prepared from human cDNA libraries.

Statistical analysis
Data were expressed as mean ±SE. Differences between experimental groups were assessed by analysis of Variance. P values of <0.05 were considered statistically significant.

RESULTS
Transepithelial cephalexin transport in Caco-2 cell with normal culture
Caco-2 cells were seeded at a high density of 600 000 cells/filter, the transport of 1 mM cephalexin across rhGH-treated Caco-2 monolayers, apical-to-basolateral, was examined at pH 6.0 in the donor and pH 7.4 in the receiver compartment. Integrity of the monolayers was monitored by TEER. The apical-to-basolateral transport of cephalexin was significantly increased compared with the controls (P=0.0045) (Figure 3).

Concentration (µg/ml)

0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120

Figure 3 Transport of 1 mM cephalexin in Caco-2 cells. Caco-2 cells were cultured with rhGH in apical side for 4 days. The controls were incubated with pH 7.0 culture medium without rhGH both in apical side and basolateral side. All the monolayers were washed in each experiment. The apical chambers were then filled with 1.5 ml solution containing 1 mM cephalexin. Transport was examined within the time range of 0, 5, 10, 30, 60, 90, and 120 min. At the end of the incubation period, cell monolayers were washed with PH 7.4 PBS to remove any extracellular cephalexin. Accumulated concentrations of cephalexin were determined by HPLC. The apical-to-basolateral transport of cephalexin was significantly increased compared with the controls (P=0.0045) (Figure 3).

Uptake of cephalexin in Caco-2 cells with normal culture
Uptake experiments with cephalexin were performed 6-8 days after seeding and 18 h after media replacement. Cephalexin uptake was measured in the presence and absence of 34 nM of rhGH. The differences were significant (P=0.0223), and the results were summarized in Figure 4.

Uptake of cephalexin in Caco-2 cells with anoxia/reoxygenation injury
Caco-2 monolayers were initially subjected to a 90-minute period of anoxia followed by a 30-minute period of reoxygenation. The effect of the presence of rhGH on cephalexin uptake via the PepT1 of anoxia/reoxygenation Caco-2 cells was examined (Figure 5). The present studies indicated that uptake and transport of cephalexin by the normal and anoxia/reoxygenation Caco-2 cells were significantly increased under the influence of rhGH.

Northern blot analysis
Northern blots of Poly (A)† RNA, prepared from anoxia/reoxygenation Caco-2 cells grown either under control conditions or in medium supplemented with 34 nM rhGH, were probed separately with 32P-labelled hPepT1 and β-actin cDNAs. The studies showed that the level of mRNA in rhGH-treated Caco-2 Cells was increased 1.3-fold compared with the controls.

Concentration dependence of cephalexin transport
Transport experiments were performed across the rhGH-treated Caco-2 cells using the different concentrations of cephalexin range of 20 to 80 μg·ml⁻¹. The results (Figure 6) indicated that the transport of cephalexin was increased both in rhGH-treated Caco-2 cells and Controls. However, the extent of upregulation of rhGH on the transport was greater than the controls followed the increase of the concentration of cephalexin.
To examine the functions of PepT1, we used Caco-2 cells (the human adenocarcinoma cell line) as a unique in vitro intestinal model. Some β-lactam antibiotics (cephalexin) and dipeptides share certain structural features, and they are generally in the D-form, which allows them to escape hydrolysis by cytoplasmic peptidases. So these compounds share a common transport mechanism. In this study, we employed cephalexin as an ideal substrate. The previous studies have shown that the insulin stimulates dipeptide transport by increasing membrane insertion of PepT1 from a preformed cytoplasmic pool, and choleratoxin decreases dipeptide transport by inhibiting the activity of PepT1 through an increase in the intracellular concentration of adenosine 3’,5’-cyclic monophosphate. It remains unclear, however, whether another key hormone, human growth hormone (hGH), also shows some significant importance. Strong evidence has demonstrated that growth hormone (GH) is an important growth factor for intestine. Complete GH depletion due to hypophysectomy causes pronounced hypoplasia of small intestinal mucosa with decreased villus height and reduced crypt cell proliferation. Simple replacement of GH can restore mucosal proliferative activity. rhGH promotes normal growth and development in the body by changing chemical activity in cells. It activates protein production in muscle cells and the release of energy from fats. rhGH significantly improves the anabolism in parenterally fed animals. It is typically used to stimulate growth in children with hormone deficiency, or to treat people with severe illness, burns or sepsis where destruction of human tissue and muscle occurs. Many of the effects of human growth hormone are brought about by the insulin-like growth factor 1 (IGF-1), which the growth hormone stimulates. IGF-1 plays an important part in growth-promoting effects of rhGH. The present studies showed that the uptake and transport of cephalexin in Caco-2 cells were greatly increased by using of rhGH. In addition, a specific injured cell model, anoxia/reoxygenation Caco-2 cells model, was established in our present experiment. The uptake of cephalexin in the injured Caco-2 cells was markedly decreased while the cephalexin uptake was significantly increased in the injured Caco-2 cells with treatment of rhGH. These results indicate that the functions of PepT1 in Caco-2 cells were upregulated by rhGH. The investigation of concentration-dependent transport of cephalexin in Caco-2 cells showed that the transport was increased both in rhGH-treated Caco-2 cells and controls. However, the upregulating extent of rhGH on the transport of cephalexin was greater than controls following the increase of the concentration of cephalexin.

**DISCUSSION**

The nutritional importance of amino acid uptake in the form of short-chain peptides (di/tripeptides) is well known. The intraluminal products of protein digestion are predominantly small peptides. A significant fraction of the dietary amino nitrogen is absorbed as intact dipeptide rather than free amino acids. Absorption of these small peptides from the gastrointestinal (GI) tract of mammals occurs via the dipeptide transporter (PepT1). Previous studies have shown that the functions of intestine (including PepT1) were changed under the influence of many factors. PepT1 seems to play important roles in nutritional and pharmacological therapies; for example, it has allowed the use of small peptide as a source of nitrogen for enteral feeding and the use of oral route for delivery of peptidomimetic drugs such as β-lactam antibiotics. PepT1 was located at the brush-border membranes of the absorptive epithelial cells along the small intestine but absent in crypt and goblet cells.

To examine the functions of PepT1, we used Caco-2 cells (the human adenocarcinoma cell line) as a unique in vitro intestinal model. One clear advantage of the Caco-2 cells is that the cells stay viable throughout the transport studies. Also, a monolayer of cells may be grown on a porous support to represent an intact epithelium. Some β-lactam antibiotics (cephalexin) and dipeptidomimetic drugs such as β-lactam antibiotics (cephalexin) and dipeptides share certain structural features, and they are generally in the D-form, which allows them to escape hydrolysis by cytoplasmic peptidases. So these compounds share a common transport mechanism. In this study, we employed cephalexin as an ideal substrate.

The previous studies have shown that the insulin stimulates dipeptide transport by increasing membrane insertion of PepT1 from a preformed cytoplasmic pool, and choleratoxin decreases dipeptide transport by inhibiting the activity of PepT1 through an increase in the intracellular concentration of adenosine 3’,5’-cyclic monophosphate. It remains unclear, however, whether another key hormone, human growth hormone (hGH), also shows some significant importance. Strong evidence has demonstrated that growth hormone (GH) is an important growth factor for intestine. Complete GH depletion due to hypophysectomy causes pronounced hypoplasia of small intestinal mucosa with decreased villus height and reduced crypt cell proliferation. Simple replacement of GH can restore mucosal proliferative activity. rhGH promotes normal growth and development in the body by changing chemical activity in cells. It activates protein production in muscle cells and the release of energy from fats. rhGH significantly improves the anabolism in parenterally fed animals. It is typically used to stimulate growth in children with hormone deficiency, or to treat people with severe illness, burns or sepsis where destruction of human tissue and muscle occurs. Many of the effects of human growth hormone are brought about by the insulin-like growth factor 1 (IGF-1), which the growth hormone stimulates. IGF-1 plays an important part in growth-promoting effects of rhGH. The present studies showed that the uptake and transport of cephalexin in Caco-2 cells were greatly increased by using of rhGH. In addition, a specific injured cell model, anoxia/reoxygenation Caco-2 cells model, was established in our present experiment. The uptake of cephalexin in the injured Caco-2 cells was markedly decreased while the cephalexin uptake was significantly increased in the injured Caco-2 cells with treatment of rhGH. These results indicate that the functions of PepT1 in Caco-2 cells were upregulated by rhGH. The investigation of concentration-dependent transport of cephalexin in Caco-2 cells showed that the transport was increased both in rhGH-treated Caco-2 cells and controls. However, the upregulating extent of rhGH on the transport of cephalexin was greater than controls following the increase of the concentration of cephalexin. Northern blot analysis showed that the level of PepT1 mRNA of injured Caco-2 cells with rhGH treatment were increased. This result also provides novel information about the mechanism of regulation action of rhGH. It has suggested that the alteration in the gene expression may be a mechanism of regulation of PepT1.

Use of this experimental design leads to the following three questions. What is the detail mechanism of upregulating the functions of PepT1 by rhGH? How does the rhGH receptor distribute in Caco-2 cells? How does the rhGH receptor change in anoxia/reoxygenation Caco-2 cells? Clearly, the present study needs to be followed by further studies on physiology and biology of hormonal regulation of PepT1.
Artursson P, Thamotharan M. Human intestinal epithelial (Caco-2) cells. AM J Physiol 1987; 253(3 Pt 1): C323-330

Biais A, Bissonnette P, Berteloot A. Common characteristics for Na+-dependent sugar transport in Caco-2 cells and human fetal colon. J Membr Biol 1987; 109: 113-125

Rouset M, Laburthe M, Pinto M, Chevalier G, Rouyer-Fessard C, Dussaux E, Trugnan G, Boige N, Brun JL, Zwebaum A. Enterocytic differentiation and glucose utilization in the human colon tumor cell line Caco-2: modulation by forskolin. J Cell Physiol 1989; 123: 377-385

Romond MJ, Martinot-Patgnoux M, Erlinger S. Dome formation in the human colon carcinoma cell line Caco-2 in culture. Influence of ouabain and permeable supports. Biol Cell 1985; 54: 89-92

Grasset E, Pinto M, Dussaux E, Zwebaum A, Desjeux JF. Epithelial properties of human colonic carcinoma cell line Caco-2: electrical parameters. AM J Physiol 1984; 247(3 Pt 1): C260-267

Milovic V, Turchanowa L, Stein J, Caspary WF. Transepithelial transport of putrescine across monolayers of the human intestinal epithelial cell line. Caco-2. World J Gastroenterol 2001; 7: 193-197

Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 1989; 96: 736-749

Artursson P. Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. J Pharm Sci 1990; 79: 476-482

Artursson P, Karlsson J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem Biophys Res Commun 1991; 175: 880-885

Artursson P. Cell cultures as models for drugs absorption across the intestinal mucosa. Crit Rev Ther Drug Carrier Syst 1991; 8: 305-330

Thamotharan M, Bawani SZ, Zhou X, Adibi SA. Hormonal regulation of oligopeptide transporter PepT1 in a human intestinal cell line. AM J Physiol 1999; 276(4 Pt 1): C821-826

Nielsen CU, Amstrup J, Steffansen B, Frokjaer S, Brodin E. Epidermal growth factor inhibits glycylsarcosine transport and hPepT1 expression in a human intestinal cell line. AM J Physiol Gastrointest Liver Physiol 2001; 281: G191-199

Ganapathy V, Mendicino JF, Lebach FH. Transport of glycyl-L-proline into intestinal and renal brush border vesicles from rabbit. J Biol Chem 1981; 256: 118-124

Rajendran VM, Ansari SA, Harig JM, Adams MB, Khan AH, Ramaswamy K. Transport of glycyl-L-proline by human intestinal brush border membrane vesicles. Gastroenterology 1985; 89: 1298-1304

Berteloot A. Membrane potential dependence of glutamic acid transport in rabbit jejunal brush-border membrane vesicles: K+ and H+ effects. Biochim Biophys Acta 1986; 861: 447-456

Dantzig AH, Bergin L. Uptake of the cephalosporin, cephalaxin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. Biochim Biophys Acta 1990; 1027: 211-217

Dantzig AH, Bergin L. Carrier-mediated uptake of cephalaxin in human intestinal cells. Biochim Biophys Res Commun 1988; 155: 1062-1087

Ratyche R, Chuknyiska RS, Bulkley GB. The primary localization of free radical generation after anoxia/reoxygenation in isolated endothelial cells. Surgery 1987; 102: 122-131

Ferraris RP, Diamond J, Kwan WW. Dietary regulation of intestinal transport of the dipeptide carnosine. AM J Physiol 1988; 255(2 Pt 1): G143-150

Ganapathy V, Brandsch M, Leibanch FH. Intestinal transport of amino acids and peptides. In: Johnson LR, ed. Physiology of the gastrointestinal tract. Volum 2. 3rd ed. New Yor: Raven 1994: 1773-1794

Li YS, Li J, Li N, Jiang ZW, Zhao YZ, Li NY, Liu FN. Evaluation of various solutions for small bowel graft preservation. World J Gastroenterol 1998; 4: 140-143

Liang LJ, Yin XY, Luo SM, Zheng JF, Lu MD, Huang JF. A study of the ameliorating effects of carnitine on hepatic steatosis induced by total parenteral nutrition in rats. World J Gastroenterol 1999; 5: 312-315

Ogihara H, Saito H, Shin BC, Terada T, Takenoshita S, Nagamachi Y, Inui K, Takata K. Immuno-localization of H+/peptide cotransporter in rat digestive tract. Biochem Biophys Res Commun 1996; 220: 948-952

Adibi SA. The oligopeptide transporter (PepT1) in human intestine: Biology and Function. Gastroenterology 1997; 113: 332-340

Dantzig AH, Bergin L. Uptake of the cephalosporin, cephalaxin, by a dipeptide transport carrier in the human intestinal cell line, Caco-2. Biochim Biophys Acta 1990; 1027: 211-217

Zhou X, Li YX, Li N, Li JS. Effect of bowel rehabiltiative therapy on structural adaptation of remnant small intestine: animal experiment. World J Gastroenterol 2002; 7: 66-73

Bastie MJ, Balas D, Laval J, Sonegas-Balas F, Bertrand C, Freixinos J, Ribet A. Histological variations of jejunal and ileal mucosa on days 8 and 15 after hypophysectomy in rat: morphometrical analysis on light and electron microscopy. Acta Anat (Basel) 1982; 112: 321-337

Scow RO, Hagan SN. Effect of testosterone Propionate and growth hormone on growth and chemical composition of muscle and other tissues in hypophysectomy sedentary male rats. Endocrinology 1965; 77: 852-859

Gu Y, Wu ZH. The abiotic effects of recombinant human growth hormone and glutamine on parenterally fed, short bowel rats. World J Gastroenterol 2002; 8: 752-757

Jeschke MG, Herndon DN, Wolf SE, Debroy MA, Rai J, Lichtenbelt BJ, Barrow RE. Recombinant human growth hormone alters acute-phase reactant proteins, cytokine expression, and liver morphology in burned rats. J Surg Res 1999; 83: 122-129

Roth E, Valentini L, Semsroth M, Holzenbein T, Winkler S, Blum LG, Yin XY, Luo SM, Zheng JF, Lu MD, Huang JF. Resistance of nitrogen metabolism to growth hormone treatment in the early phase after injury of patient with multiple injuries. J Trauma 1995; 38: 136-141

Postel-Vinay MC, Finidori J, Sotirooulos A, Dinerstein H, Martini JF, Kelly PA. Growth hormone receptor: structure and signal transduction. Ann Endocrinol 1995; 56: 209-212

Edited by Xu JY