Pharmacological Preconditioning by Incretinomimetics Exenatide and Vildagliptin: Decrement of Liver Ischemia-reperfusion Injury

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Abstract

Introduction: Experimental and clinical data accumulated over the past decade indicate a number of pleiotropic effects that are inherent in incretinomimetics. These effects are due to the wide distribution of GLP-1 receptors (GLP-1R) in many organs and tissues.

Objective: Study of hepatoprotective activity of exenatide and vildagliptin on the liver ischemia/reperfusion model, taking into account biochemical and morphological parameters.

Methods: Ischemia-reperfusion injury of the liver was reproduced by 15-minute clamping of the rat liver-duodenal ligament analogue with subsequent restoration of blood flow and removal of the animal from the experiment on the 3rd day. Exenatide at a dose of 10 μg/kg was administered subcutaneously, vildagliptin orally at a dose of 0.2 mg/kg 2 hours before the experiment.

Results: Exenatide (10 μg/kg) and vildagliptin (0.2 mg/kg) reduced hepatocellular ischemia/reperfusion injury, which resulted in preventing the increased activity of AST and ALT transaminases, reducing necrosis areas by 1.9 times (compared with the ischemia/reperfusion group), reducing the infiltration of stromal and parenchymal elements of the liver, reducing edema.

Conclusion: The hepatoprotective effects of exenatide and vildagliptin identify them as potentially effective drugs for correcting ischemic/reperfusion injury of the liver.

Keywords: Ischemia-reperfusion, Liver, Incretinomimetics, Exenatide, Vildagliptin, Hepatoprotection.

Introduction

Currently, a wide range of methods for the prevention and correction of disorders arising from ischemia/reperfusion syndrome of the liver has been developed. One of these approaches is ischemic preconditioning, the essence of which is short-term ischemic-reperfusion effect by applying/removing the clamp on afferent liver vessels before or after the main ischemia episode [1], as a result, there is an improvement in microcirculatory and metabolic processes, the activity of the LPO decreases, decreases production of pro-inflammatory cytokines [2-4, 64]. Despite the promising results of experimental studies, ischemic preconditioning showed no effect in clinical trials [5]. From a clinical point of view, preconditioning with pharmacological agents seems preferable. For this purpose, various drugs have been studied: antioxidants (glutathione, α-tocopherol, acetylcysteine, ascorbic acid, α-lipoic acid), as well as antioxidant gene therapy, cytokine receptor antagonists and inhibitors of the complement system, apoptosis inhibitors (Z-DEVD-FMK - inhibitor 3 and 7 of caspases), hormones and hormone-like substances (erythropoietin, melatonin, methylprednisolone), vasodilators (precursors and NO donators, prostacyclin analogues), immunosuppressants (cyclosporine), as well as enzyme inhibitors - cyclooxygenase-2, x antioxidase, NADH oxidase [6-13]. At the same time, none of the proposed methods and their combinations provide guaranteed protection against ischemic-reperfusion injury of the liver with potential development in the postoperative period of its dysfunction [14], and therefore the search and selection of innovative and promising compounds and preparations of this direction of action continues.

Preclinical studies at the cellular, molecular [15-17], organ [18-21, 63], systemic and organism levels [22-28, 61], including specific activity [29-35, 58-60] and toxicological studies [36, 62] in combination with bioequivalence studies [37], therapeutic equivalence and effectiveness are an integral part of the research of innovative drugs.

Experimental and clinical data accumulated over the past decade indicate a number of pleiotropic effects that are inherent in incretinomimetics, which

expand their therapeutic potential. In this regard, the study of the exenatide and omindagliptin increstinomimetics as agents of pharmacological preconditioning with the aim of possible prevention and correction of the effects of ischemic-reperfusion injury of the liver is of particular interest.

Objectives of the study: study of hepatoprotective activity of exenatide and vildagliptin in liver ischemia/reperfusion, taking into account biochemical and morphological parameters and studying the mechanism of their action.

Materials and Methods

An experimental study was conducted on 60 male Wistar rats weighing 250-300 g.

Ischemic reperfusion injury of the liver was reproduced by 15-minute clamping of the rat liver-duodenal ligament analogue with subsequent restoration of blood flow and removal of the animal from the experiment on the 3rd day, this ischemia dosing regimen was chosen as one of the most common in the study of supraliminal ischemic stimulus [10] (Figure 1).

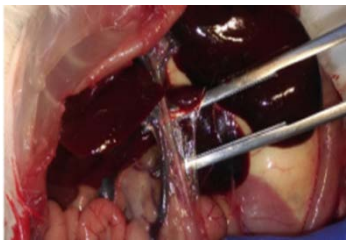

Figure-1: Isolation of the hepatic-duodenal rat ligament followed by modeling of ischemia/reperfusion

Exenatide at a dose of 10 μg/kg was administered subcutaneously, vildagliptin orally at a dose of 0.2 mg/kg 2 hours before the experiment. Ademethionine was chosen as a reference drug, which was used at a dosage of 400 mg/kg intraperitoneally 30 minutes before an episode of ischemia.

Glibenclamide, the K^+-ATP-channels blocker, was administered at a dose of 5 mg/kg, 30 minutes before the introduction of the pharmacological agent.

Blood samples were taken from all groups of animals in order to determine the biochemical parameters of AST and ALT, immediately before euthanasia (after 3 days of the experiment), and liver sites for morphological studies. To assess the severity of hepatocellular damage, the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined by a kinetic photocolorimetric method on a Vitalab Flexor E (Netherlands) automated biochemical analyzer using Biocon and Human reagents (Germany). For the histological examination of the liver, areas of 1.0x1.0x0.5 cm were taken with subsequent preparation of histological microscopic preparations and their staining with hematoxylin and eosin, according to Van Gieson, Mac Manus and Pappenheim. The study of micropreparations, photoprotocoling and morphometry was performed on a Leica DM4000B microscope with a video recording and image processing system. The obtained experimental data were processed by methods of variation statistics [38] using software. Before starting the procedures of statistical processing, data arrays were tested for normal distribution using the W Shapiro-Wilk criterion. Given the non-normal distribution pattern, the data were presented as medians (Me) and quartiles (p0.25/p0.75). In connection with the design features of the study, the non-parametric U-criterion Mann-Whitney (for independent groups) was used to identify statistical differences between groups. Differences in which the level of the confidence interval (p) was more than 95% ($p<0.05$) were considered significant.

Results and Discussion

In order to assess the effectiveness of using exenatide and vildagliptin as a cytoprotectant in conditions of liver ischemia/reperfusion, the activity of classical markers of cytolysis of hepatocytes in blood plasma was determined (Table 1). The data obtained indicate the development and increase of cytolytic syndrome in animals in groups with ischemia. Thus, in rats subjected to a 15-minute shutdown of the liver from the systemic circulation, there was an increase in the release of hepatocyte enzymes into the blood with an increase in ALT activity by 2.8 times and to a lesser extent AST - 2.0 times in comparison with the group of false-operated animals (Table 1).

Table-1: The protective effect of exenatide and vildagliptin on transaminases on the background of glibenclamide during liver ischemia/reperfusion (M±m; n=10)

Animal Group	AST (unit/l)	ALT(unit/l)
Intact	110.3±13.8[1]	61.4±19.9[1]
Sham operated	115.2±12.3[**]	64.8±27.3[**]
Control	234.1±23.2[*1]	183.1±16.8[1*]
Exenatide 10 mcg/kg	130.2±15.9[**]	107.1±11.7[**]
Vildagliptin 0.2 mg/kg	123.6±18.5[**]	89.8±27.3[**]
Ademethionine 400mg/kg	170.2±6.7[*]	149.7±5.1[*]
Glibenclamide 5 mg/kg + exenatide 10mg/kg	229.7±20.5[*1]	171.4±18.2[*1]
Glibenclamide 5 mg/kg + vildagliptin 0.2 mg/kg	232.4±19.7[*1]	174.5±22.4[*1]

Note: * - p> 0.05 compared with a group of intact animals; 1– p <0.05 compared with the group of the sham-operated; ** - p <0.05 compared with the control group.

The activity of AST and ALT with a 15-minute shutdown of the liver from the systemic circulation on the background of the administration of exenatide to animals 10 μg/kg and vildagliptin 0.2 mg/kg differed from that of rats who did not receive drugs. Thus, with vascular isolation of the liver on the background of exenatide 10 μg/kg and vildagliptin 0.2 mg/kg, AST and ALT were significantly lower than the control group and amounted to 130.2±15.9 u/ l and 107.1 ± 11 for exenatide. 7 units/l for vildagliptin: 123.6±18.5 units/ l and 89.8 ± 27.3 units/ l, respectively. Thus, the use of incretinomimetics for 3 days to correct liver ischemia/reperfusion made it possible to reduce the development of hepatocyte cytolysis.

The comparison drug ademethionin 400 mg/kg AST and ALT was lower than in the control group, but higher than in the group with exenatide 10 μg/kg and vildagliptin 0.2 mg/kg (Table 1). In order to determine the mechanism for the implementation of the protective action of exenatide and vildagliptin, 30 minutes before distant ischemic preconditioning a blocker of ATP-dependent K^+channels glibenclamide was used as a pharmacological analyzer intraperitoneally at a dose of 5 mg/kg.

Pretreatment with 5 mg/kg glibenclamide resulted in an increase in AST and ALT, which is comparable with the ischemia/reperfusion group and leveled the anti-ischemic effect of incretinomimetics (Table 1).

A morphological study in the control group revealed that significant changes in nuclei characterized by vacuolization, chromatin fragmentation until complete disappearance, with chromatin residues along the periphery of the nucleus. In hepatocytes, there are few unchanged nucleoli. Cytoplasm had significant granular and adipose degeneration.

There is significant edema, characterized by a sharp expansion of the Disse space. Foci of necrosis with regenerative hypertrophic hepatocytes along the periphery and foci of lymphocytic and thick leukocyte infiltration are expressed.

Necrosis is localized subcapsular and around the hepatic tracts. Glycogen in hepatocytes is diffuse, exists not in all cells (Figure 2).

On average, the area of necrosis in the control group was 0.217±0.014 mm^2 (Table 2).

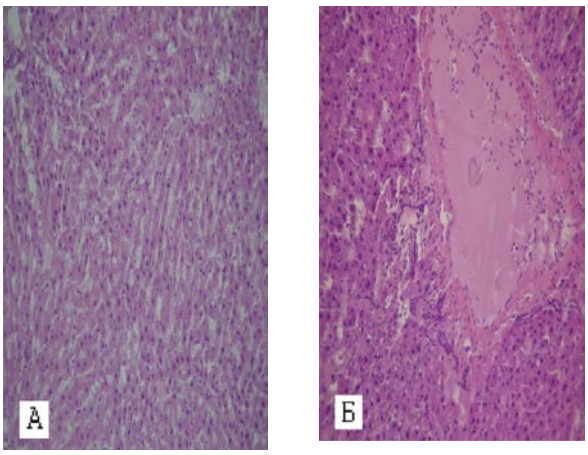

Figure-2: Morphological changes in the liver during ischemia/reperfusion.
A) The intact group - structure of the liver tissue is preserved, the lobules and portal tracts are clear.
B) The group of ischemia/reperfusion - the structure is broken, visible foci of necrosis, fat: infiltration of hepatocytes. Stained with hematoxylin and eosin. Microphoto. X 100.

By simulating ischemia/reperfusion of the liver, both studied incretinomimetics contributed to a reliable correction of hepatocyte functioning. The necrosis area when used with exenatide and vildagliptin decreased by 1.7 and 1.9 times, respectively, and averaged to 0.126±0.012 and 0.118±0.008 mm^2 (Table 2).

Table-2: The protective effect of exenatide and vildagliptin on the necrosis area on the background of glibenclamide during liver ischemia/reperfusion (M±m; n=10)

№	Animal Groups	Necrosis area, mm^2
1.	Sham operated	-
2.	Ischemia/reperfusion (control)	0.217±0.014
3.	Exenatide 10 mcg/kg	0.126±0.012*
4.	Vildagliptin 0.2 mg/kg	0.118±0.008*
5.	Ademethionine 400mg/kg	0.189±0.011*
6.	Glibenclamide 5 mg/kg + exenatide 10μg/kg	0.210±0.017
7.	Glibenclamide 5 mg/kg + vildagliptin 0.2 mg/kg	0.213±0.012

Note: * p≤0.05 when compared with the ischemia / reperfusion group.

Morphological changes were reduced to minimizing the swelling of Disse spaces, reducing infiltrative changes (Figure 3).

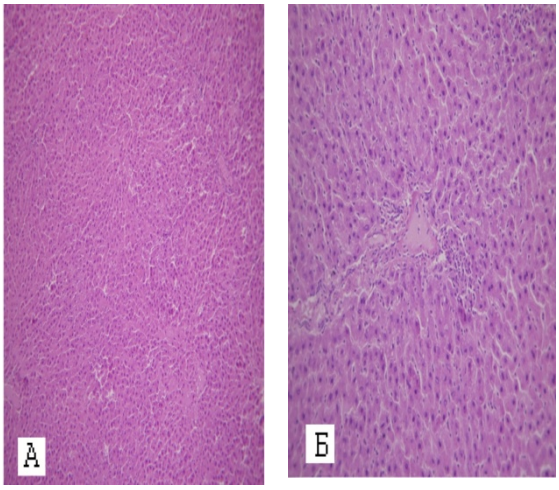

Figure-3: The effect of exenatide 10 μg/kg and vildagliptin 0.2 mg/kg on ischemia/reperfusion of the liver.

A) The group with the use of exenatide 10 μg/kg: the structure of the liver is preserved, isolated foci of necrosis.

B) Group using vildagliptin 0.2 mg/kg: micronecrosis, hepatocyte fatty infiltration. Stained with hematoxylin and eosin. Microphoto. X 100.

The comparison drug ademethionine at a dose of 400 mg/kg reduced the area of necrosis to a much lesser extent than incretinomimetics - 0.189±0.011 mm^2 (Table 2).

Using exenatide 10 μg/kg and vildagliptin 0.2 mg/kg on the background of the ATP blocker K^+ channels of glibenclamide 5 mg/kg showed a decrease of necrosis areas in their effectiveness, and approached the control group in size (Table 2).

The data obtained during the work indicate a powerful hepatoprotective effect of exenatide 10 μg/kg and vildagliptin 0.2 mg/kg, which is accompanied by a decrease in the severity of hepatocellular damage in ischemic/reperfusion injuries of the liver, manifested by a decrease in transaminase activity and a decrease in areas of necrosis.

The proposed mechanism of hepatoprotective action is realized through the activation of mitochondrial ATP-dependent K^+ channels, as evidenced by the complete abolition of the protective action against the introduction of the ATP blocker K^+ channels glibenclamide (5 mg/kg).

Due to ischemia and subsequent liver reperfusion, a cascade of metabolic, morphological and immunological changes in the liver is launched, which can potentially lead to the development of liver failure in the early postoperative period, which is especially dangerous for patients with chronic hepatitis and liver cirrhosis. In addition, there is evidence of accelerated growth of colorectal micrometastases with prolonged ischemic liver damage under vascular occlusion [39]. In addition, ischemia-reperfusion of the graft of varying severity is present at each liver transplant and contributes significantly to its early postoperative dysfunction. In this regard, it is relevant to search and use drugs that minimize the effects of ischemic/reperfusion injury of the liver.

Intensive studies of the mechanisms of the incretinomimetics anti-ischemic action have provided significant progress in understanding the implementation of the principle of their action [40].

Normally, GLP-1R is identified in the pancreas, intestines, lungs, adipose tissue, muscles, kidneys, heart, endothelial, smooth muscle cells (SMC), macrophages and monocytes, as well as in neurons and glial cells [41-43]. However, GLP-1R-independent effects of GLP-1 are not excluded, in particular, their ability to activate ATP-dependent K^+ channels [43-47]. In a number of studies, preliminary introduction of glibenclamide (an ATP-dependent potassium channel blocker) resulted in the disappearance of the cardioprotective, endothelial-protective effect of GLP-1R activation. A number of authors suggest that the protective mechanisms of action of GLP-1 are mediated including the discovery of ATP-dependent K+mitochondria. Their discovery is known to be a key element in the phenomenon of ischemic preconditioning, which involves several intracellular signaling pathways, including RISK pathways (Reperfusion injury kinase/RISK/pathway) - protein kinase A, phosphoinositol-3-kinase (PI3K), protein kinase B and extracellular signal-regulated kinases (ERK1/2). Probably, the anti-apoptotic effect of GLP-1 is associated with the RISK kinase pathway, which protects against reperfusion injury by reducing the permeability of the mitochondrial membrane [48-49]. RISK kinases inhibit the opening of highly permeable mitochondrial pores (MPTP), block cell overload with calcium ions, which triggers anti-apoptotic pathways, or blocks the initiation of apoptotic factors.

According to the authors, activation of the GLP-1 receptor is able to induce preconditioning mechanisms.

GLP-1R agonists and DPP-4 inhibitors can increase the expression of the antioxidant enzyme hemoxygenase-1 (HO-1), a stress-inducible enzyme, which limits the rate of heme degradation and protects the cell from oxidative stress. Some researchers attribute this to GPP1R-mediated activation of the transcription factor Nrf2 (GPP1R/PKA(PKB)/CREB/Nrf2), regulating gene expression of antioxidant enzymes (glutathione-S-transferase, UDP-glucuronyl transferase, heme oxygenase-1, etc.). [50-57]

Conclusion

The data obtained during the research indicate the hepatoprotective effect of incretinomimetics, which is accompanied by a decrease in the severity of hepatocellular damage in ischemic and reperfusion injuries of the liver. The effect of the complete abolition of the protective action using the mitochondrial blocker ATP-dependent K^+channels glibenclamide, suggests the implementation of the protective effect through ATP-dependent potassium channels.

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