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MORPHOLOGICAL ASSESSMENT OF APOPTOSIS AND NECROSIS SIGNS IN THE GASTRIC MUCOSA IN A MODEL OF HEPATORENAL SYNDROME

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Abstract

Background: Hepatorenal syndrome (HRS) is a severe complication of advanced liver disease characterized by functional renal failure and profound systemic hemodynamic disturbances. These alterations may significantly affect gastrointestinal organs, particularly the gastric mucosa. However, the mechanisms of epithelial injury and the balance between apoptosis and necrosis in the gastric mucosa during HRS remain insufficiently studied.

Objective: The aim of this study was to evaluate morphological manifestations of apoptosis and necrosis in the gastric mucosa in an experimental model of hepatorenal syndrome.

Materials and Methods: Sixty male Wistar rats were divided into three groups: sham-operated control (n=20), cirrhosis group induced by common bile duct ligation (n=20), and hepatorenal syndrome group (n=20), where acute-on-chronic liver failure and renal dysfunction were induced using carbon tetrachloride following bile duct ligation. Gastric tissues were collected on day 28. Histological examination was performed using hematoxylin–eosin staining to detect necrotic changes. Apoptosis was evaluated using the TUNEL assay. Immunohistochemical analysis assessed the expression of apoptosis-related proteins including Bax, Bcl-2, and cleaved caspase-3.

Results: The HRS group demonstrated the most severe gastric mucosal damage. The apoptotic index increased significantly from 4.2±1.1 in controls to 18.5±3.4 in the cirrhosis group and 34.7±5.2 in the HRS group ($p<0.01$). Necrotic lesions were observed in 75% of HRS specimens compared to 20% in the cirrhosis group. Immunohistochemical analysis revealed increased expression of pro-apoptotic proteins Bax and cleaved caspase-3 and decreased expression of anti-apoptotic Bcl-2 in the HRS group.

Conclusion: Hepatorenal syndrome significantly aggravates gastric mucosal injury and induces both apoptotic and necrotic cell death. These morphological changes may contribute to mucosal erosion and gastrointestinal bleeding observed in patients with advanced liver and renal dysfunction.

Keywords: Hepatorenal syndrome, gastric mucosa, apoptosis, necrosis, TUNEL assay, morphology, caspase-3.

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Introduction

Hepatorenal syndrome represents a serious complication of advanced liver disease and is characterized by functional renal failure in the absence of structural kidney damage. The syndrome develops as a result of severe systemic and splanchnic hemodynamic disturbances associated with portal hypertension and progressive hepatic insufficiency. These changes lead to activation of vasoconstrictor systems such as the renin-angiotensin-aldosterone system and the sympathetic nervous system, ultimately causing renal vasoconstriction and decreased glomerular filtration rate. Although renal dysfunction is the hallmark of HRS, the syndrome also affects other organs, particularly the gastrointestinal tract. The gastric mucosa is highly sensitive to hypoxia and ischemia caused by circulatory disturbances. In patients with liver cirrhosis, portal hypertensive gastropathy is frequently observed, characterized by vascular dilation, mucosal edema, and increased vulnerability of epithelial cells to injury. Recent studies have demonstrated that hypoxic conditions in the gastric mucosa activate molecular pathways associated with programmed cell death. Hypoxia-inducible factor-1α (HIF-1α) plays a key role in this process by activating p53-dependent signaling pathways that regulate the expression of apoptosis-related proteins such as Bax and Bcl-2. Increased Bax expression and reduced Bcl-2 expression shift the cellular balance toward apoptosis and lead to activation of caspase-3, an essential executor of programmed cell death.

In addition to hypoxia-induced apoptosis, inflammatory mediators released during liver failure can also promote epithelial injury. Tumor necrosis factor-alpha (TNF-α) has been identified as an important mediator of intestinal epithelial apoptosis in experimental models of fulminant hepatic failure. These inflammatory mechanisms may also contribute to gastric mucosal damage in advanced liver disease.

However, the specific morphological characteristics of gastric mucosal injury in hepatorenal syndrome remain poorly understood. Renal failure associated with HRS introduces additional pathogenic factors including uremic toxins, metabolic disturbances, and further hemodynamic instability. These conditions may aggravate epithelial damage and alter the balance between apoptotic and necrotic cell death.

Understanding the morphological mechanisms of gastric mucosal injury in HRS is important for clarifying the pathogenesis of gastrointestinal complications in patients with advanced liver disease. Experimental animal models provide a valuable opportunity to investigate these mechanisms under controlled conditions.

Purpose of the Study

The purpose of this study was to investigate morphological manifestations of apoptosis and necrosis in the gastric mucosa in an experimental model of hepatorenal syndrome using histological, TUNEL, and immunohistochemical methods.

Materials and Methods

This experimental study was conducted at the Department of Pathological Anatomy and the Central Research Laboratory of the Clinics of the Tashkent State Medical University. A total of sixty adult male Wistar rats weighing 220–280 g were used. Animals were housed under standard laboratory

conditions with controlled temperature and a 12-hour light–dark cycle, with free access to food and water. The rats were randomly divided into three groups (n=20 in each group).

The first group served as the sham-operated control. These animals underwent laparotomy with isolation of the bile duct without ligation.

The second group consisted of animals with experimentally induced cirrhosis. Secondary biliary cirrhosis was produced by common bile duct ligation.

The third group represented the hepatorenal syndrome model. In these animals, bile duct ligation was performed, and on the 21st postoperative day a single intraperitoneal injection of carbon tetrachloride (CCl_4) diluted in olive oil was administered to induce acute-on-chronic liver failure and renal dysfunction.

All surgical procedures were performed under ketamine-xylazine anesthesia. On day 28 of the experiment, the animals were euthanized and gastric tissue samples were collected for morphological analysis.

For histological evaluation, tissue specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4–5 μm thickness. Hematoxylin-eosin staining was used to assess general morphology and detect necrotic changes.

Apoptosis was evaluated using the TUNEL assay. The apoptotic index was calculated as the percentage of TUNEL-positive cells per 1000 epithelial cells in randomly selected microscopic fields. Immunohistochemical analysis was performed to evaluate the expression of Bax, Bcl-2, and cleaved caspase-3. The percentage of positively stained cells and staining intensity were assessed to determine immunoreactivity scores.

Statistical analysis was performed using standard statistical methods. Data were expressed as mean ± standard deviation, and differences between groups were evaluated using analysis of variance. A p-value less than 0.05 was considered statistically significant.

Results

Biochemical analysis confirmed the successful induction of liver cirrhosis and hepatorenal syndrome. Animals in the cirrhosis group demonstrated elevated liver enzyme levels and hyperbilirubinemia compared with the control group. The HRS group showed additional increases in serum creatinine and urea, indicating renal dysfunction.

Histological examination of the gastric mucosa revealed clear differences among the experimental groups. In the control group, the gastric mucosa displayed normal architecture with intact epithelial lining and well-organized gastric glands. No significant inflammatory changes or cell death were observed.

In the cirrhosis group, moderate mucosal alterations were present. These included vascular congestion, mild edema, and occasional epithelial cell damage. Apoptotic cells with condensed nuclei were observed in the superficial mucosal layer, but extensive necrosis was rare.

The most pronounced changes were detected in the hepatorenal syndrome group. Severe mucosal damage was observed, including disruption of glandular structure, vascular congestion, edema, and inflammatory infiltration. Coagulative necrosis of epithelial cells was detected in a majority of samples.

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The TUNEL assay demonstrated a significant increase in apoptotic cells in the cirrhosis and HRS groups compared with controls. The apoptotic index increased from 4.2±1.1 in the control group to 18.5±3.4 in the cirrhosis group and reached 34.7±5.2 in the HRS group.

Immunohistochemical analysis revealed significant changes in the expression of apoptosis-related proteins. In the control group, Bax expression was minimal, whereas Bcl-2 expression was relatively high. In contrast, the cirrhosis and HRS groups demonstrated increased Bax expression and decreased Bcl-2 expression.

The most pronounced imbalance between pro- and anti-apoptotic proteins was observed in the HRS group. Strong expression of cleaved caspase-3 confirmed active apoptotic processes in gastric epithelial cells.

These findings indicate that gastric mucosal injury in hepatorenal syndrome is characterized by a mixed pattern of cell death involving both apoptosis and necrosis.

Conclusion

The results of this experimental study demonstrate that hepatorenal syndrome significantly aggravates gastric mucosal injury compared with liver cirrhosis alone. The gastric mucosa in HRS exhibits severe structural alterations accompanied by increased apoptosis and extensive necrosis. The imbalance between pro-apoptotic and anti-apoptotic proteins, reflected by increased Bax expression and decreased Bcl-2 levels, plays an important role in the activation of apoptotic pathways. Simultaneously, severe metabolic and hemodynamic disturbances contribute to the development of necrotic cell death.

The coexistence of apoptosis and necrosis indicates that gastric epithelial cells in HRS are exposed to multiple damaging factors including hypoxia, inflammatory mediators, and metabolic disturbances associated with renal failure. These findings suggest that gastric mucosal damage may represent an important pathological component of hepatorenal syndrome and may contribute to the development of gastrointestinal bleeding in patients with advanced liver disease.

Further studies are required to investigate molecular mechanisms underlying these processes and to develop therapeutic strategies aimed at protecting the gastric mucosal barrier in hepatorenal syndrome.

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