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Long-term liver deportalisation in stable and time prolonged prehepatic portal hypertension model affects expression of hypoxia, angiogenesis, apoptosis, and autophagy markers in the young rats' liver

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Abstract: *prehepatic portal hypertension in children cause severe and life-threatening complications, that highlights the need in in-depth investigations of pathogenetic mechanisms, which contribute to prehepatic portal hypertension-associated liver pathology. In developed stable experimental prehepatic portal hypertension model in adolescent rat males we aimed to assess the expression levels of the key molecular markers of hypoxia, angiogenesis, autophagy, and apoptosis in the liver tissue after 6-month deportalization. Partial portal vein ligation was performed in 4-week old male rats. After 6-months tissue samples from PHP-model, sham operated and control animals were studied by western blot to identify protein levels of markers related to prehepatic portal hypertension -induced liver injury. Partial portal vein occlusion upregulated hypoxia inducible factor -1α (by 4.67 folds vs. control, p<0.001) and vascular endothelial growth factor protein expression levels (by 2.33 folds vs. control, p<0.001) suggesting chronic hypoxia development. Abnormally high levels of angiostatin isoforms were found (by 9.88 folds vs. control, p<0.001) to signify angiogenesis dysregulation. Significant caspase-3 (23.4-fold increase vs. control, p<0.001) overexpression is executive phase of apoptotic cell death evidence. Dramatic LC3 level expression indicates existence of crosstalk between autophagy and apoptosis that contribute to fibrotic changes. Hypoxia-induced events, impaired angiogenesis regulation, enhanced autophagy and apoptosis are contributing factors of prehepatic portal hypertension -induced liver injury. A better understanding of subtle molecular mechanisms of this pathology may pave the way for innovative treatment options.*

Keywords: Angiogenesis; Apoptosis; Autophagy; Hypertension; Hypoxia; Rats.

Introduction

Development of adequate experimental model is critical for the understanding of the most essential prehepatic portal hypertension (PPH)-related cellular and molecular events and basis of associated complications in order to translate the experimental results into clinical practice.

Rat models of PPH obtained by portal vein (PV) ligation is routinely used to assess changes in visceral blood circulation and to study the pathophysiology of hemodynamic changes that occur after PV occlusion (Wen et al., 2009). In the literature, the PV ligation model is widely used to study alterations in the systemic and splanchnic circulation related to PPH being a reliable and reproducible model to induce PPH-related changes during 2-4 weeks after ligation (Abraldes, Pasarín & García-Pagán, 2006). However, there is lack of

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information concerning PV ligation beyond this period. Moreover, many aspects of PH development, almost all the modes of therapy are based on adult trials.

Among various clinical manifestation of PPH, formation of portosystemic collaterals is an essential adaptive response of liver tissue in order to decompress the portal system and minimize the portal hypertension (Lautz et. al., 2009; Schettino et. al., 2006). It has been considered that the pivotal drivers of angiogenesis during PPH are hypoxia, inflammation, and fibrosis (Gana, Serrano & Ling, 2016). In addition, activation of autophagy has gained considerable attention to be one of the key processes during liver injury caused by PV occlusion. Although numerous studies have revealed the crucial role of autophagy in limiting hepatocyte death in response to hypoxia-induced liver injury and restricting fibrosis, excessive autophagic flux can play a cell death-promoting role via triggering apoptosis (Jung et. al. 2020). Some specific proteins can be synthesized during hypoxic condition, autophagy or apoptotic processes. Such molecular markers may serve as indispensable tools for delineating pathogenetic mechanisms of PPH-related complications, which can be used for the discovery of novel effective therapeutic options. Therefore, the next task of our study was to evaluate tissue levels of the reliable markers of hypoxia and extracellular matrix (ECM) remodeling, key angiogenic regulators, and molecular indices of autophagy and apoptosis expressed in the liver tissue under the condition of experimental long-term PPH in young rats.

Aim: in developed stable experimental prehepatic portal hypertension model in adolescent rat males we aimed to assess the expression levels of the key molecular markers of hypoxia, angiogenesis, autophagy, and apoptosis in the liver tissue after 6-month deportalization.

Materials and methods

Development of prehepatic portal hypertension model in four-week-old male Wistar rats by means of partial portal vein ligation was described in detail in earlier publication (Godik et. al., 2023). The study was approved by the Bioethics Committee of Bogomolets National Medical University (Kyiv, Ukraine), protocol no.141 27/01/2021. The rats were randomly divided into three groups: 1 – intact control (n = 3), 2 – sham operation (n = 4) – false ligation without narrowing PV; 3 – PV ligation (n = 10) – experimental group in which PPH was formed.

Protein sample preparation

Deep-frozen phosphate buffered saline (PBS)-perfused livers were ground under liquid nitrogen using a mortar and pestle, and homogenized in an ice-cold lysis buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 5 mM ethylenediaminetetraacetic acid (EDTA), containing supplemented with proteases/phosphatases inhibitor cocktail (PierceTM Protease and Phosphatase Inhibitor Mini Tablets, ThermoScientific, USA). Protein extracts for MMP assay were prepared with the use of the same extracting buffer in absence of enzyme inhibitors and EDTA. Tissue/buffer ratio was taken equal 1:5 (m/v). After homogenization steps, samples were sonicated for 60 s by ultrasonic disintegrator Sartorius (Labsonic® M, Göttingen, Germany) and centrifuged at 16,000 g for 45 min at 4 °C. Supernatants were transferred into new plastic tubes and the total protein concentration in each supernatant was determined spectrophotometrically by Stoscheck method (Stochek, 1990). The samples for western blots were diluted 1:2 in reduced Laemmli Sample Buffer, while non-reduced electrophoretic buffer was used for sample preparation for MMP assay. All protein extracts were flash frozen and stored at – 80 °C before analysis.

Western blot

The levels of regulatory proteins related to hypoxia regulatory pathways (hypoxia-inducible factor-1α, HIF-1α), the key counteracting angiogenesis regulators (vascular endothelial growth factor, (VEGF), and angiostatins (AS), markers of apoptosis (Bax, caspase-3, poly(ADP-ribose) polymerase, (PARP-1) and autophagy flux (intra-autophagosomal LC3) were measured by western blot. Tubulin was used to check an equal protein loading. Protein samples of the liver tissue were separated electrophoretically in 10% sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) loading 100 µg protein per lane. Proteins were transferred from gel onto 0.45 ± 0.2 µm pore-size nitrocellulose membranes (Amersham Biosciences, Uppsala, Sweden) by electroblot. The membranes were blocked in 5% non-fat skim milk solution

ISSN 2786-6661
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(ApexTM Bioresearch Products, USA) for 90 min at 37 °C. After blocking, the blots were probed with the following primary antibodies against proteins of interest, namely rat anti-tubulin YL1/2 (Abcam, USA, no. ab6160), rabbit anti- hypoxia inducible factor (HIF)-1α (Sigma Aldrich, USA, no. HPA001275), mouse anti-VEGF (Invitrogen, USA, no. MA5-12184), mouse anti-caspase-3 (Invitrogen, USA, no. PA5-77887), rabbit anti-LC3 (ProSci, USA, no. 79-626), or rabbit anti-angiostatins (produced as described elsewhere [14]) at 4oC overnight. The membranes were washed in PBS, containing 0.05% Triton X-100 (PBST), and incubated with the appropriate anti-species goat secondary antibodies conjugated with horseradish peroxidase (HRP) (anti-rabbit IgG, Abcam, USA, ab6721; anti-mouse IgG, Abcam, USA, ab197767; anti-rat IgG, Abcam, USA, ab97057) for 90 min at 37 °C. After washing in PBST, the membranes were incubated with horseradish peroxidase (HRP) substrate and exposed on X-ray film (Konica Minolta, Japan) by enhanced chemiluminiscence (ECL) technique. Signals were visualized, digitized, and analyzed using TL120 software (TotalLab Ltd., USA). Molecular weights were determined using standard prestained trans blot molecular weight markers (PageRuler, cat. no. 26616, Fermentas, Lithuania). Protein levels were expressed in arbitrary units after correction for α-actin.

Statistical analysis

The Shapiro-Wilk test was used to check a normal distribution of data. Data of western blots were analyzed with the use of one-way ANOVA followed by U Mann-Whitney *post-hoc* test or Sheffe multiple comparison test to evaluate differences between group mean values by «OriginPro» software (major version 9.0 SR2 Pro English) and IBM SPSS for Windows version 24.0 (IBM Corp., Armonk, NY). All variables were expressed as mean ± standard error of the mean (SEM). For all tests, $P<0.05$ was considered statistically significant.

Results

The liver biopsy was taken an all rats of all groups in 6 months since the initial operation. The ability of the model was proven in the primary study by confirming the PPH [7] via specific changes revealed by ultrasound (liver hilum collaterals formation, splenomegaly ($p<0.01$)), and later via studying the pathological morphological changes of the rats livers structure, that showed PV occlusion dramatically decreased (>2.4-folds, P=0.0003)

Next, semi-quantitative western blot analysis was performed to uncover the effects of 6-th month PV occlusion on the expression of the key protein markers, which reflect development of pathological events, including hypoxia, angiogenesis, apoptosis, and autophagy (Fig. 1).

HIF-1α- hypoxia-inducible factor-1α; VEGF – vascular endothelial growth factor; AS – angiostatins, LC3 – intra-autophagosomal protein, caspase-3 – marker of apoptosis. Tubulin – protein of control.

It was demonstrated that PV ligation led to overexpression of HIF-1α (by 4.67 folds vs. control, $P<0.001$) and VEGF levels (by 2.33 folds versus control, $P<0.001$). These results indicate hypoxia condition development, which triggers associated excessive intrahepatic angiogenesis as

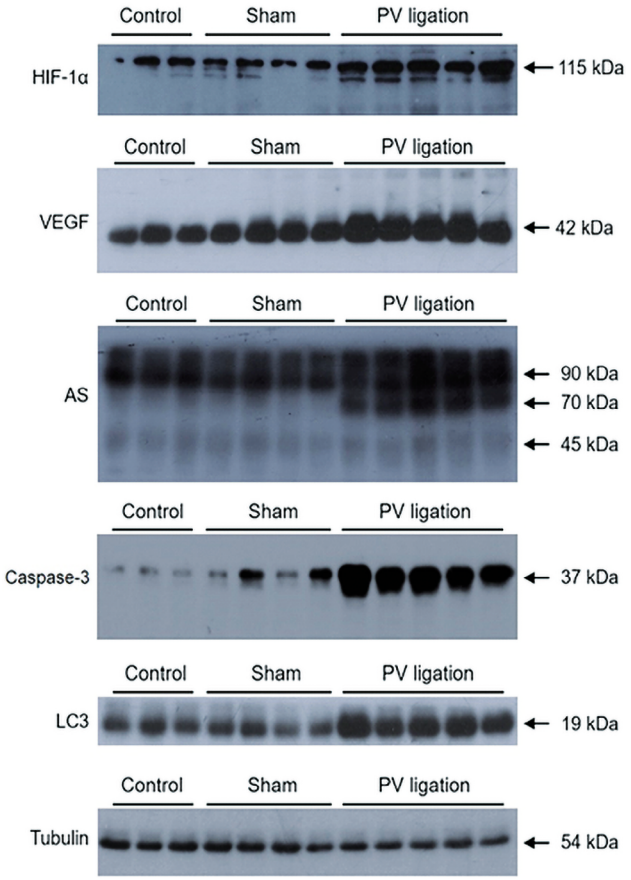

Fig. 1. Western blot analysis of expression of hypoxia-, angiogenesis-, and apoptosis/autophagy-related protein markers in the liver tissue at the 6-th month after partial portal vein ligation in the liver tissue of control, sham and PV-ligation groups

ISSN 2786-6661
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major ischemic vascular responses. Interestingly, in the false-operated group, slight but statistically significant increase in VEGF level as compared with control (by 1.71 fold, P=0.027) was identified as well suggesting even minor influence on PV environment may induce angiogenesis activation. However, the levels of plasminogen digestion products, AS, known as the main VEGF counteracting factors, were also increased in the injured livers. Content of the angiostatins (AS) variants, which corresponds to fragment of the precursor protein K1-5, was up-regulated by 9.88 folds as compared with control (P<0.001). The content of K1-4 isoform was also up-regulated (by 1.33-fold versus control), but these changes did not reach statistically significant level (P = 0.07745). Obtained results argue that partial PV ligation caused perturbations in the balance of angiogenesis modulators that can result in dysregulated blood vessel outgrowth and aberrant morphology and drive further pathology aggravation. Dramatic elevation in protein expression of caspase-3 was observed in the injured liver tissues as compared with the basal level (by 23.4 folds, P<0.001) that indicates triggering pro-apoptotic signaling. In parallel, statistically significant increase (2.13-fold, P<0.05) in the expression levels of autophagy protein marker, LC3, was determined in the liver tissue in PV ligation group suggesting possible crosstalk between apoptosis and autophagy-regulated cell death (Figure 2).

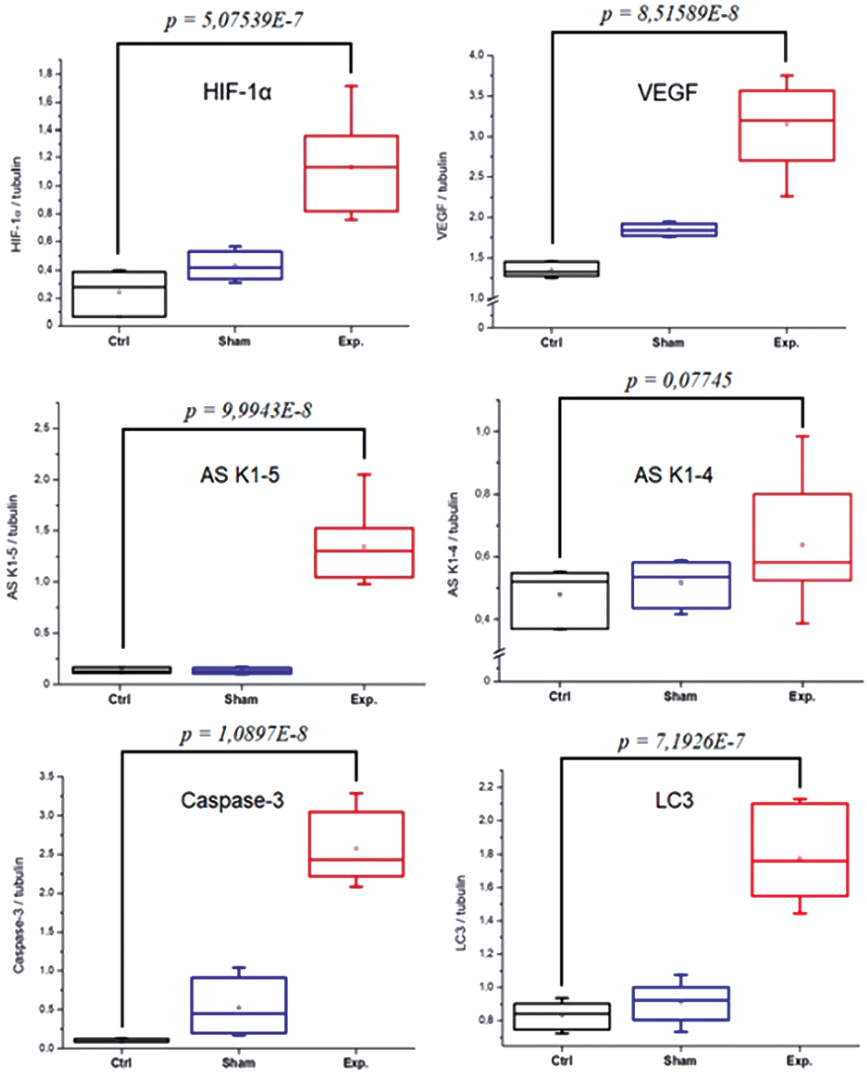

Fig. 2. Western blot analysis quantification of expression of hypoxia-, angiogenesis-, and apoptosis/autophagy-related protein markers in the liver tissue at the 6-th month after partial portal vein ligation in the liver tissue of control, sham and PV-ligation groups (Black «boxes» – control, blue «boxes» – sham; red «boxes» – PV-ligation groups).

ISSN 2786-6661
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Discussion

In pediatric patients, the most prevalent extrahepatic causes of portal perfusion reduction are obstruction at the presinusoidal or sinusoidal level, PV thrombosis, and biliary atresia, comparing with adults where liver cirrhosis is the most common cause (Lautz et. al., 2009; Schettino et. al. 2006). Chronic liver hypoperfusion leads to porto-portal anastomoses formation, which do not ensure the adequate restoration of satisfactory portal blood flow. Current methods of PH treatment in children can, on one hand, reduce the risks of life-threatening complications development, and on the other hand, porto-systemic shunting procedures increase liver deportalization. The results obtained in the present study performed on four-week-old rat males confirm that the surgical care can be considered the main way for restoring portal blood flow and improving liver decompression in pediatric patients with PH. Since intrahepatic periportal tissue is being progressively substituted by the connective tissue, restoration of portal perfusion should be considered as an urgent surgical approach in children with confirmed diagnosis of PPH. Although, several surgical therapy protocols including selective or non-selective shunts and devascularization has been implemented, there is no consensus over the most effective pharmacological option for decreasing PP or alleviating PPH consequences and reducing the risk of worsening decompensation (Gao et. al., 2020). Currently used drugs are prescribed to target the increased liver blood flow, to reduce hyperdynamic circulation and vascular hyperplasia, and to decrease the PV inflow through splanchnic vasoconstriction but their use often leads to abnormal liver microcirculation and PHT development (Lautz et. al., 2009; de Ville de Goyet et. al., 2012). For example, meta-analysis data on nonselective β-blockers demonstrated that only one-third of patients have hepatic venous pressure gradient response during treatment with β-blockers. Therefore, uncovering intricate mechanisms that underlie pathological processes in the injured hepatic tissue has an essential fundamental importance and forms the basis for efforts to develop novel targeted therapies for the pharmacological management of PHT. Moreover, the etiology of PHT in pediatric patients may differ from that of adults and may require different management strategies. In the present study, we showed for the first time that PV occlusion in the chronic settings in adolescent rats caused dramatic upregulation of the levels of hypoxia/angiogenesis, apoptosis, autophagy, and pro-fibrotic regulators, which possibly may contribute to pathological process.

It is known that angiogenesis activation plays dual role in PPH pathogenesis. It was reported (Gana et. al., 2016) that portosystemic collaterals in PPH are formed due to activation of angiogenesis, i.e. formation of portal-systemic shunts from pre-existing vasculature. On the one hand, liver tissue regeneration is angiogenesis-dependent process. However, newly formed blood vessels with abnormal structure fail to provide oxygen and nutrients to the tissues, thus worsening the course of the disease and increase hepatic vascular resistance to portal blood flow to contribute to the pathogenesis of many chronic liver diseases, including fibrosis, cirrhosis, and hepatocellular carcinoma. Inflammation and hypoxia are two major drivers of angiogenesis in all tissues (Drixler et.al., 2002). Drastic upregulation of HIF-1α expression shown in the liver tissue of rats with PV ligation clearly indicates development of hypoxic condition in the injured organ. Several animal studies suggest VEGF, the major proangiogenic mediator, to play a crucial role in intensive neovascularization caused by PHT (Gana et. al., 2016; Fernández et. al., 2009) VEGF expression is upregulated by HIF-1α, which acts as a transcriptional factor for many cytokines and MMPs responsible for both angiogenesis and fibrosis progression (Quintero-Fabián et. al., 2019). The tight correlation between increased HIF-1α and VEGF levels affirms hypoxia-induced switching of neovascularization and massive formation of portosystemic collateral vessels. Furthermore, VEGF monocyte adhesion to endothelial cells followed by their transmigration and affects monocyte chemotaxis leading to inflammation progression (Ruan et. al., 2015). Thus, in chronically damaged liver, hypoxia, inflammation, and angiogenesis develop in parallel during progression towards fibrogenesis that was confirmed in our study by histological and electronic microscopy assays. In turn, fibrosis causes an increase in hepatic vascular resistance and impairs oxygen delivery to liver cells to trigger autophagy and apoptosis, forming a «vicious circle» of disease (Kouroumalis,

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Voumvouraki & Samonakis, 2021). Moreover, if highly progressive, the fibrotic process ultimately leads to organ failure and even death. Plasma protein plasminogen, which is synthesized in liver and circulated in the bloodstream as a proenzyme form of plasmin, can by digested by tissue proteases or autolyzed by plasmin to produce a variety of kringle (K)-containing fragments referred to as angiostatins (AS). These truncated plasminogen-derived polypeptides were recently defined as the most potent physiological inhibitors of angiogenesis (Soff, 2000). Although it has been first demonstrated that AS are produced by cancerous cells and act as suppressors of solid tumor growth and anti-metastatic regulators via inhibiting tumor-induced angiogenesis and counteracting VEGF-stimulated endotheliocyte activation and migration (Niu & Chen, 2010), there is no in vivo evidence that intrahepatic AS generation occurs during PPH. We showed for the first time that prolonged PV occlusion switches on mechanisms of AS generation in the liver tissue, and AS elevated levels correlated with upregulation of active form of MMP-9, which is primarily responsible for the digestion of their precursor protein. It is of interest that, in comparison with AS 45 kDa isoform (fragment K1-4), another AS variant correspondent to K1-5 polypeptide is produced in abundancy in the injured tissue, but not in the livers from control or sham-operated rats. Several lines of evidence have been accumulated for the beneficial action of AS variants in the animal models of hepatic fibrosis and inflammation. Apart from suppressing metastatic liver cancer via inhibiting growth of neovessels and improving survival animals with hepatocellular carcinoma (Schmitz et.al., 2007), AS K1-5 expression not only reduced vessel density but also induced apoptosis and inhibited proliferation of hepatoma cells, modulated inflammation and down-regulated VEGF levels in livers. It was demonstrated that human K1-5 can act as an anti-inflammatory regulator via reducing TNF-α-induced expression of ICAM-1 and VCAM-1 on endothelial cells. Other authors have highlighted AS to be perspective antifibrotic agents based on the observation that AS inhibited the development of pathological angiogenesis and liver fibrosis in mice injected with carbon tetrachloride (Vogten et. al., 2004). Bearing in mind that angiogenesis is tightly regulated by a balance between stimulating and inhibiting factors, it can be hypothesized that enhanced AS production in the liver after 6-week-long PV occlusion can be considered as a compensatory mechanism to cease VEGF-mediated vessel outgrowth. On the other hand, AS may cause a suppressive effects of liver regeneration via inhibition of reparative angiogenesis. In the earlier study, Drixler et al. unequivocally proved that tissue repair during liver regeneration is angiogenesis-dependent process. They have found that injection of AS before partial (70%) hepatectomy in rats reduced microvascular density and inhibit liver regeneration indicating that AS may have undesirable effects of relief of liver injury. Based on these observations, which illustrate double-faced character of AS-mediated effects in damaged liver, there is a concern that suppression of VEGF-mediated angiogenesis by augmenting production of endogenous inhibitors can be an effective treatment modality for liver recovery. Nonetheless, since specificity of AS-associated signal pathways in the liver is generally unknown, the significance of AS formation in the settings of hepatic angiogenesis and fibrosis in EHPVO-related liver injury may become an important point for future in-depth investigations.

It is known that autophagy (a controlled cell self-digesting) contributes to normal hepatic functions by utilization of damaged macromolecules or organelles, and support cell survival during starvation and some pathological states. However, there are several pieces of research that report autophagy to play an essential role in many liver diseases. For example, several studies have demonstrated that autophagy promotes activation of hepatic stellate cells, which play a crucial role in the pathogenesis if hepatic fibrosis (Zhou, Wang & Shi, 2022). It has been hypothesized that inhibition of autophagy can be a novel therapeutic approach in preventing liver fibrosis (Ye, et. al., 2020). In our study, we verified autophagy progression by increasing level of LC3, a well-known marker of so-called «autophagic flux» (Schläfli et. al., 2016). As a component of autophagosome component, LC3 is related to the induction of autophagy and formation of autophagosome. It is possible that relatively slight elevation of LC3 level (two-fold increase versus basal value, $P<0.05$) in the liver tissue at the termination of experimental 6-week-long PV occlusion

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may already reflect the final stage of autophagy, in which most amount of LC3 molecules are digested in autophagosomes. In contrast to minor, though statistically significant, increase in LC3 level, we observed a drastic overexpression of caspase-3, a widely used indicator of executive phase of apoptosis, in the injured liver tissue (by 23.4 folds compared with control, $P<0.001$). It is supposed that, during the development of EHPVO-related pathology, autophagy could switch its function from pro-survival to destructive phase by triggering cell death through apoptosis activation. It has been extensively reported that liver autophagy and apoptosis may affect each other by reciprocal interactions. It is interesting that the accumulation of caspase-3 in its pro-enzyme form, which has minor proteolytic activity and can autoactivate to form active caspase-3, was found in the livers of experimental rats. This result means that at the end of 6-week-period since PV ligation, the switch of cell fate to irreversible destructive stage, which is designated by overexpression of an apoptotic executioner, caspase-3, has initiated.

The strength of this study is a good agreement between histological and ultrastructural observations and results of western blot analysis of several protein markers, which help to confirm that experimental findings are meaningful and reflect the major pathogenetic mechanisms and to prove adequacy of the developed experimental model for chronic PPH in pediatric age. Obtained results are important for pediatric hepatology since they pave the way for improved management of PPH and its complications in children and adolescents.

Conclusions. In the present study, we discovered pathophysiological changes in the hepatic tissue, including hepatocyte loss, lipid dystrophy, tissue remodeling, and fibrosis induced by chronic PV occlusion in young rats as a model of PPH. Hypoxia, angiogenesis, autophagy, and apoptosis may contribute to EHPVO-associated liver pathology, as judged by the overexpression of the correspondent protein regulators. Correlation between pathomorphological/ultrastructural changes in the liver tissue and altered levels of protein expression caused by long-term PV occlusion suggest that studied regulatory proteins may contribute to the progression of pathological process and therefore can be used as reliable markers of EHPVO-induced pathological events in the liver. Expanded knowledge of molecular mechanisms proves the optional surgical treatment of pediatric PPH must be focused on portal blood flow restoration. Further pharmacological options elaboration is to be provided to specifically target the key pathogenetic stages of chronic PV occlusion in order to reverse PPH manifestations or to improve recovery of pediatric PPH.

Conclusion

This study has some limitations. Partial PV ligation mimics general aspects of the specific liver diseases associated with PPH but may not accurately reflect the intrahepatic and splanchnic environment including inflammatory processes associated with PH in human disease. Therefore, results obtained on the given animal study must be interpreted with these limitations, and future studies are needed to translate experimental data to clinical care. Additional comparative studies are also required to clarify if some of the changes observed in the developed model of PPH have age-dependent character. Similarly, future prospective experiments should show, which of the observed EHPVO-related pathological alterations are reversible after surgical restoration of portal blood flow.

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Conflict of interest

The author declares that he has no conflict of interest.

Animal research

This study was approved by the Committee on Clinical Investigation of Bogomolets National Medical University (Kyiv, Ukraine), Protocol №141 27.01.2021. The study was conducted in accordance to implemented guidelines in consideration of GCP-ICH and the principles enshrined in the Declaration of Helsinki and Declaration of Istanbul.

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Consent to publish and contributions

Author conceived the study, performed experiments and analyzed the data, drafted the manuscript, revised it, critically reviewed and approved the final manuscript.

ORCID ID and Autor contributions

0000-0002-1084-9484 (A, B, C, D, E, F) Godik Oleg

A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of article

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Тривала депорталізація печінки в стабільній пролонгованій моделі допечінкової портальної гіпертензії впливає на експресію маркерів гіпоксії, ангіогенезу, апоптозу та аутофагії в печінці молодих щурів

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Анотація: *допечінкова форма портальної гіпертензії у дітей викликає важкі та загрозливі для життя ускладнення, що підкреслює необхідність поглибленого вивчення патогенетичних механізмів, які сприяють розвитку патології печінки, асоційованої з допечінковою формою портальної гіпертензії. Ми мали на меті оцінити рівні експресії ключових молекулярних маркерів гіпоксії, ангіогенезу, аутофагії та апоптозу в тканині печінки після 6-місячної депорталізації в розробленій стабільній експериментальній моделі допечінкової портальної гіпертензії у самців щурів-підлітків. Часткову перев'язку ворітної вени проводили у 4-тижневих самців щурів. Через 6 місяців зразки тканини печінки щурів з експериментальної групи, групи псевдо-оперованих тварин і тварин групи контролю досліджували методом Вестерн-блоттингу для визначення рівнів білка маркерів, пов'язаних з пошкодженням печінки, спричиненим допечінковою портальною гіпертензією. Часткова оклюзія ворітної вени підвищила регуляцію фактора індукції гіпоксії -1α (у 4,67 рази порівняно з контролем, $p<0,001$) та рівні експресії білка фактора росту судинного ендотелію (у 2,33 рази порівняно з контролем, $p<0,001$), що свідчить про розвиток хронічної гіпоксії. Було виявлено аномально високі рівні ізоформ ангіостатину (у 9,88 разів порівняно з контролем, $p<0,001$), що вказує на дисрегуляцію ангіогенезу. Значна гіперекспресія каспази-3 (23,4-кратне збільшення порівняно з контролем, $p<0,001$) є виконавчою фазою доказів апоптотичної смерті клітин. Значна експресія рівня LC3 вказує на наявність перехресних перешкод між аутофагією та апоптозом, які сприяють фіброзним змінам. Події, спричинені гіпоксією, порушення регуляції ангіогенезу, посилення аутофагії та апоптозу є факторами, що сприяють пошкодженню печінки, спричиненому допечінковою портальною гіпертензією. Краще розуміння тонких молекулярних механізмів цієї патології може прокласти шлях до інноваційних лікувальних підходів.*

Ключові слова: ангіогенез, апоптоз, аутофагія, гіпертензія, гіпоксія, портальна, щури