eISBN: 978-1-68108-397-1
ISBN: 978-1-68108-398-8
eISSN: 2210-268X
ISSN: 2452-3240

Anti-Angiogenesis Drug Discovery and Development

Volume 4

Editors:

Atta-ur-Rahman

M. Iqbal Choudhary

Bentham Books

Anti-Angiogenesis Drug Discovery and Development

(Volume 4)

Edited by

Atta-ur-Rahman, *FRS*

Honorary Life Fellow,
Kings College, University of Cambridge, Cambridge, UK

&

M. Iqbal Choudhary

H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

Anti-Angiogenesis Drug Discovery and Development

Volume # 4

Anti-Angiogenesis Drug Discovery and Development

Editors: Atta-ur-Rahman and M. Iqbal Choudhary

ISSN (Online): 2210-268X

ISSN (Print): 2452-3240

ISBN (Online): 978-1-68108-397-1

ISBN (Print): 978-1-68108-398-8

BENTHAM SCIENCE PUBLISHERS LTD.

End User License Agreement (for non-institutional, personal use)

This is an agreement between you and Bentham Science Publishers Ltd. Please read this License Agreement carefully before using the ebook/echapter/ejournal (**"Work"**). Your use of the Work constitutes your agreement to the terms and conditions set forth in this License Agreement. If you do not agree to these terms and conditions then you should not use the Work.

Bentham Science Publishers agrees to grant you a non-exclusive, non-transferable limited license to use the Work subject to and in accordance with the following terms and conditions. This License Agreement is for non-library, personal use only. For a library / institutional / multi user license in respect of the Work, please contact: permission@benthamscience.net.

Usage Rules:

1. All rights reserved: The Work is the subject of copyright and Bentham Science Publishers either owns the Work (and the copyright in it) or is licensed to distribute the Work. You shall not copy, reproduce, modify, remove, delete, augment, add to, publish, transmit, sell, resell, create derivative works from, or in any way exploit the Work or make the Work available for others to do any of the same, in any form or by any means, in whole or in part, in each case without the prior written permission of Bentham Science Publishers, unless stated otherwise in this License Agreement.
2. You may download a copy of the Work on one occasion to one personal computer (including tablet, laptop, desktop, or other such devices). You may make one back-up copy of the Work to avoid losing it.
3. The unauthorised use or distribution of copyrighted or other proprietary content is illegal and could subject you to liability for substantial money damages. You will be liable for any damage resulting from your misuse of the Work or any violation of this License Agreement, including any infringement by you of copyrights or proprietary rights.

Disclaimer:

Bentham Science Publishers does not guarantee that the information in the Work is error-free, or warrant that it will meet your requirements or that access to the Work will be uninterrupted or error-free. The Work is provided "as is" without warranty of any kind, either express or implied or statutory, including, without limitation, implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the results and performance of the Work is assumed by you. No responsibility is assumed by Bentham Science Publishers, its staff, editors and/or authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products instruction, advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of the U.A.E. as applied in the Emirate of Dubai. Each party agrees that the courts of the Emirate of Dubai shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Ltd.
Executive Suite Y - 2
PO Box 7917, Saif Zone
Sharjah, U.A.E.
Email: subscriptions@benthamscience.net

CONTENTS

PREFACE

Angiogenesis, the process of new blood vessel formation, is both physiological and pathological in nature. A better understanding of the role of angiogenesis in disease process has already helped in the development of several classes of anti-angiogenic agents against various diseases. Inhibition of pathological angiogenesis can help in slowing down the progression of numerous diseases, such as retinopathies, benign and malignant angiogenic tumors, progression of malignant tumors, cardiovascular and CNS disorders. Extensive research in this field is yielding an exponentially growing number of research publications, focusing on various aspects, such as characterization of new pro- and anti-angiogenic factors, their role in various diseases, and identification of natural and synthetic molecules with antiangiogenic properties. This book series entitled, **"Anti-Angiogenesis Drug Discovery and Development"** is an attempt to highlight the major developments in this dynamic interdisciplinary field of research.

Volume 4 of the book series is a compilation of seven scholarly written reviews, focusing on the molecular basis of angiogenesis in various diseases and on the development of anti-angiogenic drugs for therapeutic purposes. Rachel Knott's article is focused on retinal angiogenesis in diabetes, and other macular degeneration conditions, covering molecular initiators of angiogenesis and development of specific pharmacological inhibitors. The review by Ivan Cameron is largely based on his own studies on decline of hypoxia-driven angiogenesis in cancer through short electromagnetic field exposure, in combination with infra-red. Rathinavelu *et al.* have comprehensively reviewed the success and failures, as well as lessons learned in anti-angiogenic drug discovery and development in the last six decades. The chapter by Latrakis focused on various classes of angiogenesis regulators, both positive and negative, and their merits as well as demerits, in his review. Retinal diseases and their treatment through anti-angiogenic/anti-VEGF therapies including clinical outcomes, comprise the theme of the article by Soriano *et al.* The role of angiogenesis in multiple sclerosis (MS) has been a topic of extensive research in recent years. Kulkarni *et al.* have contributed a chapter reviewing the relationship between MS and angiogenesis, inflammation, and identification of certain targets for the development of drugs against MS. The last review in this volume is centered on the role of angiogenesis in portal hypertension (PH), and strategic directions to treat PH and associated complications through the anti-angiogenic agents.

At the end, we would like to express our gratitude to all the contributors of the above cited review articles for their excellent contributions in this promising, and exciting field of biomedical and pharmaceutical research. The efforts of the efficient team of Bentham Science Publishers for the timely production of the 4th volume. We are particularly grateful to Ms. Mariam Mehdi (Assistant Manager Publications), and the excellent management of Mr. Mahmood Alam (Director Publications).

Prof. Dr. Atta-ur-Rahman, *FRS*
Honorary Life Fellow,
Kings College,
University of Cambridge,
Cambridge
UK

&

Prof. Dr. M. Iqbal Choudhary
H.E.J. Research Institute of Chemistry,
International Center for Chemical and Biological Sciences,
University of Karachi,
Pakistan

List of Contributors

Ali Alaseem	Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
Appu Rathinavelu	Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, Nova Southeastern University, Fort Lauderdale, USA
Ankit P. Laddha	Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India
Dmitry Victorovich Garbuzenko	South Ural State Medical University, Chelyabinsk, Russia
Evgeniy Leonidovich Kazachkov	South Ural State Medical University, Chelyabinsk, Russia
Georgios M. Iatrakis	Technological Educational Institute of Athens, Agiou Spyridonos, Athens, Greece
Ivan Cameron	Department of Cell Systems and Anatomy, University of Texas Health Science Center at San Antonio 7703, Floyd Curl Drive San Antonio, USA
Jesica Dimattia	Retina Department, Ophthalmology Service, Hospital Provincial del Centenario, Rosario, Argentina
Khalid Alhazzani	Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
Mohammad Algahtani	Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, Nova Southeastern University, Fort Lauderdale, USA College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
Mitzy E. Torres Soriano	Retina Department, Ophthalmology Service, Hospital Provincial del Centenario, Rosario, Argentina Clínica de Ojos "Dr. Carlos Ferroni", Rosario, Argentina
Maximiliano Gordon	Retina Department, Ophthalmology Service, Hospital Provincial del Centenario, Rosario, Argentina Centro de la Visión Gordon-Manavella, Rosario, Argentina
Manisha J. Oza	Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India SVKM's Dr. Bhanuben Nanavati College of Pharmacy, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India

Mayuresh S. Garud	Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India
Nikolay Olegovich Arefyev	South Ural State Medical University, Chelyabinsk, Russia
Rachel M Knott	School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen, AB10 7GJ, UK
Sivanesan Dhandayuthapani	Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, USA
Sachin V. Suryavanshi	Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India
Sandip T. Auti	Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India
Thiagarajan Venkatesan	Rumbaugh-Goodwin Institute for Cancer Research, Nova Southeastern University, Fort Lauderdale, USA
Yogesh A. Kulkarni	Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (W), Mumbai-400056, India

CHAPTER 7

Angiogenesis and Portal Hypertension: An Update

Dmitry Victorovich Garbuzenko*, **Nikolay Olegovich Arefyev** and **Evgeniy Leonidovich Kazachkov**

South Ural State Medical University, Chelyabinsk, Russia

Abstract: Developing medicines for hemodynamic disorders that are characteristic of cirrhosis of the liver is a relevant problem in modern hepatology. The increase in hepatic vascular resistance to portal blood flow and subsequent hyperdynamic circulation underlie portal hypertension (PH) and promote its progression, despite the formation of portosystemic collaterals. Angiogenesis and vascular bed restructurization play an important role in PH pathogenesis as well. In this regard, strategic directions in the therapy for PH in cirrhosis include selectively decreasing hepatic vascular resistance while preserving or increasing portal blood flow, and correcting hyperdynamic circulation and pathological angiogenesis. The aim of this review is to describe the mechanisms of angiogenesis in PH, methods for studying angiogenesis in experimental research, and the perspectives of antiangiogenic therapy. Although most angiogenesis inhibitors were studied only in animal experiments, this selective therapy for abnormally growing newly formed vessels is pathogenetically reasonable to treat PH and associated complications.

Keywords: Angiogenesis, Antiangiogenic Therapy, Liver Cirrhosis, Portal Hypertension, Pathogenesis, Vascular Remodeling.

INTRODUCTION

Developing medicines to treat hemodynamic disorders that are characteristic of liver cirrhosis and promote portal hypertension (PH) and related complications is a relevant problem in modern hepatology. In accordance with the current clinical recommendations, nonselective β-blockers are the drugs of choice [1, 2]. However, their influence on portal pressure is variable. A number of studies showed that they did not lead to a clinically significant decrease in portal pressure, and the weakening of their therapeutic effect was noted in 50–70% of cases in the long-term period. Also, the question of the appropriateness of using nonselective β-adrenergic blockers in patients with decompensated cirrhosis has not been finally resolved [3].

* **Corresponding author Dmitry Victorovich Garbuzenko:** South Ural State Medical University, Chelyabinsk, Russia; E-mail: garb@inbox.ru

Atta-ur-Rahman & Mohammad Iqbal Choudhary (Eds.)

Ideally, the pharmacotherapy of PH should lessen the severity of morphofunctional disorders in the liver, contributing to the reduction of the vascular resistance to portal blood flow. Also, it should successfully correct a hyperdynamic circulatory state. As a result, the hepatic venous pressure gradient (HVPG), the most accurate equivalent of portal pressure, should be reduced to values less than 12 mmHg or be 20% lower than an original value. In addition, it is necessary to avoid arterial hypotension and at the same time reduce the influx of splanchnic blood into the portal vein, keeping unchanged the portal blood flow, which participates in liver perfusion [4].

Angiogenesis plays an important role in the pathogenesis of many chronic liver diseases, including fibrosis, cirrhosis, and hepatocellular carcinoma [5]. It can also accompany PH, underlying its development and causing related complications. Indeed, the newly formed blood vessels, which bypass sinusoids in response to the gross morphofunctional rearrangement of the liver in cirrhosis, fail to provide oxygen and nutrients to the tissues, which worsens the course of the disease and increases hepatic vascular resistance to portal blood flow [6]. Further progression of PH is a consequence of complex processes including angiogenesis, vascular remodeling, and endothelial dysfunction, which contribute to splanchnic congestion, portosystemic shunt formation, and a hyperdynamic circulatory state [7] (Fig. **1**). From this, it can be inferred that antiangiogenic therapy, which is selectively aimed at suppressing newly formed vessels' formation and growth, is a pathogenetically grounded method of treating PH and associated complications [8].

MORPHOFUNCTIONAL REARRANGEMENT OF THE HEPATIC MICROVASCULAR BED IN CIRRHOSIS-ASSOCIATED PORTAL HYPERTENSION PATHOGENESIS

It is generally accepted that pathologically modified sinusoids are the main sites of resistance to portal blood flow in cirrhosis. Endothelial cells lining sinusoids (SEC) become dysfunctional and among other features acquire a vasoconstrictor phenotype. In this situation, SEC sensitivity to endogenous vasoconstrictors (such as endothelin, norepinephrine, angiotensin II, vasopressin, leukotrienes, thromboxane A2) is elevated. At the same time, SEC produce less nitric oxide (NO), the most studied vasodilator that takes part in the regulation of hepatic vascular tone [9]. This may be due to the lower activity of endothelial nitric oxide synthase (eNOS) caused by increased interaction of eNOS with caveolin-1. Moreover, endotelin-1 stimulates G-protein-coupled receptor kinase-2, which directly interacts with and decreases protein kinase B (Akt) phosphorylation and NO production.

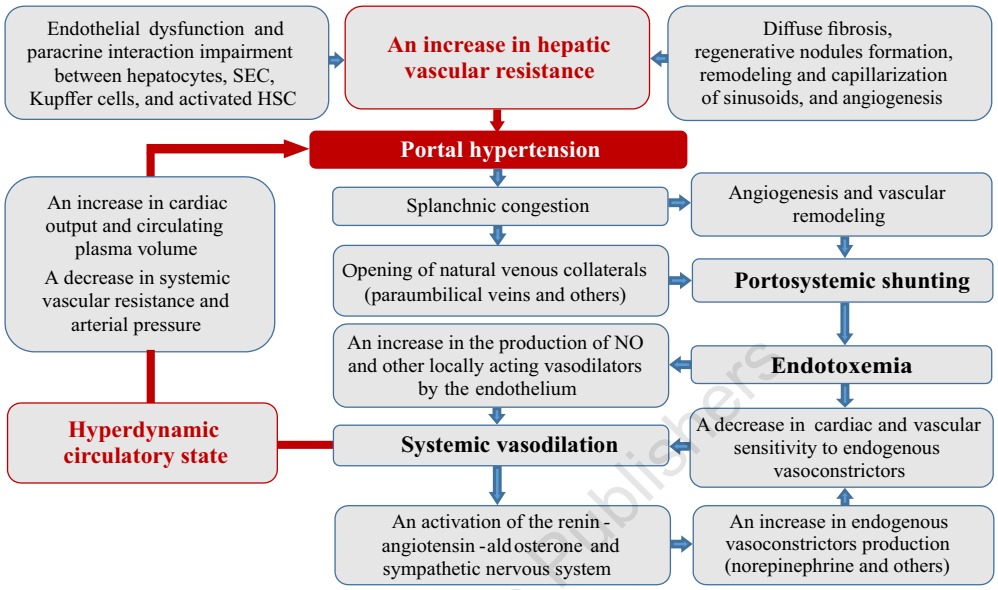


Fig. (1). Potential mechanisms of portal hypertension pathogenesis in cirrhosis. The newly formed blood vessels, which bypass sinusoids in response to the gross morphofunctional rearrangement of the liver in cirrhosis, fail to provide oxygen and nutrients to the tissues. This process is accompanied by endothelial dysfunction and impaired paracrine interaction between hepatocytes, sinusoidal endothelial cells, Kupffer cells, and activated hepatic stellate cells, and leads to an increase in hepatic vascular resistance to portal blood flow. Further progression of portal hypertension is a consequence of complex processes including angiogenesis, vascular remodeling, and endothelial dysfunction, which contribute to splanchnic congestion, systemic vasodilation, and portosystemic shunt formation. The subsequent hyperdynamic circulatory state worsens the course of the disease [7].

Intrahepatic oxidative stress is one of the main factors of sinusoidal endothelial dysfunction in cirrhosis. Oxidative stress is associated with a decrease in NO bioavailability and eNOS expression. For example, cyclooxygenase attenuates Akt-eNOS signalization by activating Rho-kinase and thromboxane A2 that inhibits Akt phosphorylation in endothelial cells. Asymmetric dimethylarginine, an endogenous inhibitor of NOS, causes uncoupling of NOS, which leads to a higher production of reactive nitrogen species such as peroxynitrite. Down-regulated tetrahydrobiopterin expression increases eNOS inability to generate NO but to produce O^2 instead, leading to a further decrease in NO production. Additionally, a probable reason for the insufficient bioavailability of NO may be a reduction of superoxide dismutase («an enzyme that saves NO») and an increase in homocysteine serum level caused by a reduced expression of cystathionine-β - synthase and cystathionine-γ-lyase [10].

Activated hepatic stellate cells (HSC) and its paracrine interaction with SEC

significantly affect the sinusoidal microcirculation in cirrhosis. In pathological conditions, structural and functional damage to HSC includes a loss of retinoids reserve and HSC transformation into myofibroblasts. Activated HSC start functioning as pericytes. This transformation is confirmed by the expression of phenotypic markers of pericytes, such as α-smooth muscle actin (α-SMA), desmin, NG2, glial fibrillary acidic protein, and emergence or increase in the amount of receptors for growth factors, endothelin and cytokines, as well as a number of cell adhesion molecules on HSC surface [11].

HSC, located in the subendothelial Disse spaces between the SEC and hepatocytes, contact with nerve endings by means of the long branching cytoplasmic processes. The nerve endings contain various neurotransmitters such as substance P, vasoactive intestinal peptide, somatostatin, cholecystokinin, neurotensin, NO, calcitonin gene-related peptide, and neuropeptide Y. Some vasoactive substances are capable of regulating HSC tone. Some substances, such as endothelin-1, substance P, angiotensin II, prostaglandin F2, norepinephrine, thromboxane A2, platelet activating factor (PAF), and thrombin trigger HSC contractility. On the contrary, acetylcholine, prostaglandin E2, NO, vasoactive intestinal peptide, carbon monoxide, hydrogen sulfide, and adrenomedullin are known for the ability to cause HSC relaxation [12].

HSC contraction involves myosin II and is mediated by Ca^{2+}-dependent and Ca^{2+}-independent pathways. In a Ca^{2+}-dependent pathway, an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and subsequent formation of the Ca^{2+}/calmodulin complex induce activation of myosin light chain kinase that phosphorylates myosin light chains. In a Ca^{2+}-independent pathway, Rho kinase and protein kinase C inhibit the activity of myosin light chain phosphatase, an enzyme that dephosphorylates phosphorylated myosin light chains and induces relaxation [13].

Endothelins (ET) are potent endogenous vasoconstrictors that may change HSC tone. ET have three kinds of isoforms: ET-1, 2, and 3. The isoforms are synthesized from their precursor "large endothelin" by endothelin-converting enzymes. ET interact with conjugated protein G receptors type A and B, which are well expressed on the HSC. Endothelin-1 is the most studied. The main site of its synthesis in cirrhosis is activated HSC. Stimulation of endothelin A receptors leads to their proliferation [14]. Angiotensin II has a similar effect. In cirrhosis, HSC positively affect angiotensin II synthesis, which is due to the increased expression of angiotensin-converting enzyme [15]. HSC constriction may also be mediated by decreased NO production and/or bioavailability in cirrhotic liver. In contrast, Kupffer cells overproduce carbon monoxide, causing the dilation of sinusoids, and hence decrease hepatic vascular resistance because of paracrine impact on HSC and SEC [16].

Mobility and migration of HSC are necessary to promote enhanced coverage of HSC around sinusoids, which is important for sinusoidal remodeling in liver cirrhosis [17]. Cellular locomotion requires dynamic but regulated actin remodeling to form membrane structures that promote cell extension. These membrane structures include lamellipodia, which are membrane protrusions that form the leading edge toward directed cell migration, and filopodia, which are thin, actin filament-structured spikes emanating from the plasma membrane. Small guanosine triphosphatases from the Rho family, including RhoA (Rho), Rac1 (Rac), and Cdc42, coordinate the formation of actin-based structures. While Rac contributes to filopodia formation and migration of HSC, Rho creates the resistance to the inhibitory action of NO and restores the chemotactic response to platelet-derived growth factor (PDGF) in the absence of a functional Rac [18].

PDGF is a key factor responsible for proliferation, migration, mobility, and recruitment of HSC, which is secreted by endothelial cells. PDGF binds to its receptor (PDGFR-β) on pericytes, which is mediated by an ephrin-B2/EphB4 signaling pathway [19]. Moreover, activated PDGFR-β stimulates Raf-1 kinase, MEK kinase, and extracellular-signal regulated kinase (ERK), leading to HSC proliferation. Phosphatidylinositol 3-kinase (PI 3-K) activation is also required for both PDGF-induced mitogenesis and chemotaxis [20]. In addition, the axonal guidance molecule neuropilin-1 takes part in the chemotactic response to PDGF as well [21].

Activated HSC are a rich source of polypeptides, eicosanoids, and various other molecules with paracrine, juxtacrine, autocrine signalization, or chemoattractant activity, which include:

- polypeptides that increase cell proliferation in an autocrine and paracrine manner: vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), transforming growth factor (TGF)-α, endothelin-1, epidermal growth factor (EGF), insulin-like growth factor (IGF), and acidic fibroblast growth factor (aFGF);
- members of the TGF-β family;
- neurotrophins; and
- hematopoietic growth factors such as erythropoietin [22].

When the liver is damaged, activated HSC proliferate and migrate to areas of inflammation and necrosis of hepatocytes, producing high amounts of extracellular matrix components. TGF-β1, PDGF, connective tissue growth factor (CTGF), and FGF regulate this process [23].

Three general sources of fibrogenic cells in the liver include:

- endogenous (resident) fibroblast or myofibroblast-like cells, mainly represented by HSC, but also by portal fibroblasts, vascular smooth muscle cells, and pericytes;
- the epithelial–mesenchymal transition that may occur in the liver as well as in other organs and lead to transdifferentiation of parenchymal cells; and
- recruitment of fibrocytes from the bone marrow [24].

Intrahepatic Angiogenesis in Cirrhosis

In 1983, Rappaport *et al* [25] were among the first to report the collateral microcirculation in the cirrhotic liver. Nowadays, pathological angiogenesis is well studied in experimental liver fibrosis [26] and in patients with chronic viral and autoimmune liver diseases and nonalcoholic steatohepatitis [27, 28].

Angiogenesis is a complicated physiological process through which new blood vessels form from pre-existing vessels. It includes endothelial cell activation, protease expression, extracellular matrix destruction, proliferation, migration, and the formation of highly permeable primary vascular structures by endothelial cells. These structures become stabilized and "mature" because of pericyte and smooth muscle cell involvement and transform into a three-dimensional vascular network [29].

Molecular Insights into the Angiogenic Process

The primary inducer of angiogenesis in physiological and pathological conditions is hypoxia. Cells respond to hypoxic stress through multiple mechanisms, including the stabilization of hypoxia-inducible factors (HIF), which directly regulate the expression of angiogenic growth factors. The family of HIF includes three α-subunits, which are associated with a common β-subunit (HIF-1β). HIF-1α appears to be ubiquitously expressed, whereas HIF-2α is detected in a more restricted set of cell types, including vascular endothelial cells, hepatocytes, type II pneumocytes, and macrophages. A third mammalian HIF-α subunit, HIF-3α, has also been described, although its role in hypoxic responses is less well understood [30].

NADPH oxidase is an important mediator of angiogenic signaling pathways. It was noted that the increased NADPH oxidase expression increases the reactive oxygen species (ROS) levels because of NADPH oxidase subunit p47phox phosphorylation, contributing to HIF-1α induction, VEGF-receptor (VEGFR) activation, and EGF-receptor transactivation [31].

Recently, an important role of miRNA has been shown in the regulation of cellular response to hypoxia. In particular, Let-7 and miR-103/107 favor the

VEGF induction by targeting argonaute 1 protein [32].

The most studied angiogenic growth factors are VEGF family consisting of five homologs: VEGF-A, -B, -C, -D, and placental growth factor (PlGF). VEGF stimulate both physiological and pathological angiogenesis. All members of this family are connected to different homologous receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt-4), of which only the first and second are responsible for angiogenic signal transmitting. Besides that, the binding of VEGF-A to VEGFR-2 and increasing vascular permeability through NO are the mechanisms that start angiogenesis and vasculogenesis.

PlGF, a homolog of VEGF binding VEGFR-1, enhances angiogenesis only in pathological conditions. PlGF directly and indirectly affects multiple cell types, including endothelial cells. Also, PlGF possibly breaks the binding of VEGF with VEGFR-1, making the binding of VEGF with VEGFR-2 more probable. Mass spectrometry studies showed that PlGF and VEGF each induce the phosphorylation of distinct tyrosine residues in VEGFR-1, further indicating that PlGF and VEGF transmit distinct angiogenic signals through VEGFR-1.

Different mechanisms are the basis of synergism between PlGF and VEGF. By activating VEGFR-1, PlGF induces an intermolecular cross talk between VEGFR-1 and VEGFR-2, which thereby is more responsive to VEGF. PlGF, as a subunit of PlGF/VEGF heterodimer, induces the formation of VEGFR-1/2 heterodimers, which transphosphorylate each other in an intramolecular reaction. By producing PlGF, endothelial cells are able to improve their own responsiveness to VEGF but adjacent stromal or inflammatory cells may also release PlGF.

PlGF directly affects smooth muscle cells and fibroblasts, which express VEGFR-1, but may also indirectly influence its proliferation and migration through cytokine release from activated endothelial cells. In this way, PlGF recruits smooth muscle cells around nascent vessels, thereby stabilizing them into mature, durable, and non-leaky vessels.

PlGF also mobilizes VEGFR-1 positive hematopoietic progenitor cells from the bone marrow and recruits (indirectly, via upregulation of VEGF expression) VEGFR-2-positive endothelial progenitor cells to the ischemic tissue. PlGF is also chemoattractive for monocytes and macrophages, which express VEGFR-1 [33].

FGF family members are also able to induce angiogenesis. Cellular response to FGF occurs through specific binding with FGF-receptors (FGFR), which have internal tyrosine kinase activity. FGFR dimerization is a prerequisite for phosphorylation and activation of signaling molecules with the participation of

heparin-binding proteins. This causes migration, proliferation, cell differentiation, and destruction of extracellular matrix. It should be noted that while VEGF family members are involved mainly in the formation of the capillaries, FGF is primarily involved in arteriogenesis [34].

Although the angiogenic effect of PDGF is not as expressed as that of VEGF, PlGF, and FGF, studies *in vivo* have shown that it may induce the formation of blood vessels and regulate their tone [35].

Tie-2 (Tek), an endothelial-specific receptor tyrosine kinase, and its ligands, the angiopoietins, have been identified as critical mediators of vascular development. Angiopoetin-1 induces migration, inhibits apoptosis, and stimulates the formation of endothelial cells, promoting stabilization of vessels. At the same time, NADPH oxidase is involved in the ang-1-mediated activation of Akt and mitogen-activated protein kinase (p42/p44 MAPK or ERK2 and ERK1) and the subsequent modulation of endothelial cell migration and angiogenesis [36]. In contrast, angiopoietin-2 causes vascular destabilization by shifting endothelial cells from stable to proliferative phenotype. However, it may also stimulate angiogenesis in the presence of VEGF [37].

Integrins αVβ3 and αVβ5 are adhesion receptors stimulating angiogenesis by mediating endothelial cell migration, proliferation, and the formation of new blood vessels [38].

Endothelial-specific adhesion molecule vascular endothelial VE-cadherin contributes to cell-cell junctions during neovascularization and controls the passage of molecules through the endothelial lining [39].

Thrombospondin-1, one of the five known thrombospondins, is an adhesive protein that controls the interaction of cells with each other and with the extracellular matrix. The increased expression of thrombospondin-1 strongly correlates with the severity of fibrosis and angiogenesis during the progression of cirrhosis. However, the precise role of thrombospondin-1 in this process is not determined. It may serve as a promoter or inhibitor of angiogenesis, which may depend on its concentration, the type of domain being activated, and the type of receptors on endothelial cells [40].

Angiostatin, a fragment of plasminogen, and endostatin, a fragment of the C-terminal part of the collagen XVIII α1-chain, inhibit the migration of human endothelial cells stimulated with FGF and VEGF and do not have an impact on intracellular signaling pathways stimulated by FGF and VEGF [41].

Toll-like receptor 4 (TLR4), which recognizes bacterial lipopolysaccharide, is

expressed by SEC involved in fibrosis-associated angiogenesis in cirrhotic liver. It acts through the cytosolic adapter protein MyD88 involved in the production of extracellular protease regulating the invasive ability of SEC [42].

Hepatic apelin system (apelin/APJ-receptor) is a connecting link between chronic inflammation and subsequent fibrogenic and angiogenic processes in cirrhosis. On the one hand, hypoxia and inflammation initiate the expression of APJ. On the other hand, the profibrogenic activation of APJ mediates the induction of profibrogenic genes, HSC proliferation, and secretion of pro-angiogenic factors [43].

Aquaporin-1 is an integral membrane channel protein, overexpressed in cirrhosis, that promotes angiogenesis by enhancing endothelial invasion [44].

It is known that chemokines from CXC family are involved in angiogenesis. ELR-positive chemokines stimulate this process, and ELR-negative suppress it [45].

Neuropilin-1 and neuropilin-2 are transmembrane glycoproteins with large extracellular domains that interact with both class 3 semaphorins, VEGF, and the classical receptors for VEGF, VEGF-R1, and -R2, mediating signal transduction. Neuropilin-1 is mainly expressed by arterial endothelium, whereas neuropilin-2 is only expressed by venous and lymphatic endothelium. Both neuropilins are commonly over-expressed in the regions of physiological and pathological angiogenesis, but the definitive role of neuropilins in angiogenic processes is not fully studied [46].

Mechanisms of Intrahepatic Angiogenesis in Cirrhosis

Hepatic angiogenesis may substantially differ from homologous processes in other organs or tissues on the basis of: (a) the rather unique phenotypic profile and functional role of activated HSC and of other liver myofibroblasts; (b) the presence of two different microvascular structures described (*i.e.*, sinusoids lined by fenestrated endothelium versus large vessels lined by a continuous one); and (c) the existence of ANGPTL3, a liver-specific angiogenic factor.

There are two main ways of angiogenesis in cirrhosis [47] (Fig. **2**).

One of them is associated with the increased expression of pro-angiogenic growth factors, cytokines, and matrix metalloproteinases on the background of chronic inflammation. Proinflammatory mediators produced by Kupffer cells, mast cells, and leukocytes may cause angiogenic response because of the induction and increased transcriptional activity of HIF-1α [48].

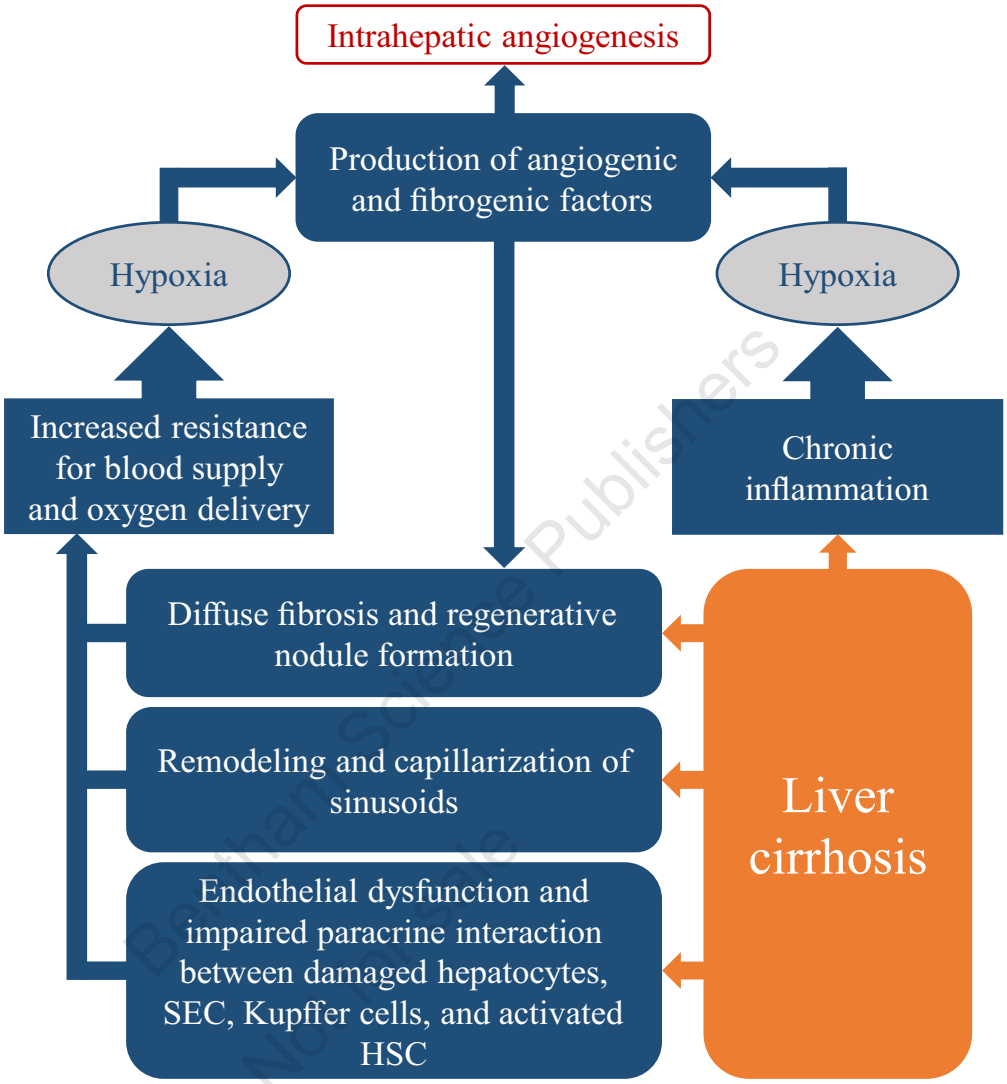


Fig. (2). Two main pathways of intrahepatic angiogenesis in liver cirrhosis [6].

It is believed that macrophages, when in the normal state, are not directly involved in angiogenesis. In contrast, activated Kupffer cells contribute to the formation of new blood vessels through the production of cytokines, ROS, and PAF in cirrhosis [49]. Kupffer cells also release tumor necrosis factor-α (TNF-α), which promotes the migration of cells and coordinates apoptosis and angiogenesis [50]. The increase of ROS in the liver induces angiogenesis via the enhanced

expression of TNF-α, NO, HIF-1, and VEGF [51]. PAF promote the development of VEGF by activating nuclear transcription factor kappa-light-chain-enhancer of activated B cells (NF-κB) [52]. Mast cells take part in angiogenesis by producing heparin, histamine, tryptase, cytokines (TGF-β1, TNF-α, interleukins), and VEGF. They are also able to increase the number of SEC *in vitro* [53]. Soluble mediators, in particular, pro-inflammatory cytokines, growth factors, proteases, and products of oxidative stress regulate the increased expression of chemokines in chronic inflammation of the liver. Due to this process, leukocytes can penetrate into the liver tissue, where they produce angiogenic factors, such as VEGF, PIGF, PDGF, FGF, TGF-β1, EGF, ang-2, and different interleukins [54].

On the one hand, hypoxia, that is caused by HIF-1α stimulation, activates HSC and leads to the production of various angiogenic and fibrogenic factors (PIGF, VEGF, NO, HGF, PDGF) [55], promoting angiogenesis and progression of hepatic fibrosis [56]. On the other hand, diffuse fibrosis, regenerative nodules formation, and sinusoidal capillarization cause an increase in hepatic vascular resistance and impair the oxygen supply to the liver cells [57]. Accumulation of HIF, in particular, HIF-1α, increases the VEGF, angiopoietin-1, and their receptors expression on activated HSC. This leads to the involvement and stimulation of SEC, stabilizing the newly formed vessels and providing them with strength [5]. In turn, SEC generate PDGF and TGF-β, attracting HSC. This process includes ROS-mediated activation of ERK and c-Jun-NH2-terminal kinase (JNK) followed by a delayed- and HIF-1α-dependent up-regulation and release of VEGF [58].

Respectively, there are two different phases of an angiogenic process occurring in cirrhosis. Initially, the formation of blood vessels occurs in developing incomplete septa, in which concomitant expression of VEGF, Flk-1, and Tie-2 is restricted by HSC. In a later phase, angiogenesis occurs in large bridging septa, and the expression of this proangiogenic panel is limited to endothelial cells and aims to stabilize the newly formed blood vessels [59]. Some of them are located mainly around and inside of the fibrous septa and probably needed for compensation of the insufficient intrahepatic blood flow. Others form intrahepatic shunts bypassing sinusoids and draining blood from the portal to the central venules. Although they decompress the portal system, this may lead to liver dysfunction because of declining oxygen delivery and nutrients to the liver tissues and limiting the free exchange between hepatocytes and sinusoids [60].

During last years it has been found that endothelial progenitor cells produced by stem cells of the bone marrow are capable of causing *in situ* neovascularization in both physiological and pathological conditions (postnatal vasculogenesis). In particular, they may stimulate angiogenesis in patients with cirrhosis by activating

SEC through the secretion of paracrine factors, such as PDGF and VEGF [61]. However, their angiogenic ability is significantly reduced in patients of this category, and especially in those with severe hepatic dysfunction. This may be due to chronic inflammation stimulating the release of angiogenic factors by resident HSC and SEC and inhibiting the endothelial progenitor cells mobilization into the bloodstream [62].

Thus, in addition to structural changes associated with diffuse fibrosis and regenerative nodule formation in cirrhosis, there are endothelial dysfunction, impaired paracrine interaction between activated HSC and SEC, and sinusoidal remodeling and capillarization, which play an important role in increasing the hepatic vascular resistance to portal blood flow. The development of intrahepatic angiogenesis may be considered as a compensatory mechanism aimed at portal system decompression. However, the newly formed vessels bypass sinusoids. Therefore, they are unable to provide oxygen and nutrients to the liver tissue, which worsens its morphofunctional state and leads to an increase in portal pressure.

ADAPTATION OF THE VASCULAR BED TO HEMODYNAMIC DISTURBANCES IN PORTAL HYPERTENSION

At the early stages of portal hypertension, a moderate increase in portal pressure leads to blood flow redistribution towards the muscle layer of the small intestine. The appearance of mucosal hypoxia causes a significant increase in NAD(P)H oxidase activity, the main source of reactive oxygen species (ROS) in the mucous membrane, and also leads to the increased production of VEGF and NO by arterioles, contributing to splanchnic vasodilation [63]. In addition, multiple signaling pathways are stimulated, such as mitogen-activated protein kinases, tyrosine kinases, and transcription factors that are involved in VEGF-induced neovascularization [64]. It was shown that overexpression of Kruppel-like factor 2 in duodenal tissue, with the assistance of microRNA, causes hemodynamic stimuli integration and VEGF-driven angiogenesis in patients with cirrhosis [65]. Besides the small intestinal wall [66], the elevated levels of VEGF, VEGFR-2, and CD31 (PECAM-1) are observed in the mesentery [47].

After PH induction, vascular stem/progenitor cells (VSPC) of the mesentery become activated and produce daughter cells (*i.e.*, proliferative progenitors or transit-amplifying cells), which divide and differentiate into endothelial cells or smooth muscle cells lineages and readily incorporate into newly formed mesenteric blood vessels, making a physical and functional contribution to neovascularization *in vivo* in PH. The differentiation potential of VSPC may be regulated by various factors including VEGF, PDGF, and their receptors, which

are increased within the precise tissue microenvironment of neovascularisation sites during PH, creating a suitable setting to promote VSPC differentiation towards either endothelial cells or smooth muscle cells [67].

Mechanism of the Formation of Portal-Systemic Collaterals

These pathophysiological disturbances may be an initial step in the development of portosystemic collateral circulation in portal hypertension [68]. Monocytes adhere to the surface of activated endothelial cells and produce growth factors and proteases, such as urokinase plasminogen activator and MMP, promoting the division and migration of smooth muscle cells. Proinflammatory cytokines (macrophage chemotactic protein-1, granulocyte-macrophage colony-stimulating factor, transforming growth factor β1 (TGF-β1), TNF-alpha) also promote the growth of blood vessels. PlGF stimulates the growth of endothelial and smooth muscle cells. FGF upregulates PDGF and VEGF-receptor expression via Ang-1. At the same time, anti-inflammatory cytokines (*e.g.*, interleukin-10) inhibit this process [33].

It was shown in animal models of prehepatic portal hypertension induced by partial portal vein ligation, that the blockade of VEGFR-2 with anti-VEGFR-2 monoclonal antibodys for 5-7 days and inhibition of VEGF/VEGFR-2 signalization using autophosphorylation inhibitor VEGFR-2 for 5 days after the operation resulted in a 50% reduction of portosystemic collateral vessel formation [69, 70]. Blockade of NAD(P)H also contributed to this owing to the reduced splanchnic expression of VEGF, VEGFR-2, and CD31 [71]. In addition, fourfold sequential intravenous administration of siRNA KDR-lipoplexes reduced the portosystemic collateralization by 73%, violated the angiogenic potential of endothelial cells and reduced pathological neovascularization in the mesenteric vascular bed [72].

It should be noted that the emerging shunts are very dynamic vascular structures because of the expression of various receptor types on the surface of the endothelial lining, for example, α and β-adrenoreceptors and 5-HT2 receptors. Furthermore, vasoactive substances such as NO, ET-1, and prostaglandins may affect vessel tonus [73]. In particular, the excessive shunting of blood through portosystemic collaterals at the time of postprandial splanchnic hyperemia promotes their dilation due to shear stress activating the overproduction of NO by endothelial cells [74].

Although natural portosystemic anastomoses are found in all patients with portal hypertension, they acquire the highest clinical significance in the development of gastroesophageal varices, because their rupture leads to life-threatening bleeding. The determining factor of their formation is the hepatofugal blood flow, and a

gastroesophageal drainage path is the most important in this situation. The left gastric vein plays the main role in this path. It drains blood from both surfaces of the stomach, ascends from right to left along the lesser curvature into the lesser omentum, to the esophageal opening of the diaphragm, where it receives esophageal veins. It then turns backward and passes from left to right behind the omental bursa and drains into the portal vein. Anastomoses between the left and right gastric veins and the left and short gastric veins, respectively indicated by the terms "coronary vein" and "posterior gastric vein", have clinical significance only in portal hypertension, because they are involved in the formation of esophageal and paraesophageal varices [75].

Immunohistochemical studies, which were conducted in patients with portal hypertension, revealed the existence of the pronounced expression of PDGF, basic fibroblast growth factors (FGF-2), EGF, and transforming growth factor-α (TGF-α) in the wall of the coronary vein of the stomach. This fact shows that the increase in pressure in this vein activates smooth muscle cells and induces the release of growth factors that stimulate their proliferation, differentiation, and migration, as well as contribute to the disruption of the metabolism of collagen and elastin fibers. Phenotypic changes of smooth muscle cells are a response to chronic mechanical stimuli. They lead to venous wall thickening and elasticity reduction [76].

Vascular Structure of the Lower Esophagus in Clinical Portal Hypertension

The venous system of the distal portion of the esophagus includes intraepithelial, subepithelial superficial, deep submucosal, and adventitial veins. The largest varices are generally localized 2-3 cm above and 2 cm below the cardia, mainly in the lamina propria of the mucous membrane. They have two types of vascular structure: palisading type and bar type. The palisading type has dilated intraepithelial channels and numerous small superficial collateral veins. The bar type has triply dilated subepithelial superficial veins and deep submucosal veins which erode the epithelium [77].

Structural changes in the veins of the distal portion of the esophagus in portal hypertension are characterized by thickening of the medial layer because of hyperplasia of elastic and collagen fibers. Elastic fibers become fragmented and sharply tortuous directly in the varicose veins of the esophagus in the background of increasing sclerosis of the vascular wall [78].

Four distinct intramural vascular zones of the gastroesophageal junction were defined as follows: gastric zone, palisade zone, perforating zone, and truncal zone. Portacaval shunts in this area are formed because of increased pressure in the portal venous system [79].

Gastric Zone

The longitudinal veins of the gastric area are located in the submucosa and the lamina propria of the proximal portion of the stomach. They are more abundant near the esophagus, have a small diameter, and form a group of several longitudinal vessels. The veins merge in the submucosa of the distal part of the gastric zone and form large tortuous trunks draining blood into the portal vein system.

Palisade Zone

The palisade zone is an extension of the gastric zone. It begins in the projection of the gastroesophageal junction and ends 2-3 cm above it. Veins in that zone are located randomly, close to each other, and are arranged longitudinally and in parallel as a palisade.

Numerous anastomoses are identified between vessels of both gastric and palisade zones. They are localized in the submucosa of the gastroesophageal junction, penetrate the muscularis mucosa, and pass into the lamina propria mainly in a longitudinal direction.

The veins of a proximal portion of the palisade zone simultaneously converge at one point and, perforating the muscularis mucosa, pass into the submucosa again as four or five big trunks. There are arched transverse anastomoses between them. Veins perforating the muscular layer of the esophagus were not detected in this zone.

Perforating Zone

Veins of the perforating zone, which is located 3-5 cm above the gastroesophageal junction, are not so homogeneous and constant. Vessels form five polygonal networks in the lamina propria of the esophageal mucosa (as a continuation of the veins of the palisade zone) and perforate the muscular layer, communicating with adventitial veins located on the outer esophageal surface. They were referred to as «treble clef» veins because of their similarity with music symbols.

The perforating zone is the “critical area” for variceal rupture in portal hypertension. This is due to increased resistance to blood flow in this anatomical area, as well as increased fragility and superficial location of perforating veins [80].

Truncal Zone

The truncal zone is a region from 8 to 10 cm in length with the bottom edge 5 cm

above the gastroesophageal junction. Large longitudinal venous trunks, discovered here in the lamina propria, constitute a continuation of the polygon vascular networks of the perforating zone. They have a small diameter in the proximal portion. Between them, there are several transversely oriented anastomoses. Perforating veins, locating randomly along the zone, pass from the submucosa of the esophagus to its outer surface and communicate with adventitial veins.

In physiological terms, palisade zone is the most important part of the vascular structure of the gastroesophageal junction. Veins are located there mainly in the lamina propria. Their superficial location decreases venous blood flow resistance to a minimum, which would otherwise arise in the high-pressure zone in the area of the lower esophageal sphincter.

Small longitudinal vessels in the palisade zone are perfectly adapted to the physiological pressure variations that lead to a bi-directional flow during breathing. When the venous outflow is caudal, the gastric zone collects and drains the blood into the portal vein system.

Deep submucosal veins are enlarged because of the blood outflow in the cranial direction in portal hypertension. They drain the blood into the enlarged adventitial veins (periesophageal collateral veins) through the numerous veins perforating the esophageal smooth muscle layer in the perforating zone. Adventitial veins, in turn, communicate with paraesophageal collateral veins, which are located in the posterior mediastinum. The blood flows from them usually into the azygos vein [81], which structural changes in response to increased blood flow are characterized by focal destruction, hyperplasia, and chaotic arrangement of elastic fibers [78].

The Systemic and Splanchnic Adaptive Response of Vascular Bed to Hemodynamic Disturbances in Portal Hypertension

The development of portosystemic collateral circulation is a compensatory mechanism aimed at decreasing portal pressure. However, this does not happen. Conversely, there is a hyperdynamic circulatory state accompanied by increased cardiac output, decreased peripheral vascular resistance, and the opening of arteriovenous communications, which exacerbate portal hypertension. The cause of these disorders may be the flow of vasodilator substances (*e.g.*, glucagon, endocannabinoid, atrial natriuretic peptide, bacterial endotoxin) through the network of portosystemic shunts, as well as the increased production of topical vasodilators by endothelium, such as NO, carbon monoxide, PGI2, endothelium-derived hyperpolarizing factor, adrenomedullin, and hydrogen sulfide. Furthermore, in spite of increased circulating levels of endogenous

vasoconstrictors (noradrenaline, ET-1, angiotensin II), vascular sensitivity to them is significantly reduced [82].

Abdominal Aorta

In the conditions of the hyperdynamic circulation, adaptive response of the abdominal aorta to shear stress induced by the blood flow may be associated with oxidative stress. The production of ROS, such as superoxide and hydrogen peroxide, which are cell toxic metabolic products, leads to non-specific damage of nucleic acids, proteins, lipids, and other cellular components. ROS regulate vascular tone, endothelial cells sensitivity to oxygen, their growth, proliferation, and apoptosis. Furthermore, they promote the expression of inducible genes *via* transcription factors, such as NF-kB. These genes contribute to the synthesis of proinflammatory cytokines, chemokines, chemokine receptors, and adhesion molecules, inducing an inflammatory response. Potential sources of ROS are various enzyme systems: NAD(P)H oxidase, xanthine oxidase, enzymes of arachidonic acid metabolism (cyclooxygenase and lipoxygenase), and the mitochondrial respiratory chain [83].

Increased levels of TNF-α, IL-1β, and IL-6 in the aorta, as a result of oxidative stress, play an important role in the induction of immune-mediated systemic vascular process in portal hypertension. The subsequent increase in expression of connective tissue growth factor (CTGF) may enhance the synthesis of extracellular matrix proteins, particularly, collagen I type, whereas the decrease of the level of MMP-2 / TIMP-2 complex (tissue inhibitor of metalloproteinase-2) contributes to reducing the degradation of extracellular matrix proteins. These processes lead to significant histological changes in the aorta. Its wall thickness decreases, as well as the ratio of medial layer thickness to lumen diameter. Elastic fibers lose their ordered arrangement, and well-marked collagen fibers become more narrow and separated because of the increase in the extracellular matrix in the media interstitium with a significant decrease in the number of smooth muscle cells [84, 85].

The left gastric artery is the first branch of the celiac artery. It is assumed that the hemodynamics in the left gastric artery in portal hypertension may act as the initiator of variceal formation, showing close linkage with variceal recurrence [86].

Mesenteric Resistance Arteries

Similar infringements also occur in mesenteric resistance arteries. The mechanical stimuli, generated by shear stress, activate endothelial cells and induce

hyperproduction of NO and prostaglandins, causing vasodilation [87]. The significantly reduced isometric stiffness of blood vessels and their increased elongation may cause structural changes in the internal elastic membrane and increase fenestrations in it [88]. This contributes to excessive NO-mediated vascular permeability and angiogenic processes in the mesentery of the small intestine because of the high VEGF and eNOS expression in microvessels located there [89].

Portal Vein and Hepatic Artery

Splanchnic congestion leads to increased portal inflow. At the same time, portal blood flow decreases as a result of the development of collateral circulation [90]. The portal vein becomes dilated, and shear stress simultaneously decreases [91]. Intima and media of the portal vein are thickened due to the high amount of collagen fibers, hypertrophy, and hyperplasia of smooth muscle cells, which significantly reduce the vascular wall elasticity [92]. *In vitro* studies have revealed that transmembrane protein 16A can be associated with the proliferation of portal vein smooth muscle cells and portal vein remodeling in PH. Upregulation of transmembrane protein 16A promotes the proliferation of portal vein smooth muscle cells, whereas inhibition reduces it [93].

In this situation, so-called hepatic arterial buffer response maintains hepatic perfusion constancy. This phenomenon, first described by Lautt in 1981 [94], was identified in physiological and in various pathological conditions, including cirrhosis. It maintains oxygen delivery to the liver, protecting its structure and function [95]. However, increased blood flow through the hepatic artery causes its remodeling and decreases its elasticity with time [96].

Splenic Artery and Vein

Significant histopathological changes also occur in the blood vessels of the spleen. Damaged splenic artery intima becomes thicker, and smooth muscle cells grow into it. The internal elastic lamina is stratified, that is accompanied by the destruction of both included in its structure and localized in media elastic fibers. Smooth muscle cells, randomly located in the media, have a different size and morphology, and the content of separating them collagen fibers, as well as the extracellular matrix, increases significantly, causing the “collagenization” of the vascular wall, thickening, and rigidity [97]. The splenic vein expanding and its intima and media thickening is due to high content of collagen fibers, hypertrophy, and hyperplasia of smooth muscle cells [98]. These pathologic changes in the blood vessels of the spleen lead to a significant reduction of their elasticity.

Thus, in addition to pathophysiological disorders related to endothelial dysfunction in cirrhosis, there is the restructuring of the splanchnic and systemic vasculature, which includes vascular remodeling and angiogenesis. In spite of the fact that these changes are the compensatory-adaptive response to the deteriorating conditions of blood circulation, taken together, they contribute to the development and progression of portal hypertension and cause severe complications, one of which is bleeding from esophageal varices.

MODERN METHODS FOR STUDYING PORTAL HYPERTENSION-ASSOCIATED ANGIOGENESIS IN EXPERIMENTAL RESEARCH

Various experimental models and methods, both *in vitro* and *in vivo*, of qualitative and quantitative evaluation are used to study angiogenesis [99].

Intrahepatic Angiogenesis Assays

Appearing simultaneously with fibrosis, intrahepatic angiogenesis causes abnormal angioarchitecture formation characteristic of cirrhosis, which is the endpoint of the disease. Therefore, evaluation of intrahepatic angiogenesis is necessary to assess disease progression and search for therapeutic targets (Table **1**).

Scanning Electron Microscopy

Scanning electron microscopy is the traditional method for studying the three-dimensional (3D) structure of microcirculation. It is visualized by an electron beam after intravascular injection of colored gelatin, latex, or plastic casting material followed by tissue clearing or corrosion. Unlike tissue sections, this approach makes it possible to not only quantify vessel dimensions, intervascular distances, branching order, and luminal surface features, but also to mathematically calculate the wall shear stress [100].

Table 1. Intrahepatic angiogenesis assays.

Assay	Advantages	Disadvantages
Scanning electron microscopy	Quantification of vessel dimensions, intervascular distances, branching order and luminal surface features, as well as the wall shear stress.	Perfusion difficulties of casting materials, especially for microvascular perfusion. Biloma formation after bile duct ligation causes holes in the cast.

(Table 1) cont.....

Assay	Advantages	Disadvantages
Intravital fluorescence microscopy	Evaluation of microcirculatory structural changes *in vivo*. HSC visualization. Possibility to estimate the number of rolling and adherent leukocytes, relative vascular density, the diameter and perfusion of sinusoids, and flow and volume velocity of blood.	Visualization of only superficial structures; not capable of estimating the diameter of portal venules. Hard to perform if intraperitoneal injections of CCl_4 or bile duct ligation are used.
Three-dimensional microcomputed tomography *in vivo*	May be re repeatedly conducted, thereby allowing gradual monitoring. 3D images of vasculature.	Resolution is relatively low, therefore it is not possible to evaluate microcirculation. Perfusion difficulties of casting materials.
Three-dimensional microcomputed tomography *ex vivo*	Higher resolution, if compared to *in vivo* microCT. Analysis of the microcirculation, as well as large vessels. 3D images of vasculature.	Perfusion difficulties of casting materials.
Immunohistochemical methods	Evaluation of newly formed vessels without a lumen. Evaluation of proangiogenic factors expression.	Analysis of microcirculation only.
Confocal laser scanning microscopy after immunohistochemical staining	3D images of vasculature. High resolution. Provides volumetric data on microcirculation.	Tissue shrinkage and deformation hampers confocal microscopy. Limited diffusion of (primary) antibody penetration.

Using scanning electron microscopy in rats with cirrhosis caused by subcutaneous injections of carbon tetrachloride (CCl_4) (0.3 mL 50% CCl_4 diluted with oil per 100 g of body weight twice a week for 3 months), it was determined that the number of sinusoidal endothelial fenestrae decreased and the sinusoids within the regenerative nodules surrounded by fibrous septa were narrow [14]. In mice with biliary cirrhosis, this technique allowed for the identification of numerous blindly terminating and chaotically located sinusoids, as well as large portosystemic collaterals bypassing them and shunting blood towards the hepatic veins. The disadvantage of bile duct ligation is biloma formation, causing irregular saccular deformation of sinusoids, which presents as holes in the cast. Moreover, limitations of the casting technique include perfusion difficulties, especially for microvascular perfusion, and bloating of the sinusoids due to the injection pressure. This makes it difficult to perform a more thorough morphometric analysis of the hepatic microvasculature [101].

Intravital Fluorescence Microscopy

Intravital fluorescence microscopy is necessary for the intravital evaluation of structural changes at the microcirculatory level in experimental cirrhosis. For this purpose, different models of cirrhosis may be used, such as bile duct ligation and subcutaneous or intraperitoneal administration of CCl_4. Although subcutaneous CCl_4 administration can induce necrosis at the site of injection and should be carried out for 16 weeks, this route of administration is preferable for intravital fluorescence microscopy. In contrast to intraperitoneal injections or bile duct ligation, subcutaneous CCl_4 administration does not cause adhesions between the liver and the neighboring organs that can later limit the possibility of carrying out *in vivo* studies in the abdominal cavity [102].

After performing midline and subcostal incisions, the hepatic ligaments are dissected, and the left liver lobe is placed on a fixed plate to minimize respiratory movements. A fluorescent dye was injected into the jugular or tail vein, and, while tissue contrast is increased, liver microcirculation was analyzed with a fluorescent microscope.

Intravital fluorescence microscopy makes it possible to visualize HSC due to autofluorescence of vitamin A; estimate the number of rolling and adherent leukocytes; and measure relative vascular density, the diameter and perfusion of sinusoids, and flow and volume velocity of blood [103].

The maximal number and diameters of portosystemic shunts were observed 3 weeks after bile duct ligation and 12 weeks after the first administration of CCl_4 (0.1 mL 50% oil solution per 100 g of body weight subcutaneously twice a week for 4 months; 5% alcohol was added to drinking water). Fibrosis and the activated HSC were located in periportal areas in biliary cirrhosis and in pericentral areas in CCl_4-induced cirrhosis. The sinusoids in these regions became narrow, whereas the distance between them increased.

The major drawback of intravital fluorescence microscopy is that it can only visualize superficial structures; therefore, it does not give an opportunity to estimate the diameter of portal venules. This may be due to the displacement of portal tracts deeper into the tissue as a result of pericentral fibrosis.

Three-Dimensional Microcomputed Tomography

Microcomputed tomography (microCT) provides high-resolution 3D images that are composed of two-dimensional (2D) trans-axial projections, or 'slices', of a target object. For live animal imaging, the slices are obtained by rotating the emitter and detector. To evaluate microcirculation, *ex vivo* microCT requires

vascular casting with contrast agents, such as microfil MV-122 and BaSO4/gelatin, whereas *in vivo* microCT can be performed with iodinated monomer-based bolus or lipid emulsion-based blood-pool contrast agents [104].

Intravital microCT is performed with a dual-energy flat-panel microCT scanner before and immediately after intravenous injection of 100 μl of specially optimised iodine-based contrast agent eXIA™160XL (Binitio Biomedical Inc., Ottawa, Canada). A Feldkamp-type algorithm is used to reconstruct 2D images into 3D with a voxel size of 35x35x35 μm. The relative blood volume value determination is based on the mean brightness of the liver tissue after a contrast agent injection. The value correlates with the number of angiogenic vessels. In this way, a statistically significant increase in the relative blood volume was observed in the murine cirrhotic liver 6 weeks after the first intraperitoneal administration of CCl_4 (0.06 mL 50% oil solution per 100 g of body weight twice a week for 6 weeks) and 2 weeks after bile duct ligation. The advantage of the technique is that it can be conducted repeatedly, thereby allowing gradual monitoring of the process.

The merit of *ex vivo* microCT is the higher resolution image obtained [105, 106]. It was used in different models of cirrhosis in mice and rats. In its classical version, the inferior vena cava is crossed above the diaphragm, and the radiopaque lead oxide diluted in a liquid silicone polymer (microfil) is injected into the portal vein at the rate of 8–10 mL/min and at a pressure of 10–12 mmHg; or alternatively, injection into the heart at a pressure not higher than that of the artery, this is carried out using an automatic pump. The specimens are kept at 4°C for 12 hours. Subsequently, the liver is taken, cut into lobes, fixed in formalin, and then dehydrated in increasing concentrations of a glycerol aqueous solution at 24-hour intervals. Using a microCT scanner and the special computer processing algorithm, 3D images of the intrahepatic microvasculature are obtained and then analyzed using software [107].

MicroCT *ex vivo* makes it possible to estimate the ratio of vascular volume to total liver volume and enables precise analysis of the branching of medium and large hepatic vessels. They are detected in the liver, particularly at the periphery, 6 weeks after the first intraperitoneal injection of CCl_4 (0.06 mL 50% oil solution per 100 g of body weight twice a week for 6 weeks) and 21 days after bile duct ligation. By Week 4 of biliary cirrhosis development, the vascular volume increased one and a half times and the number of branches doubled [108], which reflected the severity of angiogenesis [109].

The limitations of the technique include perfusion difficulties, especially for microcirculatory perfusion, and the reactivity of casting resins with other

chemical compounds and surrounding tissue. Moreover, dual casting, which is necessary for contrast-based differentiation between venous and arterial systems, is not possible. This is due to the presence of shunts between the hepatic arterioles and portal venules, functioning as a one-way valve that allows blood to flow only from the arterial to venous system [110].

To overcome this shortcoming, Peeters *et al* [111] sequentially injected yellow or blue contrast agents, PU4ii, into the abdominal aorta or portal vein, respectively, after clamping the thoracic aorta and renal arteries. In order to prevent damage to the microvessels by the pumped substance, a polyethylene drainage tube was installed into the inferior vena cava through the right atrium. The thoracic section of the inferior vena cava, the abdominal aorta, and the portal vein were clamped to eliminate leakage of the substance during polymerization. After 72 hours, the casted liver was macerated using a 25% potassium hydroxide bath for 5 days. The vascular replica was then flushed with distilled water and laid to dry under a vented hood for a further 5 days. MicroCT with 3D reconstruction was performed with a resolution of 1.89 μm for microcirculation and 40 μm for larger vessels. To morphologically analyze microcirculation, a sample with the dimensions 350x350x200 μm was virtually dissected in between portal triads. The average radius of the sinusoidal vessels, branch length, tortuosity, and porosity (the total sinusoidal volume divided by the volume of its envelope) of the vascular network was assessed with the in-house developed software. Using this technique in animals with macronodular cirrhosis induced by thioacetamide (first, 0.03% thioacetamide was added to drinking water, and then the concentration was adapted every week for 18 weeks to keep body weight within a 250–300 g range), these authors have identified the compression of the hepatic venules and an increase in the diameter of the hepatic artery [112].

Immunohistochemical Methods

Intravascular injection of contrast agents only revealed the structure of functioning vessels. Immunohistochemical staining of tissue sections is used for a more accurate evaluation of the newly formed vessels, including the nascent capillaries without a lumen. The most common specific markers are VEGF and membrane proteins CD31 and CD34 for endothelial cell detection. In particular, VEGF and angiopoietin-1 expression shows an increase in intrahepatic vascular density in rats with biliary cirrhosis [109]. Immunofluorescence is a variant of immunohistochemical staining. It requires the use of secondary antibodies such as streptavidin conjugated with carbocyanine CY2 [113].

Confocal laser scanning microscopy after immunohistochemical staining makes it possible to study 3D structures with a resolution of up to 0.2 μm. This technique

is based on improved protocols for the chemical purification of samples allowing a dye and photons to penetrate deeper into tissue before and after immunohistochemistry [114, 115]. Subsequent confocal laser scanning provides detailed volumetric data on microcirculation at a voxel size of 0.63x0.63x1.40 μm. The data are processed by a specially developed software (DeLiver). In a study involving rats with thioacetamide-induced cirrhosis, a decrease in mean radius and porosity as well as an increase in tortuosity and length of sinusoids were determined using this technique [112].

Extrahepatic Angiogenesis Assays

The advantages and disadvantages of different extrahepatic assays are listed below (Table **2**).

Intravital Microscopy of the Small Bowel Mesentery

Intravital microscopy enables imaging of structural changes in microvasculature, vascular permeability, and mesenteric vascular density, that characterizes splanchnic angiogenesis. After performing a midline laparotomy, a small intestinal

Table 2. Extrahepatic angiogenesis and portosystemic shunting assays.

Assay	Advantages	Disadvantages
Intravital microscopy of the small bowel mesentery	Enables imaging of structural changes of microvasculature, vascular permeability, adhesion and rolling of leukocytes, and mesenteric vascular density.	The need for surgical intervention, which limits repeated procedures because of adhesions formation. Rapid increase in the number of leukocyte rolling.
Teflon rings implantation	Corresponds to an *in vivo* situation with an intact circulation. Evaluation of newly formed vessels without a lumen. Evaluation of proangiogenic factors expression.	The need for surgical intervention.
Immunohistochemical methods	Evaluation of newly formed vessels without a lumen. Evaluation of proangiogenic factors expression.	Analysis of microcirculation only.
Scanning electron microscopy	Quantification of vessel dimensions, intervascular distances, branching order and luminal surface features, as well as the wall shear stress.	Perfusion difficulties of casting materials, especially for microvascular perfusion.

(Table 2) cont.....

Assay	Advantages	Disadvantages
Microsphere technique	Evaluation of total shunting degree, as well as spleno-renal shunting.	The need to sacrifice laboratory animals.
3D micro-single-photon emission computed tomography	Serial measurements of portosystemic shunting. 3D imaging *in vivo*. Pre-interventional measurement of portosystemic shunting that is important for selection of animals with similar baseline characteristics in studies evaluating anti-angiogenic therapy.	Requires the use of radioactive material.

loop should be exteriorized, placed on a heated Plexiglas plate, and continuously superfused with an Earle's balanced salt solution to prevent dehydration. Observations are carried out with the Axiotech Vario 100HD microscope (Carl Zeiss AG, Oberkochen, Germany) equipped with water immersion objectives (x10 and x40). The obtained image is recorded for the subsequent computer analysis. This enables study of all the types of mesenteric microvessels and calculation of their density, which is defined as the ratio of a vessel's length to the area it occupies [116].

Epifluorescence microscopy is used to measure vascular permeability. After the selection of a venular segment with a diameter of 20–40 mm and an unbranched length of about 150 mm, fluorescent isothiocyanate-bovine serum albumin was injected intravenously. As the intraluminal grey scale value fell, the perivascular grey scale value rose when the fluorescent isothiocyanate-bovine serum albumin molecule leaked through the vascular wall, quantified using black and white image and taking black for 0 and white for 255. Intravital microscopy revealed an increased vascular density and gross disturbance of the mesenteric microcirculation in rats with PH induced by partial portal vein ligation (PPVL) and in rats with biliary cirrhosis. Moreover, the changes in the latter were more significant, which may be due to the time required for cirrhosis development. In addition, vascular permeability was significantly increased in these animals, in contrast to permeability in animals with extrahepatic PH. This is explained by the higher levels of eNOS and VEGF [102].

In addition to the aforementioned uses, intravital microscopy is used to quantify adhesion and rolling of leukocytes, a well-known hallmark of inflammation, which in turn leads to angiogenesis. In particular, in rats with cirrhosis induced by CCl_4 (0.04 mL administered intragastrically and increased weekly in increments of 0.04 mL to a maximum dose of 0.4 mL; 35 mg/100 mL phenobarbital was added to drinking water once 2 weeks before the first administration of CCl_4), the index of leukocyte-endothelial interaction was increased in the microcirculation of

the liver and small bowel mesentery [117]. The disadvantage of this method is the need for surgical intervention and extraction of the mesentery from the abdominal cavity. This causes a rapid and pronounced increase in the number of leukocyte rolling in response to partial degranulation of perivascular mast cells and endothelium expression of P-selectin in a matter of minutes [118].

The Requirements for the Analysis of Microcirculation Images Obtained with Intravital Microscopy

Intravital microscopy requires video recording for the analysis of microvasculature. It is recommended that the following requirements are met [119]:

- At least three, or preferably five, arbitrary regions of microcirculation should be included in the analysis.
- Optical magnification should be x10 for microcirculation imaging in small laboratory animals.
- It is necessary to avoid pressure artifacts occurring when a microscope objective contacts the region of interest; excess pressure applied to the area may collapse the microcirculation and stop venous blood flow.
- Video images should be stored in full size without compression to the form of DV-AVI files in order to provide the possibility of computer frame-by-frame analysis. The optimal video recording time is 20 seconds.

A report on the analysis of images obtained with intravital microscopy must include the following parameters calculated for all vessels and capillaries separately:

- Total vascular density and perfused vessel density, which are calculated as the ratio of total vessel length to image area.
- Proportion of perfused vessels expressed as a percentage.
- Microvascular flow index.
- Heterogeneity index, which is calculated as the ratio of the difference between the maximum and minimum blood flow velocity to its average velocity in the five selected areas of an image.

Calculations can be made directly by the researcher using semiquantitative scales for visual evaluation [120, 121]. For a more accurate analysis of the required parameters, the CapImage software is used. It was specially developed for intravital microscopy [122].

In Vivo Evaluation of Angiogenesis in the Small Bowel Mesentery by Implantation of Teflon Rings

Implantation of Teflon rings is another technique for intravital evaluation of angiogenesis of the small bowel mesentery in PH. The rings have a diameter of 7 mm, a height of 3 mm, and an internal diameter of 5 mm. The rings are placed into polyester mesh bags and filled with a mixture of bovine Type I collagen and bovine serum albumin. After performing a midline incision and PPVL, the rings are implanted between the two mesenteric membranes and fixed with single sutures in rats. After 16 days, the rats are euthanized, the implant is removed, fixed in a 4% formalin, and paraffinized. Then, 3-μm-thick tissue sections are prepared. The tissue is stained for further video morphometry and vascular density calculation. This technique makes it possible to determine the number of vessels and the mechanisms of their formation [123].

Immunofluorescence Assay

Vascular network imaging can be carried out by immunological reaction of fluorescent antibodies with membrane proteins of endothelial cells. Anti-CD31 and anti-VEGF antibodies are most often used for these purposes [124]. The small intestinal wall or its mesentery is washed in sodium phosphate buffer, dried on gelatin-coated slides, and fixed in 100% methanol at -20°C for 30 minutes. The sections are then incubated with the corresponding primary murine anti-rat antibodies at 4°C for 12 hours. Streptavidin conjugated with carbocyanine CY2 is used as a secondary antibody. It is applied at room temperature and held for 1 hour. The image obtained after fluorescence microscopy may be analyzed using ImageJ software [125].

An alternative technique consists of the fixation of frozen sections in acetone at -20°C for 10 minutes. After which, the tissue is blocked with 5% bovine serum albumin solution for 45 minutes [38]. Besides CD31, endothelial cell identification is possible with the use of BSI-lectin. Perivascular cell markers include Neural/glial antigen 2, desmin, α-SMA, PDGFR-β, and class III β-tubulin [126].

Immunohistochemical Staining

Immunohistochemical staining is performed to study not only intrahepatic but also extrahepatic angiogenesis, including angiogenesis in the small bowel mesentery and the gastric wall. The presence of angiogenesis in the small bowel mesentery was confirmed by numerous experimental studies in laboratory animals with different PH models [127].

Tissue oxidative stress, which occurs in PH, aggravates the pathophysiological changes that occur in the gastric wall. The oxidative stress is detected by the reaction of antibodies with metabolites that arise during free radical oxidation, such as nitrotirosine. In particular, an increased expression of eNOS, VEGF, and nitrotyrosine was found in the gastric wall of rats with prehepatic PH induced by PPVL, indicating the presence of stimuli for further development of collateral circulation [128].

Aperio [129] or the CAIMAN algorithm [130] may be used to calculate the number of vessels on images of immunohistochemically stained samples. The program called AngioPath can quantify microvessels and determine the size and shape of all vessels as well as each vessel individually. As such, it is an important tool for characterizing angiogenesis [131].

Scanning Electron Microscopy

Scanning electron microscopy of vascular casts is helpful for imaging of the splanchnic vascular network's 3D structure with its subsequent quantitative analysis. The technique was used in animals with PPVL and biliary cirrhosis and revealed the presence of newly formed tortuous vessels serving as shunts between the branches of the inferior vena cava and the portal vein. In addition, holes were found in the walls of some capillaries, serving as a sign of intussusceptive angiogenesis, which is one of the two known types of microvessel growth [101].

Assessment of Portosystemic Shunting

Portosystemic Shunting Assay Using Microspheres

In 1981, Chojkier and Groszmann [132] proposed to use ^{51}Cr-labelled microspheres to assess the degree of portosystemic shunting. The modification of Chojkier and Groszmann's technique through the use of color polystyrene fluorescent microspheres has become widespread at the present time, because this technique excludes any contact with the dangerous radioactive material and maintains accuracy [133].

Approximately 30,000 yellow microspheres (15 μm in diameter) are slowly injected into the spleen. An injection of microspheres of a different colour into the ileocolic vein should be completed for a more detailed haemodynamic assessment of total shunting from the splanchnic area. The liver and lungs of the animal models are removed and placed in centrifuge tubes. Approximately 3,000 blue microspheres are added as an internal control. The tissue is digested in unilocular potassium hydroxide at 60°C for 12 hours and then sonicated. After centrifugation, the supernatant is removed and the pellets are washed once in 10%

Triton X-100 solution and twice in acidified ethanol.

The precipitate containing microspheres was dried for 12 hours, diluted in acidified Cellosolve™ acetate (The Dow Chemical Company, Midland, Michigan, USA), and the number of microspheres was counted using a spectrophotometer. A hemocytometer and an epifluorescent microscope are also adequate for this procedure [134]. The degree of portosystemic shunting is calculated as the ratio of the number of pulmonary microspheres to their sum in the lungs and liver [135].

The microsphere technique confirmed that portosystemic collaterals had started forming in rats with PH 2 days after PPVL and became fully developed on Day 7 post PPVL [69]. At the same time, portosystemic collaterals developed later in rats with biliary cirrhosis. Therefore, it is expedient to evaluate them 1 month after bile duct ligation [136].

Three-Dimensional Micro-Single-Photon Emission Computed Tomography

Since a significant disadvantage of the microsphere method is the need to use laboratory animals, micro-single-photon emission computed tomography (3D micro-SPECT) with technetium (^{99m}Tc) macro aggregated albumin was developed as an alternative. It provides a possibility of conducting serial measurements of portosystemic shunting at different time points after the creation of a model. ^{99m}Tc macro aggregated albumin particles are injected into the splenic pulp; accumulation of the particles in the liver and lungs is determined by using colour scales and computer processing.

3D micro-SPECT was used in mice with PPVL and biliary cirrhosis. The results correlated with the results obtained by using ^{51}Cr-labelled microspheres, and there were no lethality or changes in animals' behaviour after its reusing on the 8th, 12th, and 15th day after the model creation [137].

The application of modern techniques for studying angiogenesis in experimental research made it possible to establish the important role of new vessel formation in cirrhosis-associated PH pathogenesis and has created the prerequisites for the development of antiangiogenic therapy aimed at threating PH-associated hemodynamic disorders.

PERSPECTIVES OF ANTIANGIOGENIC THERAPY FOR PORTAL HYPERTENSION IN LIVER CIRRHOSIS

The efforts to develop angiogenesis inhibitors began in the 1970s at Harvard University under the guidance of Judah Folkman. The drugs were actively

introduced into clinical practice a decade after the first were developed [29].

Inhibitors of Intrahepatic Angiogenesis

Tyrosine Kinase Inhibitors

The introduction of antiangiogenic therapy into hepatological practice began with the treatment of hepatocellular carcinoma, a well-vascularized tumor that needs intense angiogenic activity for its development [138]. The most studied drug used for this purpose is sorafenib, a multi-targeted inhibitor of receptor and nonreceptor tyrosine kinases, which are responsible for transmitting various signals to cells, including proliferative stimuli. The antitumor and antiangiogenic effect of sorafenib is achieved mainly through the suppression of the Raf/MEK/ERK signaling pathway and blockade of signaling from the receptors of VEGF (VEGFR), PDGF (PDGFR), and c-kit (SCFR) [139].

Experimental studies have shown the antiangiogenic effect of sorafenib during the early stage of hepatic fibrosis [140]. In animals with various models of cirrhosis, it had positive effects on some pathogenetic pathways of fibrogenesis and angiogenesis in the liver by blocking the receptor tyrosine kinases located on the surface of HSC, the expression of which, especially VEGFR and PDGFR, was increased [141] (Fig. **3**):

- the suppression of activated HSC proliferation and the activation of apoptosis;
- the inhibition of cyclin D1 and cyclin-dependent kinase 4 (Cdk-4) with a simultaneous increase in the expression of Fas, Fas-L, and Caspase-3, and a decrease in the ratio of Bcl-2 to Bax;
- an increase in the ratio of matrix metalloproteinases to the tissue inhibitor of matrix metalloproteinases, and also a decrease in the synthesis of collagen by HSC;
- the inhibition of phosphorylation of ERK, Akt, and ribosomal protein kinase S6 with a molecular mass of 70 kDa (p70S6K) [143]; and
- the disturbance of the Kruppel-like factor 6–Ang1–fibronectin molecular triad functioning [108].

Sorafenib decreased the severity of inflammation, fibrogenesis, and angiogenesis in rats with biliary cirrhosis, which led to a reduction in hepatic vascular resistance to portal blood flow [144].

Another multi-targeted tyrosine kinase inhibitor sunitinib is less studied but known to block VEGFR1/2/3, PDGFR-α/β, fibroblast growth factor receptor (FGFR), and c-kit signaling [145]. In addition, an *in vitro* study by Majumder *et al* [146] showed that sunitinib can slow HSC collagen synthesis by 47%, reduce

HSC contractility by 65%, and decrease cellular migration by 28%, as well as inhibit the angiogenic capacity of SEC.

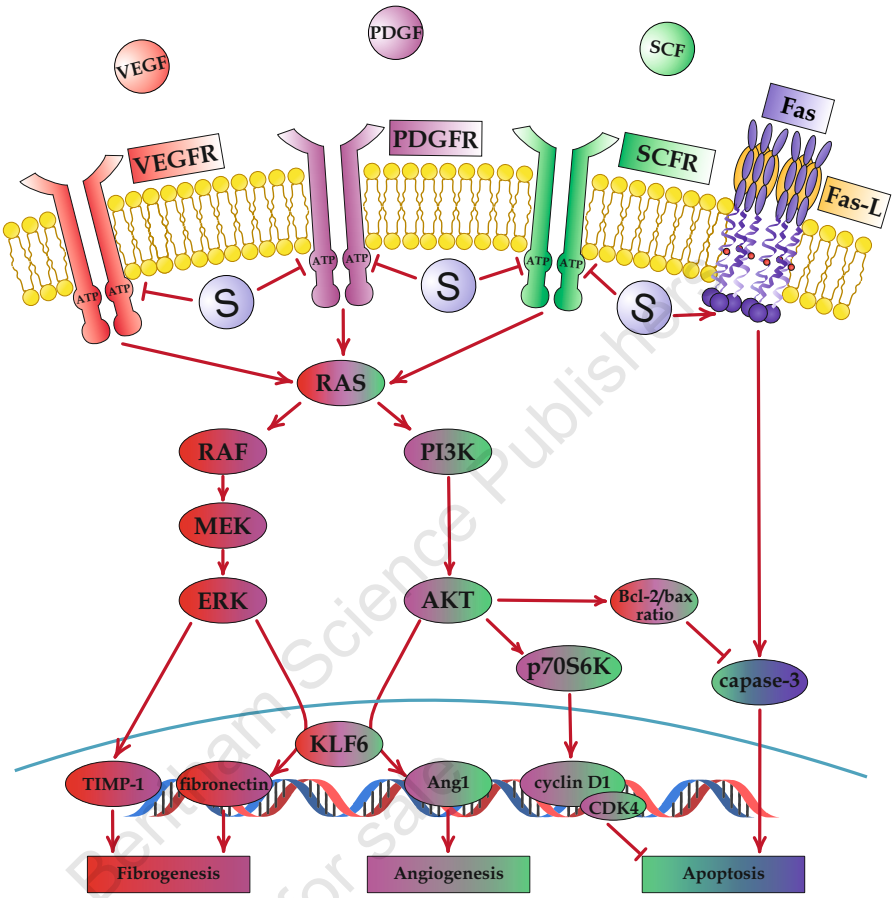


Fig. (3). Positive effects of sorafenib on some pathogenetic pathways of fibrogenesis and angiogenesis in the liver. Sorafenib (S) blocks the ATP-binding site of the vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and stem cell growth factor receptor (SCFR) tyrosine kinases located on the surface of HSC, inhibiting the two main cellular pathways of the RAS protein. At the same time, sorafenib increases the expression of Fas and its ligand. This decreases the severity of fibrogenesis and angiogenesis and increases apoptosis, leading to a reduction in hepatic vascular resistance to portal blood flow [142].

Branivib is a double inhibitor of VEGFR and FGFR signaling. It significantly suppressed intrahepatic angiogenesis and reduced PH in rats with biliary cirrhosis [109]. Additionally, it improved blood circulation in the liver and hindered the formation of ascites in rats with liver cirrhosis caused by nonalcoholic steatohepatitis [147].

Statins

The positive effect of statins on hepatic fibro- and angiogenesis in cirrhosis is associated with the induction of Krüppel-like factor 2 (KLF2) in SEC [148]. KLF2 is a member of a family of widely expressed transcription factors that regulate cell and tissue growth. KLF2 is well represented in the vascular endothelium and is necessary for the normal development of vessels; in addition, it is a well-known antiangiogenic factor that modulates the severity of many endothelial vasoprotective genes [149]. KLF2 can effectively inhibit HIF-1α, reducing the expression of such proangiogenic factors as VEGF and Ang2 [150].

The mechanical stimuli generated by shear stress are the main physiological impulse for triggering and maintaining endothelial KLF2 expression [151]. In the cirrhotic liver, KLF2 expression was elevated in both SEC [152] and activated HSC [153]. This serves as a compensatory mechanism aimed at eliminating vascular dysfunction and preventing angiogenesis by suppressing the proliferation and migration of SEC, as well as downregulating the ERK1/2 signaling pathway to inhibit the formation of tubular structures [154].

In an *in vitro* study conducted by Miao *et al* [155], simvastatin eliminated the pro-angiogenic environment for TGF-β-activated HSC as a result of the following processes:

- the reduction of cell migration and proliferation;
- the inhibition of the α-SMA expression, and the elevation of mRNA and KLF2 levels in HSC;
- an increase in the production of eNOS and suppression of the various proangiogenic proteins expression in HSC, such as VEGF, HIF-1α, and pro-inflammatory NF-κB; and
- the reduction of the hyperactivity of interferon γ, which participates in angiogenesis.

In rats with CCL4-induced liver cirrhosis, it was noted that statins (atorvastatin, mevastatin, simvastatin, and lovastatin) enhanced the effect of KLF2. By doing this, they deactivated SEC and reduced the severity of fibrosis and associated angiogenesis, thereby exerting a positive effect on PH [156].

Rifaximin

Endotoxemia, which is due to the translocation of gram-negative bacteria from the intestine, plays an important role in the pathogenesis of both cirrhosis and associated complications [157]. During the development of cirrhosis, bacterial lipopolysaccharide influences Kupffer cells and HSC. Nevertheless, SEC are

affected first. TLR4, which are located on their surface and capable of binding bacterial lipopolysaccharide, are involved in fibrosis-associated angiogenesis. These receptors manifest such properties through the related cytosolic adapter protein MyD88, which is involved in the production of extracellular protease regulating the invasive ability of SEC [42].

In mice with biliary cirrhosis, it was shown that rifaximin, a nonabsorbable antibiotic with broad antimicrobial activity against aerobic and anaerobic gram-negative bacteria, reduced the severity of fibrosis and angiogenesis in the liver by inhibiting bacterial lipopolysaccharide binding to TLR4. As a consequence, it reduced PH [158]. This drug is already used to treat hepatic encephalopathy. It has an acceptable safety profile when applied in patients with chronic liver diseases and is approved by the US Food and Drug Administration [159]. This experimental study may be a basis for evaluation of rifaximin in other complications of cirrhosis.

Largazole

The histone deacetylase inhibitor largazole is a natural compound derived from marine cyanobacteria Symploca sp. With a strong antiproliferative and cytotoxic effect, it has a wide spectrum but differential activity against several different lines of cancer cells [160]. In addition, in experimental studies *in vitro* and *in vivo*, largazole attenuated the severity of liver fibrosis and associated angiogenesis through numerous independent mechanisms:

- the reduction of VEGF production by HSC;
- the inhibition of VEGF-stimulated HSC proliferation;
- the downregulation of TGF-β1- and VEGF-induced Akt phosphorylation in activated HSC, as well as the downregulation of VEGFR2-dependent p38MAPK phosphorylation in SEC; and
- the suppression of CD34, VEGF, and VEGFR2 expression [161].

The ability of largazole to affect the main fibrogenic and angiogenic pathways in the cirrhotic liver can be used to test its effectiveness in PH.

Ribavirin

In addition to antiviral activity against certain DNA- and RNA-containing viruses, ribavirin may have a positive effect on the morphological changes underlying the development of cirrhosis [162]. In addition, at therapeutic concentrations, it is able to inhibit angiogenesis both *in vitro* and *in vivo*, which is due to the inhibition of inosine-5'-monophosphate dehydrogenase 1 activity and a decrease in tetrahydrobiopterin, NO, and cyclic guanosine monophosphate (cGMP) levels in

SEC [163].

Inhibitors of Extrahepatic Angiogenesis

It was traditionally thought that portosystemic shunts are formed when increased portal pressure "opens" pre-existing vessels in the areas of embryonic connection between the portal and systemic circulation. This paradigm was challenged by Fernandez *et al*, who first reported that portosystemic collaterals in PH are formed due to active angiogenesis. It should be noted that VEGF is of the greatest importance only at the initial stages of angiogenesis, when it activates endothelial cell proliferation and the subsequent formation of endothelial tubules. Vascular maturation is modulated mainly by PDGF, which regulates the introduction of endothelial tubules into the population of intramural cells and pericytes, thus stabilizing the newly formed vasculature [47]. The simultaneous suppression of the signaling caused by both VEGF and PDGF appears more promising than suppressing them individually.

Tyrosine Kinase Inhibitors

Fernandez *et al* [164] studied the combined effect of rapamycin (mTOR inhibitor) and glivec (tyrosine protein kinase inhibitor) on VEGF and PDGF signaling, respectively, in rats with extrahepatic PH caused by partial portal vein ligation and with well-developed portosystemic collateral circulation. It was noted that rapamycin and glivec in combination markedly reduced the splanchnic neovascularization and pericyte coverage of new vessels through the decreased expression of VEGF, VEGFR2, CD31, PDGF, PDGFR-β, and α-SMA. In addition, there was a reduction of portal pressure and blood flow along the superior mesenteric artery by 40% and 30% from the baseline level, respectively.

Similar results were obtained by Mejias *et al* [144], who found that multi-kinase inhibitor sorafenib triggered blockade of VEGF and PDGF signaling transduction and the Raf/MEK/ERK signaling pathways. Sorafenib significantly reduced intraorgan and systemic blood flow, and increased splanchnic neovascularization by 80% and portosystemic shunting by 18%. This led to a reduction in hepatic vascular resistance and decrease in portal pressure by 25% from the baseline. It was also noted that the positive effect of sorafenib on PH was more significant when it was combined with propranolol [165].

Somatostatin and its Synthetic Analogs

Somatostatin is a cyclic 14-amino acid peptide, which is secreted by nerve, endocrine, and enteroendocrine cells in the hypothalamus and digestive system (in the stomach, intestine, and pancreatic δ-cells). Somatostatin and its synthetic

analogs (octreotide, vapreotide, and others) are used in patients with cirrhosis to treat bleeding from esophageal varices by affecting both intra- and extrahepatic mechanisms of PH [166].

The ability of octreotide to inhibit cell proliferation and neovascularization through the high-affinity somatostatin subtype receptor 2 (SSTR2) was an impetus for studying its antiangiogenic properties in various diseases [167]. In studies involving rats with extrahepatic PH caused by partial portal vein ligation, octreotide significantly weakened the expression of VEGF and CD31 in the internal organs, reduced the development of splanchnic neovascularization by 64%, and lessened the severity of a portosystemic collateral circulation by 16%. At the same time, its angioinhibitory effect manifested only in the first four days of the experiment and completely disappeared after a week, as PH progressed. This is possibly due to a decrease in SSTR2 expression in mucosa, intestinal vessels, and portosystemic collaterals [168].

Spironolactone

Pathophysiological disturbances inherent to PH underlie the occurrence of ascites in cirrhosis. Systemic arterial vasodilation and the activation of various neurohormonal pathways, including the renin-angiotensin-aldosterone system, cause renal dysfunction. This decreases Na+ and water excretion and reduces the glomerular filtration rate. The drug of choice for treatment is spironolactone, an antagonist of aldosterone, a mineralocorticoid, that mediates the reabsorption of Na+ and water in the distal part of the nephron [169]. In addition to the important role in maintaining water–salt metabolism, aldosterone has angiogenic properties. In particular, it enhances ischemia-induced neovascularization [170], stimulates pathological angiogenesis in the retina [171], and promotes the proliferation of endothelial cells of the heart [172] by activating angiotensin II signaling. At the same time, its antagonist spironolactone inhibits these processes both *in vitro* and *in vivo* [173]. In rats with biliary cirrhosis, spironolactone significantly reduced the degree of mesenteric angiogenesis and portosystemic shunting by suppressing the VEGF signal transduction pathway [174].

N-acetylcysteine

Because hypoxia serves as the main inducer of angiogenesis both under physiological and pathological conditions, angiogenesis inhibitors may be drugs with antioxidant properties. One of them is N-acetylcysteine, which is a derivative of amino acid cysteine, the thiol groups of which directly interact with electrophilic groups of free radicals. N-acetylcysteine can also enhance the activity of glutathione-S-transferase, glutathione peroxidase, glutathione reductase, and a number of other enzymes involved in maintaining the

oxidant/antioxidant balance [175].

Long-term application of N-acetylcysteine in rats with biliary cirrhosis lessened oxidative stress in the mesentery of the small intestine, reduced the level of circulating inflammatory cytokines, and inhibited mesenteric angiogenesis by decreasing angiogenic marker expression (VEGF, VEGFR2, Ang1, and CD31). This eventually improved splanchnic and systemic hemodynamics.

In addition, N-acetylcysteine inhibited VEGF-induced endothelial tubule formation and endothelial cell migration by suppressing TNF-α and Akt/eNOS/NO angiogenic signaling cascade *in vitro*. It also reduced the number of reactive oxygen species (including reactive compounds of thiobarbituric acid and malondialdehyde) and inflammatory cytokines in the human umbilical vein endothelial cell supernatant [136].

Endothelin Receptor Blockers

ET-1 is one of the mediators whose synthesis is enhanced in conditions of tissue hypoxia. It is directly involved in intra- and extrahepatic mechanisms of PH pathogenesis, and its circulating level is increased in cirrhosis because of "large endothelin" hyperproduction and increased expression of endothelin-converting enzyme [176]. Experimental studies have shown that ET-1 induces angiogenic responses in cultured endothelial cells through endothelial ETB-type receptors and, in combination with VEGF, stimulates neovascularization *in vivo* [177]. The nonselective endothelin receptor blocker bosentan and the selective ETA receptor blocker ambrisentan reduced the degree of mesenteric angiogenesis and portosystemic shunting in rats with biliary cirrhosis by suppressing inducible nitric oxide synthase (iNOS), cyclooxygenase 2, VEGF and VEGFR2, and Akt signaling [178].

Pioglitazone

Pioglitazone, a potent selective agonist of peroxisome proliferator-activated receptors-γ (PPAR-γ), is able to reduce the level of systemic inflammation in patients with a high cardiovascular disease risk. It blocks the activity of pro-inflammatory genes by post-transcriptional modification of their products (by attaching small SUMO proteins to them) and suppresses NF-κB expression by transrepression. All PPAR isomers (PPAR-α, PPAR-β/-δ, and PPAR-γ) are anti-inflammatory nuclear transcription factors and NF-κB antagonists. Dominant negative mutation of PPAR-γ leads to systemic inflammation and rapid development of related diseases: arterial hypertension, atherosclerosis, type 2 diabetes, nonalcoholic steatohepatitis, psoriasis, and premature aging [179].

In addition to systemic inflammation reduction, PPAR-γ agonists are also capable of inhibiting oxidative stress and angiogenesis [180]. In rats with models of biliary cirrhosis and extrahepatic PH caused by partial portal vein ligation, pioglitazone reduced the degree of portosystemic shunting by 22–30% by suppressing angiogenic and pro-inflammatory cytokines, chemokines, and growth factors (VEGF, PDGF, and PIGF) [181].

Thalidomide

Thalidomide, a glutamic acid derivative with antiangiogenic, anti-inflammatory, and immunomodulatory properties, is able to hinder TNF-α/interleukin-1β production for which activated immune cells are responsible [182]. It was also shown in rats with biliary cirrhosis that thalidomide blocked the TNFα-VEG--NOS-NO pathway by downregulating elevated inflammasome expression in the intestinal and mesenteric tissues, which weakened mesenteric angiogenesis and portosystemic shunting [183].

Polyphenols

The possibility of influencing the pathogenetic mechanisms of extrahepatic angiogenesis was found in polyphenols, the chemicals of plant origin with a strong antioxidant effect.

The tea catechins extracted from the dried leaves of Camellia sinensis reduced the severity of mesenteric angiogenesis and portosystemic shunting in rats with biliary cirrhosis by reducing HIF-1α expression, Akt signaling, and VEGF synthesis [135].

2'-hydroxyflavonoid, which is contained in citruses, prevented the formation of new splanchnic vessels and portosystemic collaterals in rats with thioacetamide-induced liver cirrhosis by downregulating apoptosis [184].

The long-term use of curcumin, a polyphenol extracted from turmeric roots, improved the course of PH in liver cirrhosis by positively affecting liver fibrosis and reducing portal influx. These effects were achieved through inhibiting mesenteric angiogenesis and restoring mesenteric vessel contractility, as well as decreasing the degree of portosystemic collateral circulation and hyperdynamic circulatory state. Moreover, its favorable effects on the splanchnic and systemic blood flow included the suppression of VEGF, cyclooxygenase 2, and eNOS [113].

Clinical Experience of Antiangiogenic Therapy for Portal Hypertension

The effect of the drugs described above was studied only in experiments

involving animals (Tables **3**, **4**), and only tyrosine kinase inhibitors were tested as an antiangiogenic therapy in patients with cirrhosis and PH.

Coriat *et al* [185] were the first to assess the effect of sorafenib on the portal and systemic hemodynamics, in seven patients with cirrhosis and hepatocellular carcinoma. Five of them had Child–Turcotte–Pugh (CTP) class A, and two had CTP class B. Sorafenib was administered for one month at 400 mg twice a day. In one patient, this was first reduced to 400 mg once a day and then to 400 mg every two days because side effects appeared. A decrease in portal blood flow by at least 36% was noted, while no changes in blood flow were found in the azygos vein and abdominal aorta.

In a pilot study, Pinter *et al* [186] investigated the effects of sorafenib on HVPG and systemic hemodynamics, as well as the expression of mRNA genes involved in fibrogenesis, angiogenesis, and inflammation in the liver in 13 patients suffering from cirrhosis and hepatocellular carcinoma (10 patients had CTP class A and three patients had CTP class B). The drug was administered at 400 mg twice a day for two weeks. Four of the 11 patients with PH (eight of whom had clinically significant PH) had a reduction in HVPG by more than 20% from the baseline values and, in addition, a decrease in the levels of mRNA, VEGF, PDGF, PIGF, RhoA kinase, and TNF-α.

However, the results were not as optimistic in a randomized double-blind placebo-controlled study that assessed the effects of sorafenib administered at 400 mg twice a day on HVPG in nine patients with cirrhosis and hepatocellular carcinoma [187].

The main drawback of tyrosine kinase inhibitors is hepatotoxicity. A study of possibilities of their selective delivery to target cells, in particular, HSC, seems to be a promising direction in solving this problem.

Achievements in understanding the pathogenesis of PH in cirrhosis stimulated the development of new pharmacotherapeutic methods. Currently, the drugs of choice

Table 3. Drugs that can inhibit intrahepatic angiogenesis in portal hypertension.

Ref.	Drugs	Experimental models	Effects
Liu *et al* [133], Qu *et al* [134], Wang *et al* [135], Thabut *et al* [101], Mejias *et al* [136]	Sorafenib	Biliary cirrhosis, non-alcoholic steatohepatitis, thioacetamide-, diethylnitrosamine-, dimethylnitrosamine-, and CCl_4-induced cirrhosis.	Suppresses the Raf/MEK/ERK signaling pathway and blocks the signaling from the VEGFR, PDGFR, and SCFR; therefore, increases apoptosis and decreases inflammation, fibrogenesis, angiogenesis, and hepatic vascular resistance.

(Table 3) cont.....

Ref.	Drugs	Experimental models	Effects
Tugues *et al* [137], Majumder *et al* [138]	Sunitinib	CCl_4-induced cirrhosis and cell cultures (immortalized human activated HSC cell line, human HSC, and isolated primary human liver sinusoidal endothelial cells).	Blocks VEGFR1/2/3, PDGFR-α/β, FGFR, and SCFR; reduces HSC collagen synthesis, contractility, cellular migration, and SEC angiogenic capacity.
Lin *et al* [102], Yang *et al* [139]	Brivanib	Biliary cirrhosis, non-alcoholic steatohepatitis.	Inhibits VEGFR and FGFR; therefore, suppresses intrahepatic angiogenesis and portal hypertension, improves blood circulation, and hinders ascites formation.
Miao *et al* [147], Marrone *et al* [148]	Simvastatin	CCl_4-induced cirrhosis and LX-2 cell line.	Enhances KLF2, through which deactivates SEC and reduces the severity of fibrosis and associated angiogenesis.
Zhu *et al* [150]	Rifaximin	Biliary cirrhosis.	Downregulates bacterial lipopolysaccharide binding to TLR4; therefore, reduces the severity of fibrosis and associated angiogenesis.
Liu *et al* [152], Liu *et al* [153]	Largazole	Human colorectal carcinoma cells (HCT116, HT29, and HCT15), human HSC, and CCl_4-induced cirrhosis.	Suppresses the effects of CD34, VEGF, TGF-β1, and VEGFR2, blocking the main fibrogenic and angiogenic pathways.
Michaelis *et al* [155]	Ribavirin	Human umbilical vein endothelial cells.	Hinders angiogenesis by inhibiting inosine-5'-monophosphate dehydrogenase 1, tetrahydrobiopterin, NO, and cGMP.

Table 4. Drugs that can inhibit extrahepatic angiogenesis in portal hypertension.

Ref.	Drugs	Experimental models	Effects
Fernandez *et al* [156]	Rapamycin and glivec	Partial portal vein ligation	Downregulates VEGF, VEGFR2, CD31, PDGF, PDGFR-β, and α-SMA.
Mejias *et al* [136]	Sorafenib	Partial portal vein ligation and CCl_4-induced cirrhosis	Blocks VEGF, PDGF, and Raf/MEK/ERK signaling pathway; therefore, reduces intraorgan and systemic blood flow, splanchnic neovascularization, portosystemic shunting, hepatic vascular resistance, and portal pressure.
Woltering *et al* [159], Mejias *et al* [160]	Somatostatin and its synthetic analogs	Partial portal vein ligation	Reduces VEGF and CD31 expression, splanchnic neovascularization, and portosystemic collateral circulation by blocking SSTR2.

(Table 4) cont.....

Ref.	Drugs	Experimental models	Effects
Miternique-Grosse *et al* [165]	Spironolactone	Biliary cirrhosis	Suppresses the effects of aldosterone and the VEGF signal transduction pathway
Lee *et al* [129]	N-acetylcysteine	Biliary cirrhosis	Reduces oxidative stress, inflammatory cytokine levels, TNF-α, VEGF, VEGFR2, Ang1, CD31 expression, and suppresses Akt/eNOS/NO pathway.
Hsu *et al* [170]	Bosentan and ambrisentan	Biliary cirrhosis	Block endothelin receptors and suppress iNOS, cyclooxygenase 2, VEGF, VEGFR2, and Akt signaling.
Schwabl *et al* [173]	Pioglitazone	Biliary cirrhosis	Downregulates inflammatory genes and NF-κB expression, suppresses angiogenic and pro-inflammatory cytokines, chemokines, and growth factors (VEGF, PDGF, and PIGF).
Li *et al* [175]	Thalidomide	Biliary cirrhosis	Hinders TNF-α/interleukin-1β production and blocks the TNFα-VEGF-NOS-NO pathway.
Hsu *et al* [128]	Catechins of *Camellia sinensis*	Biliary cirrhosis	Reduce HIF-1α expression, Akt signaling, and VEGF synthesis.
Hsin *et al* [176]	2'-hydroxyflavonoid	Thioacetamide-induced liver cirrhosis	Downregulates apoptosis.
Hsu *et al* [106]	Curcumin	Biliary cirrhosis	Suppresses VEGF, cyclooxygenase 2, and eNOS.

are nonselective β-blockers. However, nonselective β-blockers are not recommended during the subclinical stage of the disease, when the most justified treatment is etiotropic and pathogenetic. Such treatment may be aimed at, for example, affecting fibro- and angiogenesis in the liver, as well as angiogenesis underlying the formation of portosystemic shunts. Etiopathogenic approach, as part of a complex correction of pathophysiological disorders that contribute to the development of PH, may be the key to success in preventing related complications.

CONCLUSION

When choosing the therapy for PH, one should take into account a clinical stage of cirrhosis, which largely determines the prognosis of the disease [188].

• Stage 1. An absence of esophageal varices in patients with compensated

cirrhosis. In this case, they may have both subclinical (HVPG is within 6-10 mmHg) and clinically significant PH (HVPG is more than 10 mmHg).

- Stage 2. The presence of esophageal varices in patients with compensated cirrhosis.
- Stage 3. Bleeding from esophageal varices in patients with cirrhosis in the absence of such signs of decompensation as ascites, jaundice, or encephalopathy.
- Stage 4. Patients with cirrhosis have one of the following signs of decompensation: ascites, jaundice, or encephalopathy, regardless of esophageal varices.
- Stage 5. Patients with cirrhosis have more than one of the following signs of decompensation: ascites, jaundice, or encephalopathy, regardless of esophageal varices.

Patients with the first and second stages should be divided on those who have subclinical PH and need pre-primary prophylaxis, which prevents the formation of esophageal varices, and those who have clinically significant PH and need measures aimed at variceal bleeding prevention [189] (Fig. **4**). HVPG values >10 mmHg measured using the balloon wedge method are the "gold standard" for clinically significant PH diagnosis. Alternatively, liver stiffness could be measured using transient elastography (TE). The liver stiffness values ≥20-25 kPa indicate clinically significant PH in patients with cirrhosis of viral etiology [1].

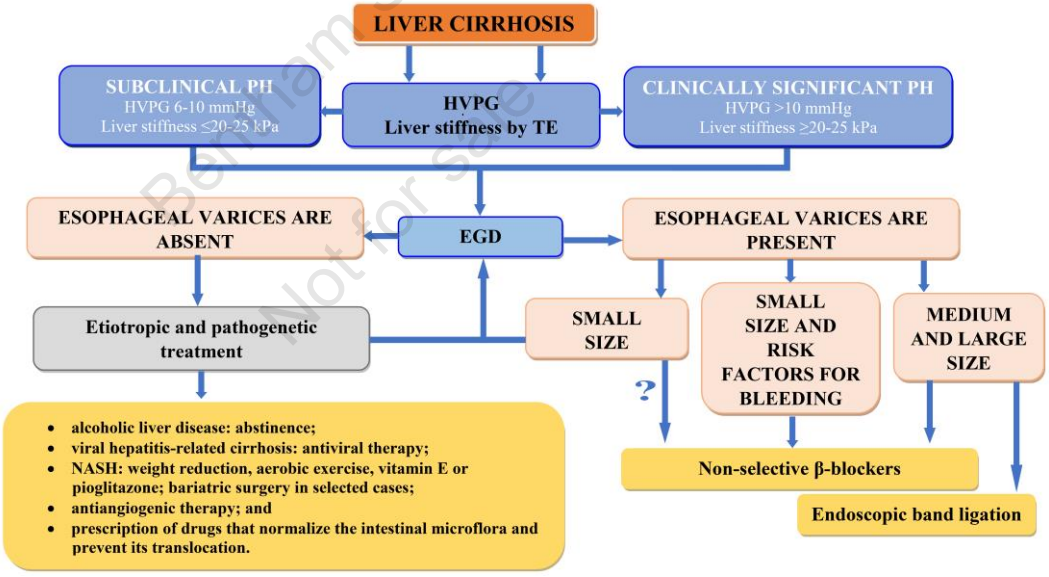


Fig. (4). The therapeutic and diagnostic algorithm in patients with cirrhosis who have not had bleeding from esophageal varices.

The ability of nonselective β-blockers to positively affect portal pressure by reducing cardiac output (blockade of β1-adrenergic receptors) and splanchnic vasodilation (blockade of β2-adrenergic receptors) allowed them to be considered the drugs of choice for primary prevention of bleeding from esophageal varices. However, the absence of a hyperdynamic circulatory status makes it inappropriate to use them in patients with subclinical PH [190]. At this stage, treatment of PH should be etiological and pathogenetic [191].

Certainly, abstinence is the most substantial and fundamental etiological treatment for alcoholic liver disease. In patients with alcoholic cirrhosis, abstinence may lead to regression of liver fibrosis, improve liver function, and help reduce portal pressure [192]. In the past decade, with the progress of antiviral therapy, viral hepatitis-related cirrhosis has undergone a radical change in its natural course following antiviral therapy. At the same time, a sustained virologic response has a positive effect on the histological structure of the liver and significantly reduces HVPG [193, 194]. Angiogenesis plays an important role in the pathogenesis of cirrhosis, underlies the development of associated PH, and is the cause of PH-related complications. Experimental studies made it possible to study the mechanism of action of drugs inhibiting angiogenesis and their effect on PH. However, only tyrosine kinase inhibitors have been tested in patients with cirrhosis as an anti-angiogenic therapy for PH [142]. Although these studies look promising, the complete blockade of the angiogenic pathways can be detrimental, since angiogenesis is also necessary to repair liver tissue and resolve fibrosis. In our opinion, the future of anti-angiogenic therapy lies in its selective effect on target cells in patients with compensated cirrhosis and subclinical PH.

Nonselective β-blockers *should be* prescribed to patients with clinically significant PH, who have small esophageal varices and risk factors for bleeding (severe liver dysfunction (CTP class B or C), alcoholic etiology of the disease, and the presence of so-called red signs on varicose veins during initial esophagogastroduodenoscopy (EGD)). The use of nonselective β-blockers is *possible* in the absence of these risk factors. The use of nonselective β-blockers is also a first-line therapy for the primary prevention of bleeding from esophageal varices of medium and large size. Endoscopic band ligation is indicated for patients who are tolerant or have contraindications to nonselective β-blockers [1].

CONFLICT OF INTEREST

Dr. Arefyev reports a grant from RFBR (Russian Foundation for Basic Research), during the writing of the manuscript. The information included in this manuscript is not related to the interests of RFBR. Prof. Garbuzenko and Prof. Kazachkov have nothing to disclose.

ACKNOWLEDGEMENT

This work is supported by RFBR according to the research project Nº 18-315-00434.

ABBREVIATION LIST

Akt	protein kinase B
Ang	angiopoietin
α-SMA	alpha smooth muscle actin
CTGF	connective tissue growth factor
cGMP	cyclic guanosine monophosphate
CTP	Child-Turcotte-Pugh
CCl_4	carbon tetrachloride
ET	endothelin-1
eNOS	endothelial nitric oxide synthase
EGF	epidermal growth factor
FGF	fibroblast growth factor
EGD	esophagogastroduodenoscopy
FGFR	fibroblast growth factor receptor
HVPG	hepatic venous portal gradient
HSC	hepatic stellate cells
HGF	hepatocyte growth factor
HIF	hypoxia-inducible factor
iNOS	inducible nitric oxide synthase
IGF	insulin-like growth factor
IL	interleukin
KLF	Krupple-like factor
KLF2	Krüppel-like factor 2
NF-κB	factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
PAF	platelet activation factor
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor-β
PIGF	placental growth factor
PPAR	peroxisome proliferator-activated receptor
PH	portal hypertension

ROS	reactive oxygen species
SEC	sinusoidal endothelial cells
SCFR	stem cell growth factor receptor
SSTR2	somatostatin receptor type 2
TGF	transforming growth factor
TNF	tumor necrosis factor
TE	transient elastography
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VSPC	vascular stem/progenitor cells

REFERENCES

[1] de Franchis R, Baveno VI. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. J Hepatol 2015; 63(3): 743-52.
[http://dx.doi.org/10.1016/j.jhep.2015.05.022] [PMID: 26047908]

[2] EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol 2019; 69(2): 406-60.
[http://dx.doi.org/10.1016/j.jhep.2018.03.024] [PMID: 29653741]

[3] Garbuzenko DV. Contemporary concepts of the medical therapy of portal hypertension under liver cirrhosis. World J Gastroenterol 2015; 21(20): 6117-26.
[http://dx.doi.org/10.3748/wjg.v21.i20.6117] [PMID: 26034348]

[4] Berzigotti A, Bosch J. Pharmacologic management of portal hypertension. Clin Liver Dis 2014; 18(2): 303-17.
[http://dx.doi.org/10.1016/j.cld.2013.12.003] [PMID: 24679496]

[5] Coulon S, Heindryckx F, Geerts A, Van Steenkiste C, Colle I, Van Vlierberghe H. Angiogenesis in chronic liver disease and its complications. Liver Int 2011; 31(2): 146-62.
[http://dx.doi.org/10.1111/j.1478-3231.2010.02369.x] [PMID: 21073649]

[6] Garbuzenko DV, Arefyev NO, Belov DV. Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis. World J Hepatol 2016; 8(16): 665-72.
[http://dx.doi.org/10.4254/wjh.v8.i16.665] [PMID: 27326313]

[7] Garbuzenko DV, Arefyev NO, Belov DV. Restructuring of the vascular bed in response to hemodynamic disturbances in portal hypertension. World J Hepatol 2016; 8(36): 1602-9.
[http://dx.doi.org/10.4254/wjh.v8.i36.1602] [PMID: 28083082]

[8] Rosmorduc O. Antiangiogenic therapies in portal hypertension: a breakthrough in hepatology. Gastroenterol Clin Biol 2010; 34(8-9): 446-9.
[http://dx.doi.org/10.1016/j.gcb.2010.05.007] [PMID: 20630674]

[9] García-Pagán JC, Gracia-Sancho J, Bosch J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. J Hepatol 2012; 57(2): 458-61.
[http://dx.doi.org/10.1016/j.jhep.2012.03.007] [PMID: 22504334]

[10] Hu LS, George J, Wang JH. Current concepts on the role of nitric oxide in portal hypertension. World J Gastroenterol 2013; 19(11): 1707-17.
[http://dx.doi.org/10.3748/wjg.v19.i11.1707] [PMID: 23555159]

[11] Hellerbrand C. Hepatic stellate cells--the pericytes in the liver. Pflugers Arch 2013; 465(6): 775-8. [http://dx.doi.org/10.1007/s00424-012-1209-5] [PMID: 23292551]

[12] Ueno T, Bioulac-Sage P, Balabaud C, Rosenbaum J. Innervation of the sinusoidal wall: regulation of the sinusoidal diameter. Anat Rec A Discov Mol Cell Evol Biol 2004; 280(1): 868-73. [http://dx.doi.org/10.1002/ar.a.20092] [PMID: 15382014]

[13] Iizuka M, Murata T, Hori M, Ozaki H. Increased contractility of hepatic stellate cells in cirrhosis is mediated by enhanced Ca2+-dependent and Ca2+-sensitization pathways. Am J Physiol Gastrointest Liver Physiol 2011; 300(6): G1010-21. [http://dx.doi.org/10.1152/ajpgi.00350.2010] [PMID: 21393429]

[14] Takashimizu S, Kojima S, Nishizaki Y, *et al.* Effect of endothelin A receptor antagonist on hepatic hemodynamics in cirrhotic rats. Implications for endothelin-1 in portal hypertension. Tokai J Exp Clin Med 2011; 36(2): 37-43. [PMID: 21769771]

[15] Lugo-Baruqui A, Muñoz-Valle JF, Arévalo-Gallegos S, Armendáriz-Borunda J. Role of angiotensin II in liver fibrosis-induced portal hypertension and therapeutic implications. Hepatol Res 2010; 40(1): 95-104. [http://dx.doi.org/10.1111/j.1872-034X.2009.00581.x] [PMID: 19737316]

[16] Reynaert H, Urbain D, Geerts A. Regulation of sinusoidal perfusion in portal hypertension. Anat Rec (Hoboken) 2008; 291(6): 693-8. [http://dx.doi.org/10.1002/ar.20669] [PMID: 18484616]

[17] Lee JS, Semela D, Iredale J, Shah VH. Sinusoidal remodeling and angiogenesis: a new function for the liver-specific pericyte? Hepatology 2007; 45(3): 817-25. [http://dx.doi.org/10.1002/hep.21564] [PMID: 17326208]

[18] Lee JS, Kang Decker N, Chatterjee S, Yao J, Friedman S, Shah V. Mechanisms of nitric oxide interplay with Rho GTPase family members in modulation of actin membrane dynamics in pericytes and fibroblasts. Am J Pathol 2005; 166(6): 1861-70. [http://dx.doi.org/10.1016/S0002-9440(10)62495-9] [PMID: 15920170]

[19] Semela D, Das A, Langer D, Kang N, Leof E, Shah V. Platelet-derived growth factor signaling through ephrin-b2 regulates hepatic vascular structure and function. Gastroenterology 2008; 135(2): 671-9. [http://dx.doi.org/10.1053/j.gastro.2008.04.010] [PMID: 18570897]

[20] Pinzani M. PDGF and signal transduction in hepatic stellate cells. Front Biosci 2002; 7: d1720-6. [http://dx.doi.org/10.2741/A875] [PMID: 12133817]

[21] Cao S, Yaqoob U, Das A, *et al.* Neuropilin-1 promotes cirrhosis of the rodent and human liver by enhancing PDGF/TGF-beta signaling in hepatic stellate cells. J Clin Invest 2010; 120(7): 2379-94. [http://dx.doi.org/10.1172/JCI41203] [PMID: 20577048]

[22] Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008; 88(1): 125-72. [http://dx.doi.org/10.1152/physrev.00013.2007] [PMID: 18195085]

[23] Seki E, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. J Hepatobiliary Pancreat Sci 2015; 22(7): 512-8. [http://dx.doi.org/10.1002/jhbp.245] [PMID: 25869468]

[24] Svegliati-Baroni G, De Minicis S, Marzioni M. Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition. Liver Int 2008; 28(8): 1052-64. [http://dx.doi.org/10.1111/j.1478-3231.2008.01825.x] [PMID: 18783548]

[25] Rappaport AM, MacPhee PJ, Fisher MM, Phillips MJ. The scarring of the liver acini (Cirrhosis). Tridimensional and microcirculatory considerations. Virchows Arch A Pathol Anat Histopathol 1983;

402(2): 107-37.
[http://dx.doi.org/10.1007/BF00695054] [PMID: 6420982]

[26] Lemos QT, Andrade ZA. Angiogenesis and experimental hepatic fibrosis. Mem Inst Oswaldo Cruz 2010; 105(5): 611-4.
[http://dx.doi.org/10.1590/S0074-02762010000500002] [PMID: 20835605]

[27] Elpek GÖ. Angiogenesis and liver fibrosis. World J Hepatol 2015; 7(3): 377-91.
[http://dx.doi.org/10.4254/wjh.v7.i3.377] [PMID: 25848465]

[28] Guido M, Sarcognato S, Russo FP, *et al.* Focus on histological abnormalities of intrahepatic vasculature in chronic viral hepatitis. Liver Int 2019; 38(10): 1770-6.
[http://dx.doi.org/10.1111/liv.13718] [PMID: 29427537]

[29] Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 2007; 6(4): 273-86.
[http://dx.doi.org/10.1038/nrd2115] [PMID: 17396134]

[30] Skuli N, Majmundar AJ, Krock BL, *et al.* Endothelial HIF-2α regulates murine pathological angiogenesis and revascularization processes. J Clin Invest 2012; 122(4): 1427-43.
[http://dx.doi.org/10.1172/JCI57322] [PMID: 22426208]

[31] Brandes RP, Miller FJ, Beer S, *et al.* The vascular NADPH oxidase subunit p47phox is involved in redox-mediated gene expression. Free Radic Biol Med 2002; 32(11): 1116-22.
[http://dx.doi.org/10.1016/S0891-5849(02)00789-X] [PMID: 12031896]

[32] Chen Z, Lai TC, Jan YH, *et al.* Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. J Clin Invest 2013; 123(3): 1057-67.
[http://dx.doi.org/10.1172/JCI65344] [PMID: 23426184]

[33] Carmeliet P. Manipulating angiogenesis in medicine. J Intern Med 2004; 255(5): 538-61.
[http://dx.doi.org/10.1111/j.1365-2796.2003.01297.x] [PMID: 15078497]

[34] Klein S, Roghani M, Rifkin DB. Fibroblast growth factors as angiogenesis factors: new insights into their mechanism of action. EXS 1997; 79: 159-92.
[http://dx.doi.org/10.1007/978-3-0348-9006-9_7] [PMID: 9002232]

[35] Hellberg C, Ostman A, Heldin CH. PDGF and vessel maturation. Recent Results Cancer Res 2010; 180: 103-14.
[http://dx.doi.org/10.1007/978-3-540-78281-0_7] [PMID: 20033380]

[36] Chen JX, Zeng H, Lawrence ML, Blackwell TS, Meyrick B. Angiopoietin-1-induced angiogenesis is modulated by endothelial NADPH oxidase. Am J Physiol Heart Circ Physiol 2006; 291(4): H1563-72.
[http://dx.doi.org/10.1152/ajpheart.01081.2005] [PMID: 16679392]

[37] Pauta M, Ribera J, Melgar-Lesmes P, *et al.* Overexpression of angiopoietin-2 in rats and patients with liver fibrosis. Therapeutic consequences of its inhibition. Liver Int 2015; 35(4): 1383-92.
[http://dx.doi.org/10.1111/liv.12505] [PMID: 24612347]

[38] Patsenker E, Popov Y, Stickel F, *et al.* Pharmacological inhibition of integrin alphavbeta3 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. Hepatology 2009; 50(5): 1501-11.
[http://dx.doi.org/10.1002/hep.23144] [PMID: 19725105]

[39] Kevil CG, Payne DK, Mire E, Alexander JS. Vascular permeability factor/vascular endothelial cell growth factor-mediated permeability occurs through disorganization of endothelial junctional proteins. J Biol Chem 1998; 273(24): 15099-103.
[http://dx.doi.org/10.1074/jbc.273.24.15099] [PMID: 9614120]

[40] Elpek GO, Gokhan GA, Bozova S. Thrombospondin-1 expression correlates with angiogenesis in experimental cirrhosis. World J Gastroenterol 2008; 14(14): 2213-7.
[http://dx.doi.org/10.3748/wjg.14.2213] [PMID: 18407596]

[41] Eriksson K, Magnusson P, Dixelius J, Claesson-Welsh L, Cross MJ. Angiostatin and endostatin inhibit

endothelial cell migration in response to FGF and VEGF without interfering with specific intracellular signal transduction pathways. FEBS Lett 2003; 536(1-3): 19-24.
[http://dx.doi.org/10.1016/S0014-5793(03)00003-6] [PMID: 12586331]

[42] Jagavelu K, Routray C, Shergill U, O'Hara SP, Faubion W, Shah VH. Endothelial cell toll-like receptor 4 regulates fibrosis-associated angiogenesis in the liver. Hepatology 2010; 52(2): 590-601.
[http://dx.doi.org/10.1002/hep.23739] [PMID: 20564354]

[43] Melgar-Lesmes P, Pauta M, Reichenbach V, *et al.* Hypoxia and proinflammatory factors upregulate apelin receptor expression in human stellate cells and hepatocytes. Gut 2011; 60(10): 1404-11.
[http://dx.doi.org/10.1136/gut.2010.234690] [PMID: 21450694]

[44] Huebert RC, Jagavelu K, Hendrickson HI, *et al.* Aquaporin-1 promotes angiogenesis, fibrosis, and portal hypertension through mechanisms dependent on osmotically sensitive microRNAs. Am J Pathol 2011; 179(4): 1851-60.
[http://dx.doi.org/10.1016/j.ajpath.2011.06.045] [PMID: 21854740]

[45] Sahin H, Borkham-Kamphorst E, Kuppe C, *et al.* Chemokine Cxcl9 attenuates liver fibrosis-associated angiogenesis in mice. Hepatology 2012; 55(5): 1610-9.
[http://dx.doi.org/10.1002/hep.25545] [PMID: 22237831]

[46] Staton CA, Kumar I, Reed MW, Brown NJ. Neuropilins in physiological and pathological angiogenesis. J Pathol 2007; 212(3): 237-48.
[http://dx.doi.org/10.1002/path.2182] [PMID: 17503412]

[47] Fernández M, Semela D, Bruix J, Colle I, Pinzani M, Bosch J. Angiogenesis in liver disease. J Hepatol 2009; 50(3): 604-20.
[http://dx.doi.org/10.1016/j.jhep.2008.12.011] [PMID: 19157625]

[48] Chaparro M, Sanz-Cameno P, Trapero-Marugan M, Garcia-Buey L, Moreno-Otero R. Mechanisms of angiogenesis in chronic inflammatory liver disease. Ann Hepatol 2007; 6(4): 208-13.
[PMID: 18007549]

[49] Steib CJ. Kupffer cell activation and portal hypertension. Gut 2011; 60(10): 1307-8.
[http://dx.doi.org/10.1136/gut.2011.242560] [PMID: 21708827]

[50] Lochhead PA, Gilley R, Cook SJ. ERK5 and its role in tumour development. Biochem Soc Trans 2012; 40(1): 251-6.
[http://dx.doi.org/10.1042/BST20110663] [PMID: 22260700]

[51] Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat Rev Cancer 2008; 8(6): 425-37.
[http://dx.doi.org/10.1038/nrc2397] [PMID: 18500244]

[52] Ko HM, Seo KH, Han SJ, *et al.* Nuclear factor kappaB dependency of platelet-activating factor-induced angiogenesis. Cancer Res 2002; 62(6): 1809-14.
[PMID: 11912159]

[53] Franceschini B, Ceva-Grimaldi G, Russo C, Dioguardi N, Grizzi F. The complex functions of mast cells in chronic human liver diseases. Dig Dis Sci 2006; 51(12): 2248-56.
[http://dx.doi.org/10.1007/s10620-006-9082-8] [PMID: 17103041]

[54] Marra F. Chemokines in liver inflammation and fibrosis. Front Biosci 2002; 7(7): d1899-914.
[http://dx.doi.org/10.2741/A887] [PMID: 12161342]

[55] Copple BL, Bai S, Burgoon LD, Moon JO. Hypoxia-inducible factor-1α regulates the expression of genes in hypoxic hepatic stellate cells important for collagen deposition and angiogenesis. Liver Int 2011; 31(2): 230-44.
[http://dx.doi.org/10.1111/j.1478-3231.2010.02347.x] [PMID: 20880076]

[56] Lemoinne S, Cadoret A, El Mourabit H, Thabut D, Housset C. Origins and functions of liver myofibroblasts. Biochim Biophys Acta 2013; 1832(7): 948-54.
[http://dx.doi.org/10.1016/j.bbadis.2013.02.019] [PMID: 23470555]

[57] Yokomori H, Oda M, Yoshimura K, Hibi T. Enhanced expressions of apelin on proliferative hepatic arterial capillaries in human cirrhotic liver. Hepatol Res 2012; 42(5): 508-14.
[http://dx.doi.org/10.1111/j.1872-034X.2011.00945.x] [PMID: 22502744]

[58] Novo E, Povero D, Busletta C, *et al.* The biphasic nature of hypoxia-induced directional migration of activated human hepatic stellate cells. J Pathol 2012; 226(4): 588-97.
[http://dx.doi.org/10.1002/path.3005] [PMID: 21959987]

[59] Novo E, Cannito S, Zamara E, *et al.* Proangiogenic cytokines as hypoxia-dependent factors stimulating migration of human hepatic stellate cells. Am J Pathol 2007; 170(6): 1942-53.
[http://dx.doi.org/10.2353/ajpath.2007.060887] [PMID: 17525262]

[60] Vanheule E, Geerts AM, Van Huysse J, *et al.* An intravital microscopic study of the hepatic microcirculation in cirrhotic mice models: relationship between fibrosis and angiogenesis. Int J Exp Pathol 2008; 89(6): 419-32.
[http://dx.doi.org/10.1111/j.1365-2613.2008.00608.x] [PMID: 19134051]

[61] Kaur S, Tripathi D, Dongre K, *et al.* Increased number and function of endothelial progenitor cells stimulate angiogenesis by resident liver sinusoidal endothelial cells (SECs) in cirrhosis through paracrine factors. J Hepatol 2012; 57(6): 1193-8.
[http://dx.doi.org/10.1016/j.jhep.2012.07.016] [PMID: 22824816]

[62] Chen CH, Chang LT, Tung WC, *et al.* Levels and values of circulating endothelial progenitor cells, soluble angiogenic factors, and mononuclear cell apoptosis in liver cirrhosis patients. J Biomed Sci 2012; 19: 66.
[http://dx.doi.org/10.1186/1423-0127-19-66] [PMID: 22809449]

[63] Abraldes JG, Iwakiri Y, Loureiro-Silva M, Haq O, Sessa WC, Groszmann RJ. Mild increases in portal pressure upregulate vascular endothelial growth factor and endothelial nitric oxide synthase in the intestinal microcirculatory bed, leading to a hyperdynamic state. Am J Physiol Gastrointest Liver Physiol 2006; 290(5): G980-7.
[http://dx.doi.org/10.1152/ajpgi.00336.2005] [PMID: 16603731]

[64] Angermayr B, Mejias M, Gracia-Sancho J, Garcia-Pagan JC, Bosch J, Fernandez M. Heme oxygenase attenuates oxidative stress and inflammation, and increases VEGF expression in portal hypertensive rats. J Hepatol 2006; 44(6): 1033-9.
[http://dx.doi.org/10.1016/j.jhep.2005.09.021] [PMID: 16458992]

[65] Kobus K, Kopycinska J, Kozlowska-Wiechowska A, *et al.* Angiogenesis within the duodenum of patients with cirrhosis is modulated by mechanosensitive Kruppel-like factor 2 and microRNA-126. Liver Int 2012; 32(8): 1222-32.
[http://dx.doi.org/10.1111/j.1478-3231.2012.02791.x] [PMID: 22574900]

[66] Huang HC, Haq O, Utsumi T, *et al.* Intestinal and plasma VEGF levels in cirrhosis: the role of portal pressure. J Cell Mol Med 2012; 16(5): 1125-33.
[http://dx.doi.org/10.1111/j.1582-4934.2011.01399.x] [PMID: 21801303]

[67] Garcia-Pras E, Gallego J, Coch L, *et al.* Role and therapeutic potential of vascular stem/progenitor cells in pathological neovascularisation during chronic portal hypertension. Gut 2017; 66(7): 1306-20.
[http://dx.doi.org/10.1136/gutjnl-2015-311157] [PMID: 26984852]

[68] Gana JC, Serrano CA, Ling SC. Angiogenesis and portal-systemic collaterals in portal hypertension. Ann Hepatol 2016; 15(3): 303-13.
[http://dx.doi.org/10.5604/16652681.1198799] [PMID: 27049484]

[69] Fernandez M, Vizzutti F, Garcia-Pagan JC, Rodes J, Bosch J. Anti-VEGF receptor-2 monoclonal antibody prevents portal-systemic collateral vessel formation in portal hypertensive mice. Gastroenterology 2004; 126(3): 886-94.
[http://dx.doi.org/10.1053/j.gastro.2003.12.012] [PMID: 14988842]

[70] Fernandez M, Mejias M, Angermayr B, Garcia-Pagan JC, Rodés J, Bosch J. Inhibition of VEGF

receptor-2 decreases the development of hyperdynamic splanchnic circulation and portal-systemic collateral vessels in portal hypertensive rats. J Hepatol 2005; 43(1): 98-103.
[http://dx.doi.org/10.1016/j.jhep.2005.02.022] [PMID: 15893841]

[71] Angermayr B, Fernandez M, Mejias M, Gracia-Sancho J, Garcia-Pagan JC, Bosch J. NAD(P)H oxidase modulates angiogenesis and the development of portosystemic collaterals and splanchnic hyperaemia in portal hypertensive rats. Gut 2007; 56(4): 560-4.
[http://dx.doi.org/10.1136/gut.2005.088013] [PMID: 16854998]

[72] Gallego J, Garcia-Pras E, Mejias M, Pell N, Schaeper U, Fernandez M. Therapeutic siRNA targeting endothelial KDR decreases portosystemic collateralization in portal hypertension. Sci Rep 2017; 7(1): 14791.
[http://dx.doi.org/10.1038/s41598-017-14818-7] [PMID: 29093528]

[73] Chan CC. Portal-systemic collaterals and angiogenesis. J Chin Med Assoc 2009; 72(5): 223-4.
[http://dx.doi.org/10.1016/S1726-4901(09)70060-7] [PMID: 19467943]

[74] Albillos A, Bañares R, González M, *et al.* The extent of the collateral circulation influences the postprandial increase in portal pressure in patients with cirrhosis. Gut 2007; 56(2): 259-64.
[http://dx.doi.org/10.1136/gut.2006.095240] [PMID: 16837532]

[75] Arora A, Rajesh S, Meenakshi YS, Sureka B, Bansal K, Sarin SK. Spectrum of hepatofugal collateral pathways in portal hypertension: an illustrated radiological review. Insights Imaging 2015; 6(5): 559-72.
[http://dx.doi.org/10.1007/s13244-015-0419-8] [PMID: 26337049]

[76] Yang Z, Tian L, Peng L, Qiu F. Immunohistochemical analysis of growth factor expression and localization in gastric coronary vein of cirrhotic patients. J Tongji Med Univ 1996; 16(4): 229-33.
[http://dx.doi.org/10.1007/BF02888113] [PMID: 9389088]

[77] Hashizume M, Kitano S, Sugimachi K, Sueishi K. Three-dimensional view of the vascular structure of the lower esophagus in clinical portal hypertension. Hepatology 1988; 8(6): 1482-7.
[http://dx.doi.org/10.1002/hep.1840080603] [PMID: 3192160]

[78] Turmakhanov ST, Asadulaev ShM, Akhmetkaliev MN. Morpho-functional Changes of the Azygos Vein and Other Veins of the Gastroesophageal Zone in Portal Hypertension. Ann hir gepatol 2008; 13(2): 58-65.

[79] Vianna A, Hayes PC, Moscoso G, *et al.* Normal venous circulation of the gastroesophageal junction. A route to understanding varices. Gastroenterology 1987; 93(4): 876-89.
[http://dx.doi.org/10.1016/0016-5085(87)90453-7] [PMID: 3623028]

[80] Noda T. Angioarchitectural study of esophageal varices. With special reference to variceal rupture. Virchows Arch A Pathol Anat Histopathol 1984; 404(4): 381-92.
[http://dx.doi.org/10.1007/BF00695222] [PMID: 6437071]

[81] Gaba RC, Couture PM, Lakhoo J. Gastroesophageal Variceal Filling and Drainage Pathways: An Angiographic Description of Afferent and Efferent Venous Anatomic Patterns. J Clin Imaging Sci 2015; 5: 61.
[http://dx.doi.org/10.4103/2156-7514.170730] [PMID: 26713177]

[82] Gracia-Sancho J, Maeso-Díaz R, Bosch J. Pathophysiology and a Rational Basis of Therapy. Dig Dis 2015; 33(4): 508-14.
[http://dx.doi.org/10.1159/000374099] [PMID: 26159267]

[83] Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. Nutr Rev 2007; 65(12 Pt 2): S140-6.
[http://dx.doi.org/10.1301/nr.2007.dec.S140-S146] [PMID: 18240538]

[84] de Las Heras N, Aller MA, Martín-Fernández B, *et al.* A wound-like inflammatory aortic response in chronic portal hypertensive rats. Mol Immunol 2012; 51(2): 177-87.
[http://dx.doi.org/10.1016/j.molimm.2012.03.016] [PMID: 22463791]

[85] Fernández-Varo G, Ros J, Morales-Ruiz M, *et al.* Nitric oxide synthase 3-dependent vascular remodeling and circulatory dysfunction in cirrhosis. Am J Pathol 2003; 162(6): 1985-93.
[http://dx.doi.org/10.1016/S0002-9440(10)64331-3] [PMID: 12759254]

[86] Kiyono S, Maruyama H, Kondo T, *et al.* Hemodynamic effect of the left gastric artery on esophageal varices in patients with cirrhosis. J Gastroenterol 2016; 51(9): 900-9.
[http://dx.doi.org/10.1007/s00535-015-1157-x] [PMID: 26781661]

[87] Piva A, Zampieri F, Di Pascoli M, Gatta A, Sacerdoti D, Bolognesi M. Mesenteric arteries responsiveness to acute variations of wall shear stress is impaired in rats with liver cirrhosis. Scand J Gastroenterol 2012; 47(8-9): 1003-13.
[http://dx.doi.org/10.3109/00365521.2012.703231] [PMID: 22774919]

[88] Resch M, Wiest R, Moleda L, *et al.* Alterations in mechanical properties of mesenteric resistance arteries in experimental portal hypertension. Am J Physiol Gastrointest Liver Physiol 2009; 297(4): G849-57.
[http://dx.doi.org/10.1152/ajpgi.00084.2009] [PMID: 19696142]

[89] Geerts AM, De Vriese AS, Vanheule E, *et al.* Increased angiogenesis and permeability in the mesenteric microvasculature of rats with cirrhosis and portal hypertension: an *in vivo* study. Liver Int 2006; 26(7): 889-98.
[http://dx.doi.org/10.1111/j.1478-3231.2006.01308.x] [PMID: 16911473]

[90] Bolognesi M, Di Pascoli M, Verardo A, Gatta A. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. World J Gastroenterol 2014; 20(10): 2555-63.
[http://dx.doi.org/10.3748/wjg.v20.i10.2555] [PMID: 24627591]

[91] Wei W, Pu YS, Wang XK, *et al.* Wall shear stress in portal vein of cirrhotic patients with portal hypertension. World J Gastroenterol 2017; 23(18): 3279-86.
[http://dx.doi.org/10.3748/wjg.v23.i18.3279] [PMID: 28566887]

[92] Wen B, Liang J, Deng X, Chen R, Peng P. Effect of fluid shear stress on portal vein remodeling in a rat model of portal hypertension. Gastroenterol Res Pract 2015; 2015: 545018.
[http://dx.doi.org/10.1155/2015/545018] [PMID: 25892988]

[93] Zeng X, Huang P, Chen M, *et al.* TMEM16A regulates portal vein smooth muscle cell proliferation in portal hypertension. Exp Ther Med 2019; 15(1): 1062-8.
[PMID: 29434696]

[94] Lautt WW. Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. Am J Physiol 1985; 249(5 Pt 1): G549-56.
[PMID: 3904482]

[95] Moeller M, Thonig A, Pohl S, Ripoll C, Zipprich A. Hepatic arterial vasodilation is independent of portal hypertension in early stages of cirrhosis. PLoS One 2015; 10(3): e0121229.
[http://dx.doi.org/10.1371/journal.pone.0121229] [PMID: 25793622]

[96] Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. World J Gastroenterol 2010; 16(48): 6046-57.
[http://dx.doi.org/10.3748/wjg.v16.i48.6046] [PMID: 21182219]

[97] Li T, Ni JY, Qi YW, Li HY, Zhang T, Yang Z. Splenic vasculopathy in portal hypertension patients. World J Gastroenterol 2006; 12(17): 2737-41.
[http://dx.doi.org/10.3748/wjg.v12.i17.2737] [PMID: 16718761]

[98] Yang Z, Zhang L, Li D, Qiu F. Pathological morphology alteration of the splanchnic vascular wall in portal hypertensive patients. Chin Med J (Engl) 2002; 115(4): 559-62.
[PMID: 12133298]

[99] Garbuzenko DV. Experimental methods of portal hypertension studying. Ros zhurn gastrojenterol gepatol koloproktol 2010; 20(2): 4-12.

[100] McDonald DM, Choyke PL. Imaging of angiogenesis: from microscope to clinic. Nat Med 2003; 9(6): 713-25.
[http://dx.doi.org/10.1038/nm0603-713] [PMID: 12778170]

[101] Van Steenkiste C, Trachet B, Casteleyn C, *et al.* Vascular corrosion casting: analyzing wall shear stress in the portal vein and vascular abnormalities in portal hypertensive and cirrhotic rodents. Lab Invest 2010; 90(11): 1558-72.
[http://dx.doi.org/10.1038/labinvest.2010.138] [PMID: 20714322]

[102] Niggemann P, Murata S, Naito Z, Kumazaki T. A comparative study of the microcirculatory changes in the developing liver cirrhosis between the central and peripheral parts of the main lobe in mice. Hepatol Res 2004; 28(1): 41-8.
[http://dx.doi.org/10.1016/j.hepres.2003.08.004] [PMID: 14734150]

[103] Yang YY, Huang YT, Lin HC, *et al.* Thalidomide decreases intrahepatic resistance in cirrhotic rats. Biochem Biophys Res Commun 2009; 380(3): 666-72.
[http://dx.doi.org/10.1016/j.bbrc.2009.01.160] [PMID: 19285019]

[104] Boerckel JD, Mason DE, McDermott AM, Alsberg E. Microcomputed tomography: approaches and applications in bioengineering. Stem Cell Res Ther 2014; 5(6): 144.
[http://dx.doi.org/10.1186/scrt534] [PMID: 25689288]

[105] Ehling J, Bartneck M, Wei X, *et al.* CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. Gut 2014; 63(12): 1960-71.
[http://dx.doi.org/10.1136/gutjnl-2013-306294] [PMID: 24561613]

[106] Bartneck M, Fech V, Ehling J, *et al.* Histidine-rich glycoprotein promotes macrophage activation and inflammation in chronic liver disease. Hepatology 2016; 63(4): 1310-24.
[http://dx.doi.org/10.1002/hep.28418] [PMID: 26699087]

[107] Jorgensen SM, Demirkaya O, Ritman EL. Three-dimensional imaging of vasculature and parenchyma in intact rodent organs with X-ray micro-CT. Am J Physiol 1998; 275(3 Pt 2): H1103-14.
[PMID: 9724319]

[108] Thabut D, Routray C, Lomberk G, *et al.* Complementary vascular and matrix regulatory pathways underlie the beneficial mechanism of action of sorafenib in liver fibrosis. Hepatology 2011; 54(2): 573-85.
[http://dx.doi.org/10.1002/hep.24427] [PMID: 21567441]

[109] Lin HC, Huang YT, Yang YY, *et al.* Beneficial effects of dual vascular endothelial growth factor receptor/fibroblast growth factor receptor inhibitor brivanib alaninate in cirrhotic portal hypertensive rats. J Gastroenterol Hepatol 2014; 29(5): 1073-82.
[http://dx.doi.org/10.1111/jgh.12480] [PMID: 24325631]

[110] Kline TL, Knudsen BE, Anderson JL, Vercnocke AJ, Jorgensen SM, Ritman EL. Anatomy of hepatic arteriolo-portal venular shunts evaluated by 3D micro-CT imaging. J Anat 2014; 224(6): 724-31.
[http://dx.doi.org/10.1111/joa.12178] [PMID: 24684343]

[111] Peeters G, Debbaut C, Laleman W, *et al.* A multilevel framework to reconstruct anatomical 3D models of the hepatic vasculature in rat livers. J Anat 2017; 230(3): 471-83.
[http://dx.doi.org/10.1111/joa.12567] [PMID: 27995631]

[112] Peeters G, Debbaut C, Friebel A, *et al.* Quantitative analysis of hepatic macro- and microvascular alterations during cirrhogenesis in the rat. J Anat 2019; 232(3): 485-96.
[http://dx.doi.org/10.1111/joa.12760] [PMID: 29205328]

[113] Hsu SJ, Lee JY, Lin TY, *et al.* The beneficial effects of curcumin in cirrhotic rats with portal hypertension. Biosci Rep 2017; 37(6): 1-13.
[http://dx.doi.org/10.1042/BSR20171015] [PMID: 29162665]

[114] Renier N, Wu Z, Simon DJ, Yang J, Ariel P, Tessier-Lavigne M. iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. Cell 2014; 159(4): 896-910.

[http://dx.doi.org/10.1016/j.cell.2014.10.010] [PMID: 25417164]

[115] Susaki EA, Tainaka K, Perrin D, *et al.* Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis. Cell 2014; 157(3): 726-39.
[http://dx.doi.org/10.1016/j.cell.2014.03.042] [PMID: 24746791]

[116] Arefyev NO, Garbuzenko DV, Emelyanov IV, Khasanov LR, Mineeva LV. Changes in the microvasculature of small bowel mesentery in rats with prehepatic portal Hypertension: the preliminary study *in vivo.* Abdomen 2017; 4: e1580.

[117] Maksan SM, Ryschich E, Ulger Z, Gebhard MM, Schmidt J. Disturbance of hepatic and intestinal microcirculation in experimental liver cirrhosis. World J Gastroenterol 2005; 11(6): 846-9.
[http://dx.doi.org/10.3748/wjg.v11.i6.846] [PMID: 15682478]

[118] Yamaki K, Lindbom L, Thorlacius H, Hedqvist P, Raud J. An approach for studies of mediator-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery. Br J Pharmacol 1998; 123(3): 381-9.
[http://dx.doi.org/10.1038/sj.bjp.0701617] [PMID: 9504377]

[119] De Backer D, Hollenberg S, Boerma C, *et al.* How to evaluate the microcirculation: report of a round table conference. Crit Care 2007; 11(5): R101.
[http://dx.doi.org/10.1186/cc6118] [PMID: 17845716]

[120] De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med 2002; 166(1): 98-104.
[http://dx.doi.org/10.1164/rccm.200109-016OC] [PMID: 12091178]

[121] Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF. Nitroglycerin in septic shock after intravascular volume resuscitation. Lancet 2002; 360(9343): 1395-6.
[http://dx.doi.org/10.1016/S0140-6736(02)11393-6] [PMID: 12423989]

[122] Klyscz T, Jünger M, Jung F, Zeintl H. [Cap image--a new kind of computer-assisted video image analysis system for dynamic capillary microscopy]. Biomed Tech (Berl) 1997; 42(6): 168-75.
[http://dx.doi.org/10.1515/bmte.1997.42.6.168] [PMID: 9312307]

[123] Sumanovski LT, Battegay E, Stumm M, van der Kooij M, Sieber CC. Increased angiogenesis in portal hypertensive rats: role of nitric oxide. Hepatology 1999; 29(4): 1044-9.
[http://dx.doi.org/10.1002/hep.510290436] [PMID: 10094944]

[124] Anderson CR, Ponce AM, Price RJ. Immunohistochemical identification of an extracellular matrix scaffold that microguides capillary sprouting *in vivo.* J Histochem Cytochem 2004; 52(8): 1063-72.
[http://dx.doi.org/10.1369/jhc.4A6250.2004] [PMID: 15258182]

[125] Huang HC, Wang SS, Hsin IF, *et al.* Cannabinoid receptor 2 agonist ameliorates mesenteric angiogenesis and portosystemic collaterals in cirrhotic rats. Hepatology 2012; 56(1): 248-58.
[http://dx.doi.org/10.1002/hep.25625] [PMID: 22290687]

[126] Yang M, Stapor PC, Peirce SM, Betancourt AM, Murfee WL. Rat mesentery exteriorization: a model for investigating the cellular dynamics involved in angiogenesis. J Vis Exp 2012; 63(63): e3954.
[PMID: 22643964]

[127] McConnell M, Iwakiri Y. Biology of portal hypertension. Hepatol Int 2019; 12 (Suppl. 1): 11-23.
[http://dx.doi.org/10.1007/s12072-017-9826-x] [PMID: 29075990]

[128] Licks F, Hartmann RM, Marques C, *et al.* N-acetylcysteine modulates angiogenesis and vasodilation in stomach such as DNA damage in blood of portal hypertensive rats. World J Gastroenterol 2015; 21(43): 12351-60.
[http://dx.doi.org/10.3748/wjg.v21.i43.12351] [PMID: 26604642]

[129] Aperio Technologies I. Microvessel Analysis Algorithm, User'S Guide @ONLINE. 2008.

[130] Reyes-Aldasoro CC, Williams LJ, Akerman S, Kanthou C, Tozer GM. An automatic algorithm for the

segmentation and morphological analysis of microvessels in immunostained histological tumour sections. J Microsc 2011; 242(3): 262-78.
[http://dx.doi.org/10.1111/j.1365-2818.2010.03464.x] [PMID: 21118252]

[131] Fernández-Carrobles MM, Tadeo I, Bueno G, *et al.* TMA vessel segmentation based on color and morphological features: application to angiogenesis research. Sci World J 2013; 2013: 263190.
[http://dx.doi.org/10.1155/2013/263190] [PMID: 24489494]

[132] Chojkier M, Groszmann RJ. Measurement of portal-systemic shunting in the rat by using γ-labeled microspheres. Am J Physiol 1981; 240(5): G371-5.
[PMID: 7235023]

[133] Hodeige D, de Pauw M, Eechaute W, Weyne J, Heyndrickx GR. On the validity of blood flow measurement using colored microspheres. Am J Physiol 1999; 276(4): H1150-8.
[PMID: 10199837]

[134] Theodorakis N, Maluccio M, Skill N. Murine study of portal hypertension associated endothelin-1 hypo-response. World J Gastroenterol 2015; 21(16): 4817-28.
[http://dx.doi.org/10.3748/wjg.v21.i16.4817] [PMID: 25944995]

[135] Hsu SJ, Wang SS, Hsin IF, *et al.* Green tea polyphenol decreases the severity of portosystemic collaterals and mesenteric angiogenesis in rats with liver cirrhosis. Clin Sci (Lond) 2014; 126(9): 633-44.
[http://dx.doi.org/10.1042/CS20130215] [PMID: 24063570]

[136] Lee PC, Yang YY, Huang CS, *et al.* Concomitant inhibition of oxidative stress and angiogenesis by chronic hydrogen-rich saline and N-acetylcysteine treatments improves systemic, splanchnic and hepatic hemodynamics of cirrhotic rats. Hepatol Res 2015; 45(5): 578-88.
[http://dx.doi.org/10.1111/hepr.12379] [PMID: 24961937]

[137] Van Steenkiste C, Staelens S, Deleye S, *et al.* Measurement of porto-systemic shunting in mice by novel three-dimensional micro-single photon emission computed tomography imaging enabling longitudinal follow-up. Liver Int 2010; 30(8): 1211-20.
[http://dx.doi.org/10.1111/j.1478-3231.2010.02276.x] [PMID: 20497314]

[138] Dufour JF. Anti-angiogenic therapy for HCC. Minerva Gastroenterol Dietol 2012; 58(1): 81-6.
[PMID: 22419006]

[139] Llovet JM, Ricci S, Mazzaferro V, *et al.* Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359(4): 378-90.
[http://dx.doi.org/10.1056/NEJMoa0708857] [PMID: 18650514]

[140] Liu L, You Z, Yu H, *et al.* Mechanotransduction-modulated fibrotic microniches reveal the contribution of angiogenesis in liver fibrosis. Nat Mater 2017; 16(12): 1252-61.
[http://dx.doi.org/10.1038/nmat5024] [PMID: 29170554]

[141] Qu K, Huang Z, Lin T, *et al.* New Insight into the Anti-liver Fibrosis Effect of Multitargeted Tyrosine Kinase Inhibitors: From Molecular Target to Clinical Trials. Front Pharmacol 2016; 6: 300.
[http://dx.doi.org/10.3389/fphar.2015.00300] [PMID: 26834633]

[142] Garbuzenko DV, Arefyev NO, Kazachkov EL. Antiangiogenic therapy for portal hypertension in liver cirrhosis: Current progress and perspectives. World J Gastroenterol 2019; 24(33): 3738-48.
[http://dx.doi.org/10.3748/wjg.v24.i33.3738] [PMID: 30197479]

[143] Wang Y, Gao J, Zhang D, Zhang J, Ma J, Jiang H. New insights into the antifibrotic effects of sorafenib on hepatic stellate cells and liver fibrosis. J Hepatol 2010; 53(1): 132-44.
[http://dx.doi.org/10.1016/j.jhep.2010.02.027] [PMID: 20447716]

[144] Mejias M, Garcia-Pras E, Tiani C, Miquel R, Bosch J, Fernandez M. Beneficial effects of sorafenib on splanchnic, intrahepatic, and portocollateral circulations in portal hypertensive and cirrhotic rats. Hepatology 2009; 49(4): 1245-56.
[http://dx.doi.org/10.1002/hep.22758] [PMID: 19137587]

[145] Tugues S, Fernandez-Varo G, Muñoz-Luque J, *et al.* Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and portal pressure in cirrhotic rats. Hepatology 2007; 46(6): 1919-26.
[http://dx.doi.org/10.1002/hep.21921] [PMID: 17935226]

[146] Majumder S, Piguet AC, Dufour JF, Chatterjee S. Study of the cellular mechanism of Sunitinib mediated inactivation of activated hepatic stellate cells and its implications in angiogenesis. Eur J Pharmacol 2013; 705(1-3): 86-95.
[http://dx.doi.org/10.1016/j.ejphar.2013.02.026] [PMID: 23454556]

[147] Yang YY, Liu RS, Lee PC, *et al.* Anti-VEGFR agents ameliorate hepatic venous dysregulation/microcirculatory dysfunction, splanchnic venous pooling and ascites of NASH-cirrhotic rat. Liver Int 2014; 34(4): 521-34.
[http://dx.doi.org/10.1111/liv.12299] [PMID: 23998651]

[148] Bieker JJ. Krüppel-like factors: three fingers in many pies. J Biol Chem 2001; 276(37): 34355-8.
[http://dx.doi.org/10.1074/jbc.R100043200] [PMID: 11443140]

[149] Taniguchi H, Jacinto FV, Villanueva A, *et al.* Silencing of Kruppel-like factor 2 by the histone methyltransferase EZH2 in human cancer. Oncogene 2012; 31(15): 1988-94.
[http://dx.doi.org/10.1038/onc.2011.387] [PMID: 21892211]

[150] Kawanami D, Mahabeleshwar GH, Lin Z, *et al.* Kruppel-like factor 2 inhibits hypoxia-inducible factor 1alpha expression and function in the endothelium. J Biol Chem 2009; 284(31): 20522-30.
[http://dx.doi.org/10.1074/jbc.M109.025346] [PMID: 19491109]

[151] Doddaballapur A, Michalik KM, Manavski Y, *et al.* Laminar shear stress inhibits endothelial cell metabolism *via* KLF2-mediated repression of PFKFB3. Arterioscler Thromb Vasc Biol 2015; 35(1): 137-45.
[http://dx.doi.org/10.1161/ATVBAHA.114.304277] [PMID: 25359860]

[152] Gracia-Sancho J, Russo L, García-Calderó H, García-Pagán JC, García-Cardeña G, Bosch J. Endothelial expression of transcription factor Kruppel-like factor 2 and its vasoprotective target genes in the normal and cirrhotic rat liver. Gut 2011; 60(4): 517-24.
[http://dx.doi.org/10.1136/gut.2010.220913] [PMID: 21112949]

[153] Das A, Shergill U, Thakur L, *et al.* Ephrin B2/EphB4 pathway in hepatic stellate cells stimulates Erk-dependent VEGF production and sinusoidal endothelial cell recruitment. Am J Physiol Gastrointest Liver Physiol 2010; 298(6): G908-15.
[http://dx.doi.org/10.1152/ajpgi.00510.2009] [PMID: 20338920]

[154] Zeng XQ, Li N, Pan DY, *et al.* Kruppel-like factor 2 inhibit the angiogenesis of cultured human liver sinusoidal endothelial cells through the ERK1/2 signaling pathway. Biochem Biophys Res Commun 2015; 464(4): 1241-7.
[http://dx.doi.org/10.1016/j.bbrc.2015.07.113] [PMID: 26212440]

[155] Miao Q, Zeng X, Ma G, *et al.* Simvastatin suppresses the proangiogenic microenvironment of human hepatic stellate cells *via* the Kruppel-like factor 2 pathway. Rev Esp Enferm Dig 2015; 107(2): 63-71.
[PMID: 25659387]

[156] Marrone G, Russo L, Rosado E, *et al.* The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. J Hepatol 2013; 58(1): 98-103.
[http://dx.doi.org/10.1016/j.jhep.2012.08.026] [PMID: 22989565]

[157] Garbuzenko DV, Mikurov AA, Smirnov DM. [Bacterial endotoxinemia and risk of hemorrhage from oesophageal varicose veins in patients with liver cirrhosis]. Klin Med (Mosk) 2012; 90(7): 48-51.
[PMID: 23019976]

[158] Zhu Q, Zou L, Jagavelu K, *et al.* Intestinal decontamination inhibits TLR4 dependent fibronectin-mediated cross-talk between stellate cells and endothelial cells in liver fibrosis in mice. J Hepatol

2012; 56(4): 893-9.
[http://dx.doi.org/10.1016/j.jhep.2011.11.013] [PMID: 22173161]

[159] DuPont HL. Biologic properties and clinical uses of rifaximin. Expert Opin Pharmacother 2011; 12(2): 293-302.
[http://dx.doi.org/10.1517/14656566.2011.546347] [PMID: 21226639]

[160] Liu Y, Salvador LA, Byeon S, *et al.* Anticolon cancer activity of largazole, a marine-derived tunable histone deacetylase inhibitor. J Pharmacol Exp Ther 2010; 335(2): 351-61.
[http://dx.doi.org/10.1124/jpet.110.172387] [PMID: 20739454]

[161] Liu Y, Wang Z, Wang J, *et al.* A histone deacetylase inhibitor, largazole, decreases liver fibrosis and angiogenesis by inhibiting transforming growth factor-β and vascular endothelial growth factor signalling. Liver Int 2013; 33(4): 504-15.
[http://dx.doi.org/10.1111/liv.12034] [PMID: 23279742]

[162] Fontaine H, Vallet-Pichard A, Equi-Andrade C, *et al.* Histopathologic efficacy of ribavirin monotherapy in kidney allograft recipients with chronic hepatitis C. Transplantation 2004; 78(6): 853-7.
[http://dx.doi.org/10.1097/01.TP.0000128911.87538.AA] [PMID: 15385804]

[163] Michaelis M, Michaelis R, Suhan T, *et al.* Ribavirin inhibits angiogenesis by tetrahydrobiopterin depletion. FASEB J 2007; 21(1): 81-7.
[http://dx.doi.org/10.1096/fj.06-6779com] [PMID: 17135367]

[164] Fernandez M, Mejias M, Garcia-Pras E, Mendez R, Garcia-Pagan JC, Bosch J. Reversal of portal hypertension and hyperdynamic splanchnic circulation by combined vascular endothelial growth factor and platelet-derived growth factor blockade in rats. Hepatology 2007; 46(4): 1208-17.
[http://dx.doi.org/10.1002/hep.21785] [PMID: 17654489]

[165] D'Amico M, Mejías M, García-Pras E, *et al.* Effects of the combined administration of propranolol plus sorafenib on portal hypertension in cirrhotic rats. Am J Physiol Gastrointest Liver Physiol 2012; 302(10): G1191-8.
[http://dx.doi.org/10.1152/ajpgi.00252.2011] [PMID: 22403792]

[166] Garbuzenko DV. Current approaches to the management of patients with liver cirrhosis who have acute esophageal variceal bleeding. Curr Med Res Opin 2016; 32(3): 467-75.
[http://dx.doi.org/10.1185/03007995.2015.1124846] [PMID: 26804426]

[167] Woltering EA. Development of targeted somatostatin-based antiangiogenic therapy: a review and future perspectives. Cancer Biother Radiopharm 2003; 18(4): 601-9.
[http://dx.doi.org/10.1089/108497803322287691] [PMID: 14503956]

[168] Mejias M, Garcia-Pras E, Tiani C, Bosch J, Fernandez M. The somatostatin analogue octreotide inhibits angiogenesis in the earliest, but not in advanced, stages of portal hypertension in rats. J Cell Mol Med 2008; 12(5A): 1690-9.
[http://dx.doi.org/10.1111/j.1582-4934.2008.00218.x] [PMID: 18194463]

[169] Garbuzenko DV. The principles of management of patients with liver cirrhosis complicated by ascites. Klin Med (Mosk) 2017; 95(9): 789-6.

[170] Michel F, Ambroisine ML, Duriez M, Delcayre C, Levy BI, Silvestre JS. Aldosterone enhances ischemia-induced neovascularization through angiotensin II-dependent pathway. Circulation 2004; 109(16): 1933-7.
[http://dx.doi.org/10.1161/01.CIR.0000127112.36796.9B] [PMID: 15078792]

[171] Wilkinson-Berka JL, Tan G, Jaworski K, Miller AG. Identification of a retinal aldosterone system and the protective effects of mineralocorticoid receptor antagonism on retinal vascular pathology. Circ Res 2009; 104(1): 124-33.
[http://dx.doi.org/10.1161/CIRCRESAHA.108.176008] [PMID: 19038868]

[172] Gravez B, Tarjus A, Pelloux V, *et al.* Aldosterone promotes cardiac endothelial cell proliferation *in*

vivo. J Am Heart Assoc 2015; 4(1): e001266.
[http://dx.doi.org/10.1161/JAHA.114.001266] [PMID: 25564371]

[173] Miternique-Grosse A, Griffon C, Siegel L, Neuville A, Weltin D, Stephan D. Antiangiogenic effects of spironolactone and other potassium-sparing diuretics in human umbilical vein endothelial cells and in fibrin gel chambers implanted in rats. J Hypertens 2006; 24(11): 2207-13.
[http://dx.doi.org/10.1097/01.hjh.0000249698.26983.4e] [PMID: 17053542]

[174] Hsu SJ, Wang SS, Huo TI, *et al.* The Impact of Spironolactone on the Severity of Portal-Systemic Collaterals and Hepatic Encephalopathy in Cirrhotic Rats. J Pharmacol Exp Ther 2015; 355(1): 117-24.
[http://dx.doi.org/10.1124/jpet.115.225516] [PMID: 26260462]

[175] Chikina SYu. Antioxidant effects of N-acetylcysteine in modern clinical practice. Jeffektivnaja farmakoterapija 2011; 32: 19-24.

[176] Garbuzenko DV. Pathophysiological mechanisms and new directions of therapy of portal hypertension at liver cirrhosis. Klin persp gastrojenterol gepatol 2010; 6: 11-20.

[177] Salani D, Taraboletti G, Rosanò L, *et al.* Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization *in vivo.* Am J Pathol 2000; 157(5): 1703-11.
[http://dx.doi.org/10.1016/S0002-9440(10)64807-9] [PMID: 11073829]

[178] Hsu SJ, Lin TY, Wang SS, *et al.* Endothelin receptor blockers reduce shunting and angiogenesis in cirrhotic rats. Eur J Clin Invest 2016; 46(6): 572-80.
[http://dx.doi.org/10.1111/eci.12636] [PMID: 27091078]

[179] Gerasimenko ND, Degtyar NI, Racin MS. Systemic inflammation and aging: the role of nuclear transcription factors and therapeutic possibilities (Review of the literature). Probl starenija i dolgoletija 2016; 25: 554-61.

[180] Dana N, Javanmard SH, Rafiee L. Role of peroxisome proliferator-activated receptor alpha and gamma in antiangiogenic effect of pomegranate peel extract. Iran J Basic Med Sci 2016; 19(1): 106-10.
[PMID: 27096071]

[181] Schwabl P, Payer BA, Grahovac J, *et al.* Pioglitazone decreases portosystemic shunting by modulating inflammation and angiogenesis in cirrhotic and non-cirrhotic portal hypertensive rats. J Hepatol 2014; 60(6): 1135-42.
[http://dx.doi.org/10.1016/j.jhep.2014.01.025] [PMID: 24530596]

[182] Enomoto N, Takei Y, Hirose M, *et al.* Thalidomide prevents alcoholic liver injury in rats through suppression of Kupffer cell sensitization and TNF-α production. Gastroenterology 2002; 123(1): 291-300.
[http://dx.doi.org/10.1053/gast.2002.34161] [PMID: 12105857]

[183] Li TH, Huang CC, Yang YY, *et al.* Thalidomide Improves the Intestinal Mucosal Injury and Suppresses Mesenteric Angiogenesis and Vasodilatation by Down-Regulating Inflammasomes-Related Cascades in Cirrhotic Rats. PLoS One 2016; 11(1): e0147212.
[http://dx.doi.org/10.1371/journal.pone.0147212] [PMID: 26820153]

[184] Hsin IF, Lee JY, Huo TI, *et al.* 2′-Hydroxyflavanone ameliorates mesenteric angiogenesis and portal-systemic collaterals in rats with liver fibrosis. J Gastroenterol Hepatol 2016; 31(5): 1045-51.
[http://dx.doi.org/10.1111/jgh.13197] [PMID: 26474184]

[185] Coriat R, Gouya H, Mir O, *et al.* Reversible decrease of portal venous flow in cirrhotic patients: a positive side effect of sorafenib. PLoS One 2011; 6(2): e16978.
[http://dx.doi.org/10.1371/journal.pone.0016978] [PMID: 21340026]

[186] Pinter M, Sieghart W, Reiberger T, Rohr-Udilova N, Ferlitsch A, Peck-Radosavljevic M. The effects of sorafenib on the portal hypertensive syndrome in patients with liver cirrhosis and hepatocellular carcinoma--a pilot study. Aliment Pharmacol Ther 2012; 35(1): 83-91.

[http://dx.doi.org/10.1111/j.1365-2036.2011.04896.x] [PMID: 22032637]

[187] Garcia-Tsao G, Fallon M, Reddy K, *et al.* Placebo-controlled, randomized, pilot study of the effect of sorafenib on portal pressure in patients with cirrhosis, portal hypertension and ablated hepatocellular carcinoma (HCC). Hepatology 2015; 62 (Suppl. S1): 580A.

[188] D'Amico G. Stages classification of cirrhosis: Where do we stand? In: de Franchis R, Ed. Portal Hypertension V Proceedings of the Fifth Baveno International Consensus Workshop. 132-9.

[189] Vorobioff JD, Groszmann RJ. Hepatic venous pressure gradient measurement in pre-primary and primary prophylaxis of variceal hemorrhage. Ann Hepatol 2013; 12(1): 22-9.
[PMID: 23293190]

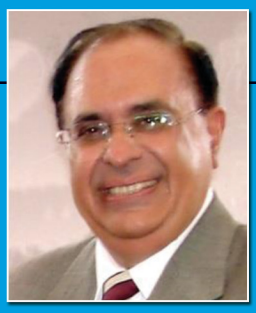
[190] Villanueva C, Albillos A, Genescà J, *et al.* Development of hyperdynamic circulation and response to β-blockers in compensated cirrhosis with portal hypertension. Hepatology 2016; 63(1): 197-206.
[http://dx.doi.org/10.1002/hep.28264] [PMID: 26422126]

[191] Vorobioff JD, Groszmann RJ. Prevention of portal hypertension: from variceal development to clinical decompensation. Hepatology 2015; 61(1): 375-81.
[http://dx.doi.org/10.1002/hep.27249] [PMID: 24913395]

[192] Jaurigue MM, Cappell MS. Therapy for alcoholic liver disease. World J Gastroenterol 2014; 20(9): 2143-58.
[http://dx.doi.org/10.3748/wjg.v20.i9.2143] [PMID: 24605013]

[193] Chung GE, Lee JH, Kim YJ. Does antiviral therapy reduce complications of cirrhosis? World J Gastroenterol 2014; 20(23): 7306-11.
[http://dx.doi.org/10.3748/wjg.v20.i23.7306] [PMID: 24966601]

[194] Libânio D, Marinho RT. Impact of hepatitis C oral therapy in portal hypertension. World J Gastroenterol 2017; 23(26): 4669-74.
[http://dx.doi.org/10.3748/wjg.v23.i26.4669] [PMID: 28765688]



PROF. DR. ATTA-UR-RAHMAN, *FRS*

Atta-ur-Rahman, Ph.D. in organic chemistry from Cambridge University (1968), has 1080 international publications in several fields of organic chemistry including 751 research publications, 37 international patents, 69 chapters in books and 221 books published largely by major U.S. and European presses. He is the Editor-in-Chief of eight European Chemistry journals. He is Editor of the world's leading encyclopedic series of volumes on natural products "Studies in Natural Product Chemistry" 54 volumes of which have been published under his Editorship by Elsevier during the last two decades.

Prof. Rahman won the UNESCO Science Prize (1999) and was elected as Fellow of the prestigious Royal Society (London) in July 2006. He has been conferred honorary doctorate degrees by many universities including (Sc.D.) by the Cambridge University (UK) (1987). He was elected Honorary Life Fellow of Kings College, Cambridge University, UK, conferred the TWAS (Italy) Prize and the Austrian government has honoured him with its high civil award ("Grosse Goldene Ehrenzeischen am Bande") (2007). He is Foreign Fellow of Chinese and Korean Academy of Sciences, Foreign Fellow of the Chinese Chemical Society and former President of Pakistan Academy of Sciences.

PROF. DR. M. IQBAL CHOUDHARY

Dr. M. Iqbal Choudhary is a Professor of Organic/Bioorganic Chemistry and Director at the International Center for Chemical and Biological Sciences (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research). He is among the most prominent scientists of Pakistan, recognized for his original contributions in the fields of natural products and bioorganic chemistry. He has written and edited 27 books, most of which have been published in USA and Europe. He is also the author of over 900 research papers and chapters in top international science journals of the West as well as 27 US patents. He is the Volume Editor of many international book series and journals. He has served as a visiting faculty in many prestigious universities of the world including Cornell University (New York), Purdue University (Indiana), Pennsylvania State University (Pennsylvania), Scripps Institution of Oceanography (San Diego, California), The University of Rhode Island (Rhode Island), and other top Universities.