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Review Article

Alteration of intrahepatic microcirculation in cirrhotic livers

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Abstract

From a hemodynamic point of view, hepatic vascular resistance and portal inflow determine the level of portal pressure. Factors that determine hepatic vascular resistance include both structural and dynamic components. Among the structural components are histological characteristics such as steatosis, fibrosis, regeneration nodules, and neo-angiogenesis. Dynamic structures include cells with contractile properties such as hepatocytes, hepatic stellate cells, sinusoidal endothelial cells, and Kupffer cells. The contributions of the interactions between four cells in cirrhotic livers resulted in hepatic endothelial dysfunction, hepatic microcirculatory dysfunction, hepatic venous dysregulation, hepatic fibrogenesis, and subsequently increased intrahepatic resistance and portal hypertension in cirrhosis. The pathogenic mechanisms that trigger the associated abnormalities in hepatic microcirculations include persistent endotoxemia, increased hepatic oxidative stress, activated endocannabinoids substances, pathogenic sinusoidal remodeling, and hypoperfusion in cirrhotic livers. Cumulative data suggested that various therapeutic strategies targeting hepatic microcirculation provided effective improvement of the systemic abnormalities of cirrhosis. Accordingly, the mechanistic and therapeutic approaches focusing on the disarrangement of hepatic microcirculation will be introduced in this article.

Keywords: cirrhosis; endotoxemia; fibrosis; hepatic microcirculation

1. Mechanical and dynamic components of increased intrahepatic resistance in cirrhotic livers

Increased intrahepatic resistance (IHR) is an initial event in the development of portal hypertension (PH) in cirrhosis.[1–4] Portal hypertension has traditionally been viewed as a progressive process, involving ultrastructural changes including fibrosis, nodule formation, and vascular thrombosis, leading to an increased intrahepatic resistance to flow.[1–4] Recent studies have demonstrated that there are contractile elements in cirrhotic livers that are able to constrict, in a reversible and graded manner, in response to vasoactive substances (Figure 1).[5–8] Chronic liver injury can activate Kupffer cells (KCs), sinusoidal endothelial cells (SECs), and hepatic stellate cells (HSCs) to modulate hepatic blood flow, IHR, and hepatic fibrogenesis.[5,7,9,10] In particular, HSCs contract and proliferate to regulate the microvascular tone in response to vasoconstrictors or vasodilators and increase extracellular matrix production in cirrhotic livers.[9,10]

It has been increasingly recognized that the dynamic component of cirrhotic livers was regulated by complex interactions between the injured hepatocytes, the SECs, the KCs, and the HSCs, which impact sinusoidal caliber. A recent study suggested that systemic inflammation and portal hypertension are linked by endothelial dysfunction and innate immune interactions within the sinusoidal niche of the injured liver.[11] Moreover, recent findings suggest these hemodynamic findings are most marked in patients with superimposed inflammation.

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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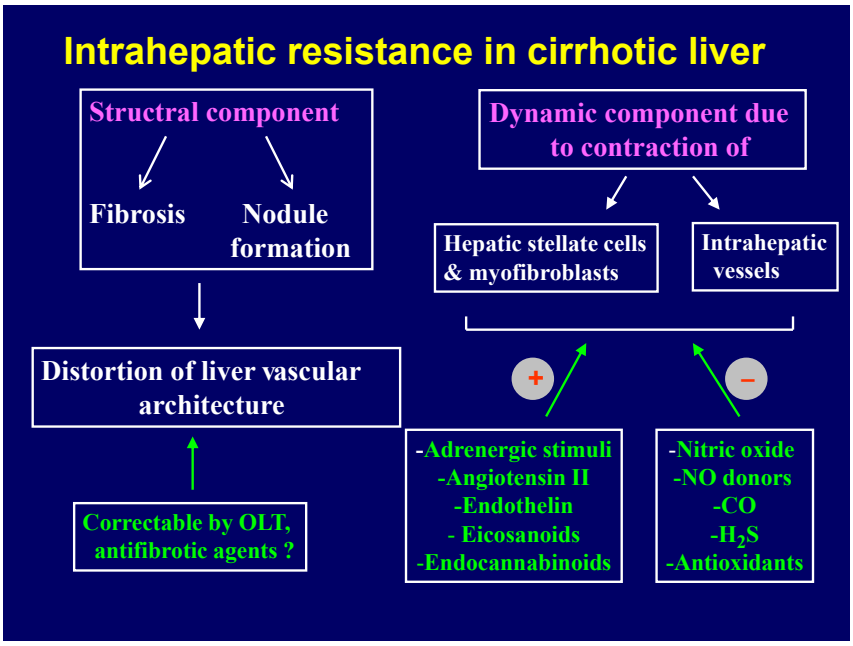

Figure 1. The illustration of the mechanistic components of increased intrahepatic resistance (IHR) in cirrhotic livers.

2. Abnormal neo-angiogenesis in cirrhotic livers

In cirrhotic livers, the microcomputed tomography-based evaluation of hepatic microvasculatures revealed the increased abnormal neo-angiogenesis which is characterized by distorted vessels of varying diameter that impair sinusoidal perfusion and promote hepatic fibrogenesis.[10–15] In fact, activated HSCs secrete angiopoietin-1 to promote interactions between HSCs and SECs and lead to fibrosis-associated angiogenesis and sinusoidal remodeling in cirrhotic livers.[15–20] Meanwhile, activation of KCs is accompanied by increased inflammatory cytokine and chemokine release which further stimulates HSC/SEC and angiogenesis.[21] Inflammatory mediators accelerate angiogenesis to maintain the chronic inflammatory state in the tissue by transporting leukocytes and supplying nutrients/oxygen. Subsequently, angiogenesis-related increased endothelial surface area creates an enormous capacity for inflammatory cytokines and adhesion molecules productions. Actually, all fibrogenic and inflammatory markers measured in our study were also classified as angiogenic factors due to their ability to stimulate the vascular endothelial growth factor (VEGF) production from vascular endothelial cells and inflammatory cells.[22–25]

Angiogenesis is regulated by the net balance of proangiogenic factors and angiogenic inhibitors. To date, many positive and negative angiogenic-modulating factors have been identified. Among these, VEGF and fibroblast growth factor (FGF) are the most potent factors in neo-angiogenesis.[25,26] VEGF is not only an angiogenic factor, it is also known as a survival factor for SECs. VEGF and FGF expression increases stepwise during liver fibrosis development and suppression of the VEGF/FGF signaling cascade attenuates these pathologic sequences.[25,26] It has become increasingly evident that VEGF and FGF expression are a common response to liver injury.[25,26] Increased serum and hepatic VEGF and FGF levels had been reported in cirrhosis.[27,28] During hepatic fibrogenesis, FGF-2 mediates the mitogenic effects of transforming growth factor-beta1 (TGFβ_1) for HSCs.[18,28] Impaired sinusoidal perfusion, intrahepatic shunt, and capillarization of the sinusoids, leading to hypoxia stress, VEGF expression, and angiogenesis in fibrotic livers.[29,30] Further studies suggest that FGF-2 and TGFβ1 synergistic with hypoxia stress to stimulate VEGF expression.[25,29,30–32]

In experimental models of PH, coordination between VEGF and FGF-2 initiate and maintain hyperdynamic systemic and splanchnic circulations.[25,29,30,32] Accordingly, chronic anti-VEGF and FGF targeting therapy attenuates hepatic fibrogenesis, improved hepatic blood flow and associated portal hypertensive syndrome in cirrhotic rats.[10,12–14,33,34]

3. Hepatic venous dysregulation in cirrhosis

Under normal conditions, the hepatic vein is well able to accommodate acute increased splanchnic blood volume, such as variceal bleeding and food intake, and still keep the portal venous pressure (PVP) at normal levels.[8,35–37] However, an immediately abnormal increase in PVP induced by acute increased splanchnic blood volume (hepatic venous dysregulation) results from improper dilatation of poor-compliance intrahepatic microvasculatures (abnormal neo-angiogenic hepatic microvessels) in NASH-cirrhotic PH rats.[10] So, repeated postprandial-increased PVP may aggravate progressive dilation of portal-systemic collateral and varices that eventually lead to variceal bleeding in cirrhosis.[38] It has been shown that blood infusion after controlled hemorrhage and hemodynamic stabilization in cirrhotic rats but not in normal rats causes a

PVP increase beyond the baseline value while the arterial pressure returns to normal.[37]

Exaggerated splanchnic vasodilatation/hyperemia and increased portal blood flow and PVP (hepatic venous dysregulation) are mainly mediated by nitric oxide (NO) overproduction in cirrhosis.[12,37,39] NO donors have been reported to improve postprandial hyperemia.[39] In cirrhotic PH patients with varices, attenuation of NO-mediated postprandial hyperemia, increased portal blood flow, and PVP should also depend on the improvement in the compliance of intrahepatic microvasculatures. Remarkably, anti-VEGFR agents improve hepatic venous dysregulation through the suppression of abnormal hepatic neo-angiogenesis and VEGF-eNOS-NO cascades. These anti-VEGFR-related effects further prevent the elevation of PVP secondary to transfusion and food intake-increased portal blood flow in cirrhosis.[10] Clinically, the above beneficial effects of anti-VEGFR agents might reduce the risk of rebleeding in cirrhotic patients after hemodynamic stabilization by controlling variceal bleeding.

4. Cirrhotic livers are characterized by vasodilator hyporesponsiveness and vasoconstrictor hyperresponsiveness

Under normal physiological conditions, vascular tone is maintained by the balance between the activity of vasodilators and vasoconstrictors.[5–8,40–43] Previous studies had demonstrated that increased IHR in cirrhosis is caused by the decrease in endothelium-dependent vasodilators (such as NO) and the increase in endothelium-dependent vasoconstrictors [such as endothelin-1 (ET-1), angiotensin II (Ang II), thromboxanes, and endocannabinoids] in liver.[5–8,40–43]

4.1. Hepatic endothelial dysfunction-vasodilators (NO) hyporesponsiveness

Oxidative stress is defined as a loss of the physiologic equilibrium due to increased ROS production and/or decreased antioxidants.[44,45] Indeed, increased oxidative stress had been documented during cirrhosis, fully developed cirrhosis, and following the development of cirrhosis.[44–46] NO is a critical vasodilator which modulates IHR.[39–41,43] An impaired endothelium-dependent response to vasodilators, which is called hepatic endothelial dysfunction (HED), due to a reduction in NO bioavailability has been noted in cirrhotic livers.[39–41,43] So, any therapeutic approach improving intrahepatic NO bioavailability is clinically relevant and thus sufficiently important for the treatment of PH.[45,47] Abraldes et al[47] had reported that administration of simvastatin can decrease IHR through increasing hepatic NO in cirrhotic rats. Furthermore, it has been reported that increased oxidative stress is involved in the depletion of NO and the development of HED in cirrhotic livers.[39–41,43]

Cumulative studies had suggested that chronic administration of antioxidants N-acetylcysteine, ursodeoxycholic acid, and vitamin E can suppress hepatic oxidative stress, therefore increases NO bioavailability to improve HED and decrease IHR in cirrhotic rats.[39–41,43] Recent studies also revealed that chronic administration of liver specific NO donor-NCX-100 and antioxidants markedly decrease hepatic collagen deposition, IHR and portal pressure in cirrhotic animals.[39–41,43,48,49]

5. HED-vasoconstrictors hyper-responsiveness

(1) ET-1

ET-1 is a potent vasoconstrictor involved in the regulation of hepatic microcirculation and in the development of PH.[7,50] Remarkably, hepatic ET-1 is overexpressed in human and rat cirrhotic livers to induce HSCs contraction and increased IHR.[7,50,51] In PH rat livers, pre-incubation with an endothelin receptor type A (ET_AR) antagonist markedly abolished the ET-1-induced increased IHR.[50,51]

(2) Angiotensin II

As IHR increases, the increased portal resistance results in dilatation of the mesenteric and systemic vasculatures and decreases the effective blood volume. A compensatory change that restores the effective blood volume is the activation of the renin-angiotensin-aldosterone system (RAS).[8,52–55] The RAS plays an important role in the regulation of local hemodynamics in cirrhotic livers.[52] The primary effector peptide of the system is angiotensin II (Ang II).[47,52] Administration of Ang II receptor blockers decreased the IHR of cirrhotic rat livers.[3,8,52] Renin, which cleaves liver-produced angiotensinogen to Ang I, is the first rate-limiting enzyme in the synthesis of Ang II. Inhibition of renin activity has been a potential way to downregulate the RAS. A recent study reported that acute administration of aliskiren, a direct renin inhibitor, reduced IHR and portal pressure by amelioration of the Ang II-induced intrahepatic vasoconstriction in cirrhotic rat livers.[8]

(3) Thromboxanes A_2

Our recent study using the single-photon emission computed tomography/positron emission tomography/computed tomography (SPECT/PET/CT)-based evaluation revealed the increased hepatic Kupffer cell activity and mass in NASH-cirrhotic rats.[10,12] However, cumulative studies also displayed that increased local production of thromboxanes A_2 is also the main factor for the increased IHR in cirrhosis.[5] In cirrhosis, activated Kupffer cells can produce a large amount of hepatic thromboxanes A_2 in livers.[52] Moreover, a positive feedback regulation loop has been noted between increased oxidative stress and activated Kupffer cells, which further stimulate the release of reactive oxygen species (ROS) and arachidonic acids (Figure 2). Meanwhile, increased ROS can stimulate Kupffer cells-derived arachidonic acid release.[55,56] In hepatic microcirculation, the Kupffer cell-derived thromboxanes A_2-mediated methoxamine hyper-responsiveness, increased IHR, and marked portal hypertension in NASH-cirrhotic rats.

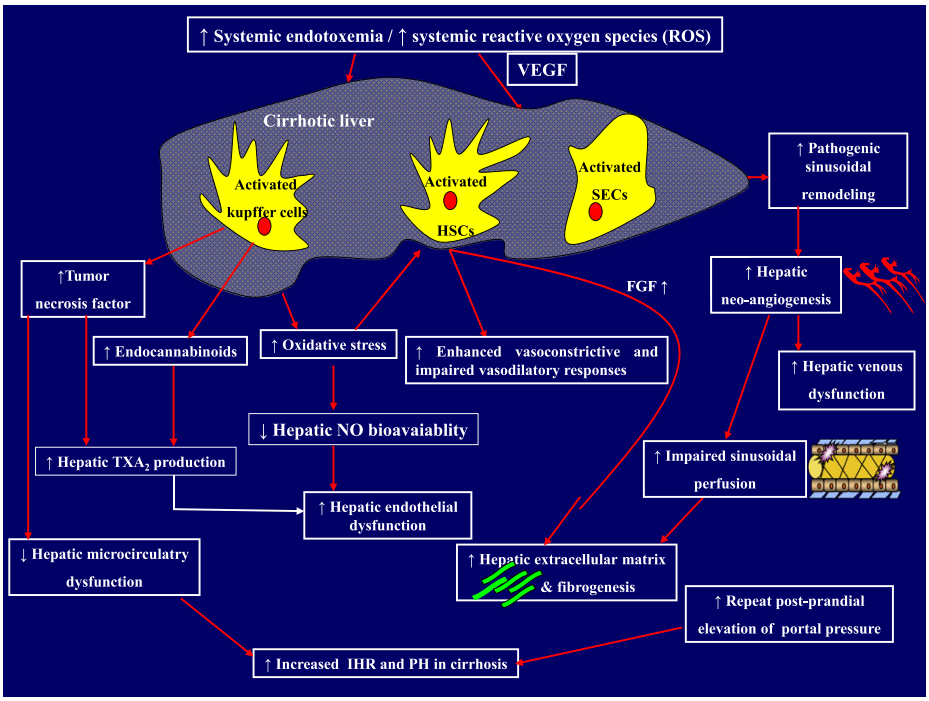

Figure 2. A summary of the systemic endotoxemia and increased oxidative stress-related pathogenic mechanisms for the increased intrahepatic resistance (IHR) and portal hypertension (PH) in cirrhosis. FGF = fibroblast growth factor; HSCs = hepatic stellate cells; NO = nitric oxide; SECs = sinusoidal endothelial cells; TXA_2 = thromboxane A_2; VEGF = vascular endothelial growth factor.

(4) Endocannabinoids

Cannabinoids are members of the fatty acid amide that bind with cannabinoid receptors to regulate many physiological and pathological functions in the body.[6,7,57–59] Endocannabinoid was present in the peripheral and central nervous system to modulate emotion, memory, pain, muscle tone, and cardiovascular function in experimental animals and humans.[6,7,57–59] It has been reported that endocannabinoids-cannabinoid receptors binding induce the vasorelaxation responses in cirrhotic splanchnic vessels.[60,61] Additionally, the significant enhancement of anandamide, being the most extensively investigated endocannabinoids, where vasoconstrictive response has been reported in cirrhotic livers.[6] Accordingly, endocannabinoids have become the potential targets for the treatment of portal hypertensive syndrome including increased IHR and associated complications in cirrhosis.[6,7,9,59–63]

6. Interaction between endotoxemia and endocannabinoids in cirrhosis-hepatic microcirculatory dysfunction

Microcirculatory dysfunction in cirrhotic livers is characterized by an increased adherence of leukocytes to the sinusoidal lining (sticky leukocytes) and impaired sinusoidal perfusion in the chronic endotoxemia state.[5,7]

Patients with cirrhosis usually have persistent endotoxemia due to impaired Kupffer cell function. Subsequently, large amounts of gut-derived bacteria and endotoxin cannot be effectively removed in cirrhotic animals.[64,65] The increased endotoxemia will promote hepatic fibrogenesis and elevate portal venous pressure in cirrhosis. Our previous study showed that intestinal decontamination with oral ciprofloxacin is effective in the prevention of rebleeding in patients with cirrhosis who were suffering from acute gastrointestinal hemorrhage.[66] It has been reported that chronic administration of thalidomide, through inhibition of endotoxin-related acceleration of hepatic fibrogenesis, significantly decreased portal pressure and IHR in cirrhotic animals (Figure 3).[67]

It is well-established that endotoxemia will stimulate macrophage and platelet activity associated with the release of inflammatory cytokines, tumor necrosis factor (TNF)α, and thromboxane A_2 in cirrhosis.[59,68–70] Further studies have shown that endotoxin-induced TNFα production is the key factor to induce liver injury and promote hepatic fibrogenesis.[68,70] Additionally, thromboxane A_2 has already proved to be a potent vasoconstrictor that is involved in the mechanism of increased IHR in cirrhosis.[6,54] Anandamide is an endocannabinoid that demonstrates increasing IHR through the release of thromboxanes A_2 in cirrhotic livers.[6] Our study also revealed that chronic inhibition of endogenous TNFα by thalidomide will attenuate the anandamide-mediated vasoconstriction in cirrhotic livers.[68,69]

7. Interaction between endotoxemia and endocannabinoids in cirrhosis-HED

Endotoxemia-related activated endocannabinoid systems induce HED and subsequently increases IHR in

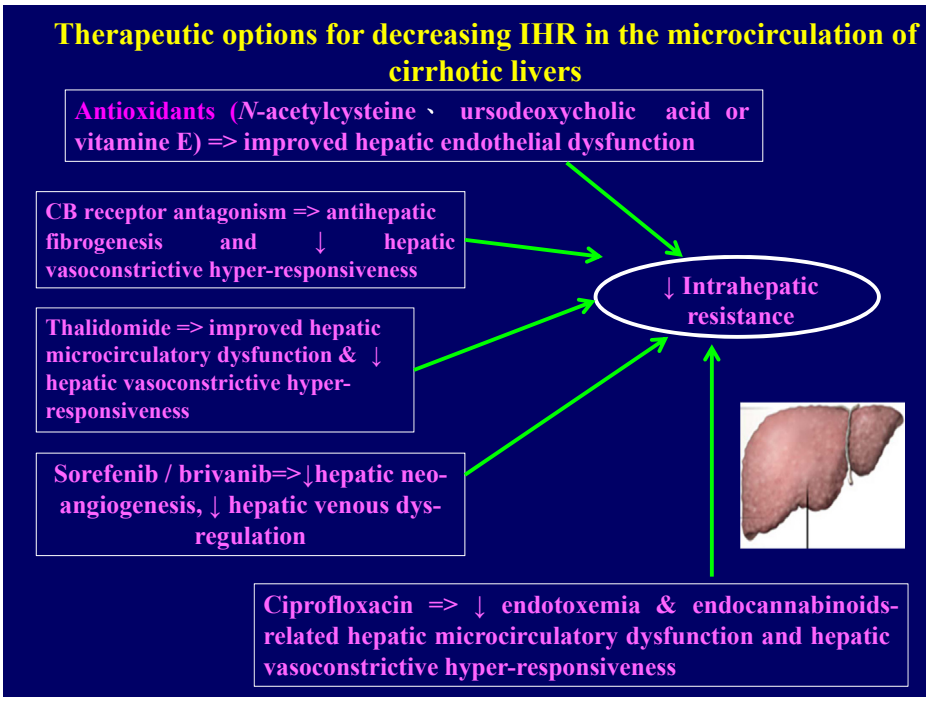

Figure 3. Therapeutic options for decreasing intrahepatic resistance (IHR) in the microcirculation of cirrhotic livers. A summary of the potential agents used to decrease intrahepatic resistance (IHR) in cirrhosis.

cirrhosis.[11,58,70] HED is characterized by an increased pressure response to vasoconstrictors including endocannabinoids in hepatic microvasculatures.[40,41,48,49] Chronic administration of ciprofloxacin had also been found to inhibit endotoxemia, improve HED, and decrease IHR in cirrhotic animals.[70] Ciprofloxacin has the advantage over the majority of fluoroquinolones because it is well-tolerated with low hepatotoxicity, which had been used to effectively prevent bacterial infection in patients with cirrhosis after upper gastrointestinal bleeding.[70,71] The same study also observed an inhibition of the activated hepatic endocannabinoid system in ciprofloxacin-treated cirrhotic animals, which enhanced the concepts of endotoxemia and activate hepatic endocannabinoid system in cirrhosis.[11,58,70] Actually, chronic administration of the cannabinoid receptor antagonist inhibits the hepatic inflammation and fibrogenesis in cirrhotic animals.[9,63] However, increased hepatic endocannabinoid production enhanced the hepatic vasoconstrictive response to ET-1 and subsequently increased IHR and portal hypertension in nonalcoholic steatohepatitis (NASH)-cirrhotic rats.[7] Accordingly, an activated hepatic endocannabinoid system is critical in the development of hepatic inflammation and fibrogenesis, and increased IHR in cirrhotic portal hypertension.

8. Interaction between bacterial translocation, endocannabinoid system, and hemodynamic abnormalities in cirrhosis

Enteric bacteria overgrowth can stimulate intestinally and systemically (mainly from MNLs) TNFα production that results in increased intestinal bacterial adherence, systemic bacteremia, and local infection [so called bacterial translocation (BT) and SBP] in cirrhosis.[72–74] The elevated circulating TNFα levels have been implicated in the BT-associated hemodynamic disturbances, including the splanchnic vasodilatation and hyperdynamic circulation associated with cirrhosis.[75,76] It has been reported that the increased TNFα levels, by binding to the TNF receptor, inhibit phagocytosis of macrophages in cirrhosis.[77] Intestinal hyperpermeability, impaired peritoneal macrophages (PMs) phagocytosis, and, BT resulting in increased systemic and local infection/inflammation such as spontaneous bacterial peritonitis (SBP), together with increased TNFα levels, are all implicated in the pathogenesis of cirrhosis-related complications.[72–76,78,79] Manipulation of cannabinoid receptors (CB_1R and CB_2R), which are expressed on the gut mucosa and PMs, have been reported to modulate intestinal inflammation and systemic inflammatory cytokines release.[80,81] Especially, CB_2R activation reduces oxidative stress and endotoxin-induced inflammatory cytokines release in various experimental models of inflammation and sepsis.[80] A recent study suggests that CB_2R agonist has the potential to treat BT and various relevant abnormalities through the inhibition of systemic/intestinal oxidative stress, inflammatory cytokines, and TNFα releases in cirrhosis.[82]

In conclusion, the regulation of IHR in cirrhosis depends on the balance between different factors. The reduction of IHR can effectively decrease the clinical complications of cirrhotic portal hypertension. The decrease in these clinical complications, such as variceal bleeding, will subsequently reduce the

mortalities in cirrhotic patients. Theoretically, effective treatments will prolong survival of cirrhotic patients by increasing their chances to receive liver transplantation during long waiting times. Thus, elucidation of the complicated mechanism in the modulation of hepatic microcirculation is crucial for the development of therapeutic agents in the future.

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