HEMORHEOLOGY AND MICROCIRCULATION IN HEALTHY AND DISEASED LIVER AND AFTER LIVER TRANSPLANTATION: A REVIEW

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ABSTRACT

A good knowledge of the liver microcirculation, spanning the arterioles, the venules and the hepatic sinusoids that maintain perfusion in continuous homeostasis in healthy liver, is essential. This vascular system also includes the portal vein and it is on this blood that the liver works as a filter, protecting the subject from toxic substances derived from food, drugs, and fluids present in blood. Another liver function is to increase the general circulation output within 3-4 minutes following an emergency (shock). About 350 ml of blood are present in special hepatic storage areas.

During liver diseases (e.g. cirrhosis) there is a strong decrease in the blood hemoglobin concentration, with anemia and impairment of the liver perfusion causing a high level of tissue hypoxia. Alterations in erythrocytes and leukocytes deformability and aggregability are also present, that contribute to the impairment of the liver microcirculation.

In liver transplantation the main risk is the reperfusion injury syndrome. In fact, a sudden gush of oxygenated blood with large amounts of xanthine, in the presence of xanthine oxidase, can stimulate reactive oxygen species (ROS) production.

More studies are needed to evaluate the liver hemorheology and microcirculation and improve the diagnosis and treatment of liver disease.

Key-words: Microcirculation, hemorheology, cirrhosis, liver transplantation, ischemic reperfusion injury.

LIVER MICROCIRCULATION

Introduction

The liver microcirculation includes the terminal portal venules and the terminal hepatic arterioles (afferent vessels), the sinusoids (capillaries) and the terminal hepatic venules (efferent vessels) [1-2]. Portal blood accounts for 70-75% of the blood flow through the liver, while 25% derives from the hepatic arterioles and is modulated by the liver metabolism. A decreased oxygenation of the liver will increase the arterial blood flow

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because the need for greater liver oxygenation induces an important local vasodilator effect[3-4]. The sinusoidal blood flow presents as a series of rays stemming from the periportal area (zone 1) and the intermediate zone (zone 2) and reaching the pericentral area (zone 3), where it drains into the terminal hepatic venules[1]. This circuit, named the "liver acinus", is formed by the portal vessel in the center and the terminal hepatic venules at the periphery[2-5].

Blood Flow through the liver

About 1100 ml of portal blood enters the liver per minute. This blood flows through the hepatic sinusoids, closely linked with the hepatic parenchymal cells, and into the central liver veins, and then finally into the inferior vena cava. In addition to the portal blood, a further 350 ml of blood per minute reaches the liver from the hepatic artery, so the total liver blood flow is nearly 1500 ml per minute, equal to about 29% of cardiac output. The hepatic artery blood guarantees the supply of nutrients to the connective tissue (loss of this blood flow can be lethal, inducing necrosis of the main liver structures), and the final outflow is to the hepatic sinusoids, where it combines with the portal blood. Stimulation of the sympathetic nervous system induces vasoconstriction in the hepatic storage areas, especially the large veins, less so in the sinusoids.

In cases of a circulation emergency with an intense sympathetic reaction, a large amount of hepatic blood is put out into the general circulation within 3 or 4 minutes. In a healthy male, this blood volume amounts to about 350 ml.[6]

Liver Sinusoids

The hepatic sinusoids are sheathed by two types of cells:"endothelial cells" and large Kupffer cells (reticulo-endothelial cells), that are able to engulf bacteria and other foreign materials present in blood[4-7]. The hepatic sinusoid walls have a similar endothelium to that of the common capillaries but are much more permeable (due to the presence of much wider pores), so the plasma proteins can pass by diffusion into the liver extravascular spaces almost as easily as fluids. Normally, the pressure in the sinusoids is about 6-8 mmHg, so the proteins can easily spread inside and out. The flow of solutes from the endothelial windows of the sinusoids to Disse's Space (a very small space between the endothelial cells and the liver cells, directly connected with the lymphatic system) is mediated by cytosolic calcium, that modulates the actin-myosin--calmodulin system[8-11].

Hepatic lobules

The functional liver unit is the hepatic lobule. Human liver has from 50 000–100 000 lobules. The hepatic lobule surrounds a central vein whose outflow is to the hepatic veins and finally the vena cava. It is formed by many hepatic cell strands radiating out from the central vein to the lobule periphery, like the rays of a wheel. Each strand is formed by a two-cell thickness and between two cells rows lay the biliary channels which flow into the terminal biliary ducts, in the septi between adjacent hepatic lobules. The portal venules also run in the septi, carrying blood from the portal vein. From these venules the blood goes to the hepatic sinusoids that are flattened and branched, running between the liver trabeculae hepatic strands. Thus, the liver cells are in continuous contact with the portal venous blood.

With the portal venules in the interlobular septi lie the hepatic arterioles: these arterioles supply arterial blood to septal tissues; some of these flow into the hepatic sinusoids, next to the interlobular septi. In the hepatic vein the blood pressure is normally 0 mmHg, while the portal vein blood pressure is about 9 mmHg. This means that the resistance to blood flow from the portal vein system to the systemic veins through the hepatic sinusoids is normally low.

However, several diseases can increase this resistance, especially liver cirrhosis[12-13]; in this condition the blood flow through the liver is seriously hampered by fibrotic constriction or total obstruction or consumption of the sinusoids.

LIVER MICROCIRCULATION REGULATION

The mechanisms underlying the sinusoidal hepatic blood flow are not yet completely known, but it is important to note that the adrenergic and cholinergic nerves completely control the portal veins and arterioles, modulating sinusoidal blood flow. Moreover, some peptides such as vasoactive intestinal neuropeptide (VIP) and neuropeptide Y (NPY) are present together with noradrenaline and acetylcholine in the nerve endings. In fact, the VIP and NPY, substance P and calcitonin have been identified adjacent to afferent vessels, portal veins and hepatic arterioles, indicating a role in modulating the neurocontrol of the hemodynamics system [14-16]. The Ito Cells in the perisinusoidal space also have an important role, since in the portal venules and hepatic terminal venules these cells collaborate in the control of the sinusoidal blood flow [17-18]. Endothelin I increases the Ito Cells contractility [14]. Another type of cell present here is the Kupffer cell[7] that can temporarily arrest the blood flow through sinusoids.

OXIDATIVE STRESS

Introduction

To fulfil their functions, the cells of aerobic organisms need energy; this energy originates from oxygen as a natural electron acceptor. In physiological conditions many cellular reactions involve oxygen, producing many highly reactive molecules deriving from oxygen itself.

These molecular species include the reactive oxygen species (ROS) or reactive oxygen metabolites (ROM) [19-20]. Anion Superoxide (O^-_2) and the hydroxylic radical (OH) are the most important oxygen-derived free radicals (ROS), but also hydrogen peroxide (H_2O_2) and single oxygen ($^1O_2$), although not chemically definable as free radicals, possess similar features to these latter.

The ROM are essential in the life of all cells, since they superintend all the redox reactions. Normally, the ROM production is completely under metabolic control, and in fact many blocking systems exist, defined as "the antioxidant biological systems", localized inside and outside the cells, that control the ROMs, delaying or preventing excessive oxidation of the substrates[21-22].

The control mechanisms consist of: (a) neutralizing triggering radicals; (b) preventing inhibitory binding of metallic ions (preventing the generation of triggering radicals); (c) decomposing peroxides to block the reconversion to triggering radicals; (d) blocking the radical cascade to prevent the extraction of other H^+ ions in the lipoperoxidation process.

In disease conditions or simply as a result of certain human lifestyles (cigarette smoking, overeating, physical stress, pollution, etc.) the ROS and other free radicals escape the control of the neutralizing systems and generate toxic phenomena. The set of oxidative alterations induced by ROM (alterations in ion homeostasis, the cytoskeleton, the DNA, loss of the membrane potential, etc.) is denominated "oxidative stress" and is the main cause of cellular ageing[23-24].

Oxidative stress can be defined as the manifestation of exposure of the cells, tissues or organs to an excess of oxidants, especially anion superoxide and ROM. An oxidative stress situation occurs when the production of O_2 and its metabolites exceeds the cell defence and detoxification mechanisms, resulting in problems of gene regulation, signals translation, necrosis, fibrosis and carcinogenesis[25-26]. Normally, the mitochondria are the main resource of reactive oxygen intermediates, since 2-3% of the oxygen consumed is converted to O_2, especially as a result of the auto-oxidation of ubisemiquinone which is able to transfer electrons from complex I and II to complex III [27-28].

Ischaemic/Reperfusion Syndrome increases superoxides production, decreasing the function of complex III[29], tumor necrosis factor alpha[30], and, through an unknown intracellular signal, decreases the function of complex III. Blockage of complex III causes an increased oxygen production due to the auto-oxidation of accumulated ubisemiquinone radicals. The phagocytic cells are an important source of superoxide and other oxidants and can contribute to the reperfusion injury damage[31-32] and to tissue damage during inflammatory processes. The action of nitric oxide (NO) on the endothelium is very important, in that low levels of NO are a relaxant factor, whereas high levels are proinflammatory. The production of NO by macrophages has a bactericide action, but is potentially harmful to the surrounding cells.

Hemorheological alterations during oxidative stress

Oxidative stress plays an important role in the physiopathology of liver damage and fibrosis, in alcohol– or heavy metal-induced diseases. It is also critical to the liver damage caused by ischemia/reperfusion injury[23,31-32] and by sepsis. In chronic liver disease oxidative stress, together with chronic inflammation, can induce hepatocarcinogenesis[35].

Oxidative stress also induces alterations in erythrocyte aggregability and in endothelial adherence[36-37]: the intercellular interactions between RBC autoaggregation and adhesion of the cells to the endothelium have a very important role in the microcirculation[38-39].

Oxidative stress and Erythrocytes (RBC)

The RBCs are formed only by a membrane and a cytosol, they are denucleated and lack sub cellular organelles. The membrane is highly flexible and deformable and allows these cells to flow even into capillaries with a smaller diameter (3.5 microns) than their own (5-7 microns). The erythrocyte membrane has a peculiar lipid and protid composition; the lipids are responsible for its physical characteristics such as permeability and flexibility, while the proteins, largely glycoproteins, show a 1:1 ratio with the lipids.

Circulating erythrocytes are the cells subjected to the highest oxidative stress and also to oxidant substances produced by the metabolism of some food, drugs, or by bacterial or viral infections. This oxidative stress can induce serious damage due to peroxidation of the membrane lipid matrix and protein polymerisation, leading to lysis or an altered hemoglobin function. In the conversion of hemoglobin to meta-hemoglobin the formation of anion superoxide occurs, which is highly toxic. This can also be brought about by chemical compounds, some drugs, or the action of nitrites derived from food preservatives. The toxicity of anion superoxide is quickly "buffered" by superoxide dysmutase, an oxido-reductase which in RBC is called erythrocuprein. Among non enzymatic detoxification systems, the main substance blocking anion superoxide formation is ascorbic acid and Vit. C.

In physiological conditions, the RBC aggregability induces the formation of rouleaux[38-40]. Normally the blood flow is able to scatter these aggregates before they flow into narrow capillaries, thus ensuring tissue perfusion. In conditions of delayed blood flow (and high plasma viscosity) the RBC aggregability is increased and rouleaux become more plentiful, as well as RBC adhesion to the endothelium. This increases the whole blood viscosity, inducing microcirculatory alterations and the risk of microplugging. Moreover, the ROS present in oxidative stress conditions induce the oxidation of the lipids on the erythrocyte membrane and alterations of their internal and external distribution (resulting in exposure of phosphatidylserine on the cell surface), protein oxidation and disruption, the degradation of surface proteoglycans, hemoglobin oxidation and adherence to the cellular membranes. These alterations have a strong influence on the composition and physical properties of the cell membrane, and modify the RBC aggregability and deformability, increasing RBC adherence to endothelial cells in the capillaries[38-39].

Oxidative stress and platelets

The main functions of the platelets are their mechanical and biochemical actions that play an important role in hemostasis and maintaining the capillaries intact. They are also able to accomplish a process similar to phagocytosis of foreign particles, as well as the active transport (of serotonin, adrenalin, K^+) and the repair of even minor tissue damage.

When vascular damage occurs, the narrow arterioles and the precapillaries contract, and local vasoconstriction is stimulated by fibrinopeptide B, adrenalin, plasma kinines and serotonin, released by the platelets. In this way, surface phospholipase C is activated, inducing hydrolysis of phosphatidylinositol 4, 5 diphosphate to inositol 4, 5 triphosphate (second messenger) and lipoprotein. This releases calcium ions in the cytosol, causing a contraction of the myosin portion of thromboastenine (contractile cytoskeleton vacuoles), together with the production of serotonin and adenosine-diphosphate (ADP).

Instead, the activation of phospholipase A releases fatty acids (especially arachidonic acid), originating cyclic endoperoxides that, in turn, are converted to thromboxane A_2 (TXA_2), stimulating platelets aggregation and prostaglandins (PGE_2) and prostacyclin (PGI_2), the inhibitors of platelets aggregation.

Oxidative stress, producing free radicals, causes massive changes in the cytoskeleton, inducing irreversible cross-linking between actin and other proteins and thus a decrease in structural peptides. In addition, an important increase in cytosolic calcium can occur, activating transglutaminase that fosters the creation of bridges which stiffen the cytoskeleton, and thus prevent normal platelet functions.

Oxidative Stress and Leukocytes

The leukocytes (WBC) can be subdivided into granulocytes or polymorphonuclear cells (65%) and leukocytes (25%): T, B lymphocytes, plasmocyte precursors, K cells and macrophages precursors, monocytes. The leukocytes have different mechanical properties from the erythrocytes: both can undergo repeated deformation as they pass through the capillaries, but while the RBC have no nuclei or other granules, the leukocytes contain all the cellular components (nucleus, granules, liposomes), that increase the cell rigidity. This degree of rigidity is due to their actin cytoskeleton and actin binding protein and their degree of binding, which allows them to respond to particular situations by taking on long term rigidity or short term elasticity. This property allows the leukocytes to intermittently arrest the capillary blood flow in cases of a fall in perfusion pressure.

One of the main functions of the leukocytes is phagocytosis; when this occurs there is a huge increase in oxygen consumption (respiratory burst), followed by an increased production of anion superoxide and free radicals. At the same time anaerobic glycolysis is stimulated, and phospholipid synthesis, especially of phosphatide acids, phosphatidyl–inositol and phosphatidyl-serine. This could be related to the altered cellular membrane organization required to engulf foreign microorganisms. The oxidative system involved in this microorganisms killing process, characteristic of neutrophil leukocytes, is catalyzed by the NADPH-oxidase enzyme (respiratory burst) localized on the neutrophil membrane in a quiescent state.

Upon stimulation, this system intervenes by activating the neutrophils, a mechanism that involves the membrane receptors, G protein, phospholipase, proteinkinase C and alterations in the interacellular calcium concentration. In this case anion superoxide, that is usually harmful to the cells, is able to mount a strong attack against the bacteria. After stimulation by pro-inflammatory factors, the neutrophils are activated, undergoing a change in their cell body so that their diameter can increase to 10 times its normal size, and thanks to the polymerization of the actin strands, they put out pseudopods. The increased cell rigidity will slow the blood flow in the capillaries. The margination phenomenon of the leukocytes in the bloodstream and their collision with the vessel walls depend on a direct hemodynamic interaction with the RBC in the post-capillary venules. The RBC, in fact, pushes the WBC against the endothelium. An important role in correct endothelial adhesion is played by the "selectins", "integrins" and an immunoglobulin superfamily known as molecules modulating endothelium adhesion[41-43].

Many studies[44-45] have shown an important role of the WBC in disease conditions, especially in conditions of oxidative stress and inflammation, indicating that they are responsible for a higher incidence of vascular disease, since their properties have a strong influence on the microcirculation.

HEMORHEOLOGICAL ALTERATIONS IN LIVER DISEASE

Liver Cirrhosis

The most important hemorheological and functional alterations in the liver microcirculation have been described in the course of cirrhosis[12-13] and ischemic-reperfusion injury syndrome[46].

Cirrhosis is defined as damage to the hepatic acini in zone 3 or zone 1 or both, which is followed by nodular regeneration and the production of fibrous septi. The damage can be such as to induce an impairment of the liver microcirculation, resulting in obstruction and/or blockage of the blood flow. Collagen deposits in Disse's Spaces, with the apparent build-up of an inferior basal membrane in the sinusoidal endothelial cells and the creation of intra-hepatic-porto-hepatic shunts, are the main anatomopathological alterations observed in the liver microcirculation. This picture is also known as hepatic sinusoids "capillarization"[47]. Not only the collagen fibers deposits in the perisinusoidal space, but also the reduction of "endothelial windows", in both number and diameter, will impede the perfusion of the perisinusoidal space, reducing the oxygenation of the remaining hepatocytes and thus triggering a vicious circle that causes the progression of cirrhosis[12]. The structural alterations of the hepatic sinusoids, moreover, contribute to increase the microvasculature resistance, inducing portal hypertension.

An important pathognomonic sign of the evolution of liver disease to cirrhosis is a significant decrease in the blood hemoglobin concentration, resulting in an impairment of liver perfusion and oxygenation of the cirrhotic liver tissue, which was already hypoxic.

Fulminant Hepatitis

In fulminant hepatitis massive bridges of necrotic cells build up from zone 3 to zone 1, causing major hepatocytic collapse. This situation can induce platelet aggregation in the sinusoids, decreasing or even entirely blocking the blood flow and thus inducing severe hepatic cellular necrosis.

Liver Shock

Physiologically, the liver microcirculation has a self-regulating role that serves to maintain a constant flow through the liver, in response to hemorrhagic shock. In severe cases of shock that overwhelm the self--regulating mechanism, the sinusoidal blood flow diminishes, causing hepatocellular damage in area 3 (centrolobular necrosis) due to an oxygen gradient decrease. Using epi--fluorescence microscopy, an altered sinusoidal perfusion and leukocyte margination have been demonstrated after hemorrhagic shock, together with the production of inflammation mediators (leukotrienes and TNF)[25], which contribute to a further impairment of the liver microcirculation.

The first liver transplantation in humans was performed by Starzl and Coll. in 1963 at the University of Colorado – Denver – USA[48]. In the following years, but especially in the last 19 years, advances in technology, and the use of specially designed drugs (i.e. cyclosporin, tacrolimus, ribavirin, cortisone, etc.) during the postoperative course have stimulated the performance of increasing numbers of liver transplantation procedures, improving disease-free survival. Many liver diseases can only be treated by liver transplantation[49-50]: if no absolute or relative contraindications are present, both children and adults are candidates for liver transplantation if they develop severe, irreversible liver disease that is no longer responsive to medical therapy. Liver transplantation should be considered in patients with end-stage liver disease and life-threatening complications, and with liver failure likely to cause porto-systemic encephalopathy and irreversible damage to the CNS.

In children the most frequent indication for liver transplantation is atresia of the biliary tract, causing progressive alterations of the biliary ducts and cirrhosis, liver failure and death.

Other important indications for liver transplantation in children and adolescents are genetically transmitted diseases with associated progressive liver failure, such as secondary progressive cirrhosis due to an alpha-1-antitrypsin deficit, Wilson's Syndrome, liver failure secondary to Byler's disease, Alagille's disease, Wolman's disease, some forms of glycogenosis, etc.

In adults the most important indications for liver transplantation are chronic active hepatitis of a presumed autoimmune nature, non viral cirrhosis associated with liver failure, end-stage primitive biliary cirrhosis, Caroli's disease, or primitive sclerosing cholangitis with liver failure, irreversible Budd-Chiari Syndrome, primitive hepatocarcinoma. During liver transplantation, hemorheological alterations occur, that vary pre-, peri– and postoperatively.

The hemorheological alterations present **before** transplantation are due to the basal pathology. In cirrhosis, for example, the structural chaos induces increased blood flow resistances and hence decreased tissue perfusion and hypo-oxygenation, an altered RBC deformability, oxidative stress and the formation of many reactive oxygen substances (ROS).

During transplantation there is generalized ischemia following surgical maneuvers to remove the damaged liver. At the clamped vessels level the blood flow is temporarily arrested, causing an increase in RBC and WBC aggregability and adhesion to the endothelium, platelets activation and hypertension up-stream to the occluded vessel[38,44-45]. This ischemic state must be kept very brief to prevent irreversible damage to the hepatocytes. The hemorheological alterations occurring when these vessels are declamped are vital to the functional recovery of the transplanted liver. T**he ischemic reperfusion injury syndrome** is particularly important[46], as the tissue reoxygenation after prolonged ischemia can compound the damage to the microcirculation induced by the ischemic phase. Capillary alterations and WBC adhesion to the endothelium have been shown in experimental models (rats) after liver transplantation[15].

Later studies revealed an altered relationship between the endothelium and the WBC in the liver sinusoids that was directly correlated to the ischemia and reperfusion time. These studies demonstrated the role of the ROS in fostering WBC adhesion to the sinusoidal endothelium. Activation of the Kuppfer[7] cells, during the post ischemic reperfusion phase, has a direct impact on the adhesion of leukocytes to the transplanted liver endothelium[42]. Calcium and inflammation mediators are also involved. It has been shown that these phenomena are particularly marked in zones 1 and 2 of the liver acini, where there is a greater abundance of Kupffer cells.

An important hemorheological alteration in liver disease and after liver transplantation is a decreased RBC deformability[38]. This phenomenon has been detected using the Cell Transit Analyzer by Erythrocyte Transit Time (ETT)[51]. Before and after liver resection, Adenosine triphosphate (ATP) the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), and liver paramaterss (GOT; GPT; gammaGT) have been evaluated. In addition, pre-surgery the green indocyanine level of retention after 15' ($ICGR_{15}$) has been studied, as well as whether RBC deformability alterations were related to this value, and if this evaluation ($ICGR_{15}$) was useful to prevent surgical complications. Green-indocyanine is able to evaluate the total liver blood flow because when injected into the vascular system it can be removed only by the liver. In this way we can calculate the liver blood flow using Fick's formula[52]: Liver Blood Flow = speed of indocyanine removal from the blood/ arterovenous indocyanine difference. The ETT is higher in cirrhotic patients before transplantation than in healthy controls. Moreover, post-surgery complications are higher in patients with high ETT levels on the first, second and third day after transplantation as compared with patients with lower levels [53-54].

ISCHEMIC REPERFUSION (I/R) INJURY.

Organ transplantation (liver, kidney, heart, etc.) raises a number of important issues regarding microcirculation conditions, hemorheology, tissue oxygenation and how they relate to ischemic-reperfusion injury (I/R), which is the major complication after liver transplantation. I/R injury is a non specific antigen--independent process that can significantly affect the outcome of organ transplantation and constitutes one of the principal risk factors for the development of long-term dysfunction of the transplanted organ.

Paradoxically, reperfusion promotes a series of very complicated pathological events that can injure the tissues[55-62]. The main target of these events is the microcirculation. During I/R injury, xanthine oxidase activity, increased oxygen free radicals (ROS), neutrophil activation and altered adhesion all play important roles. The neutrophils adhere[63] to the microvascular endothelium and induce microcirculatory permeability, which increases transcapillary fluid in the reperfused tissues[58, 64-69].

Additionally, neutrophil adhesion causes post ischemic capillary "no reflow" and has been implicated in the reduced arteriolar sensitivity to vasoactive substances induced by I/R injury[56-58, 70-74.]

I/R injury gives rise to a highly complex cascade of phenomena. During ischemia, hypoxia is rapidly induced, rapidly followed by a drop in the intracellular levels of ATP. Aerobic glycolysis is converted to anaerobic glycolysis (piruvate → lactate) resulting in a reduced NADH production. With prolonged ischemia, ATP production drops and energy--dependent functions (Na^+/K^+ dependent ATPase) are arrested, inducing cellular edema and hemoconcentration. Consequently there is a rise in capillary compression, causing reduced capillary perfusion with decreased tissue O_2. The expression of adhesion molecules increases and these in turn increase polymorphonuclear (PMN) leukocytes adhesion to vessel walls. Hypoxia can induce a decreased pH and red blood cells (RBC) deformability; this also contributes to the impairment of capillary perfusion. In such a situation, stasis with microthrombosis will occur and finally, a further deterioration of capillary perfusion.

Ischemia is associated with an important increase in hypoxanthine. When this high concentration of hypoxanthine comes in contact with the oxygen carried by the gush of blood during the early stage of reperfusion, hypoxanthine oxidase gives rise to a high quantity of oxygen derived free radicals (ROS) from the leukocytes, and uric acid (McCord reaction). Via mediators, these ROS could affect the tissue pressure, favouring interstitial edema and microvascular permeability, thus further increasing ROS release from leukocytes. This increase in ROS can directly damage the tissues through the peroxidation of fats. I/R injury can contribute to acute or chronic organ rejection. Leukocytes can contribute to tissue dysfunction during reperfusion. A role in leukocyte adhesion is played by upregulated endothelial ICAM-1 expression during rejection in cardiac, renal, hepatic and corneal allografts[75-81].

This suggests that inflammatory cell infiltration could play a role in transplantation-induced tissue injury. Moreover, I/R injury increases the risk of Delayed Graft Function (DGF) and of acute or chronic rejection. We may consider that even nowadays, the possible causes of DGF are related only to immunomediated events, but it is possible that non immunological factors such as alterations in the microcirculation could also contribute to I/R injury and to DGF. The high quantity of ROS produced during I/R injury under the effect of xanthine oxidase and a sudden rush of Oxygen and xanthine could play a central role in sustaining the tissue damage via ROS.

After ischemia another important phenomenon is the so-called "capillary no reflow". In fact, a large number of capillaries fail to reperfuse[56-58, 69-70,82-85]. The mechanism remains unclear. One hypothesis is that I/R injury and microvascular thrombus production could induce the "no reflow" phenomenon[86]. However, heparin treatment is unable to restore capillary perfusion after I/R[87]. In any case, microvascular thrombosis is unlikely to be present in post ischemic tissues[88-89]. The most credible hypothesis is that post ischemic capillary no reflow is induced by activated neutrophils[90-93]. Following I/R, leukocyte rolling (P-selectin-mediated) occurs, followed by firm adhesion (CD18/ICAM--dependent). These phenomena can induce endothelial cell swelling and finally, capillary no reflow via a reduction of the capillary diameter[94].

CONCLUSIONS

A through knowledge of the hemorheological situation and the microcirculation in liver disease is essential, especially in diseases such as cirrhosis that can completely subvert the liver structure and decrease the blood flow to this organ.

Liver transplantation can often be the only way to save patients with severe liver disease; post-transplant, in these patients we have observed a marked improvement of the peripheral microvasculature, studied by video capillaroscopy and compared with the observations immediately before liver transplantation. The blood, evaluated directly on the liver surface of all patients, also revealed absence of the no reflow phenomenon and an arterial reperfusion level corresponding to physiological values (an increase of approximately 20% of the total liver blood present at the moment of the hepatic artery connection). We found only a reversible, mild cerebral hypoxia that was resolved by increasing the inhaled oxygen. As to the RBC deformability, the situation has been shown to return to normal some days later; this could be explained by resolution of the high levels of bilirubinemia that can induce RBC rigidity. This condition could also explain the low number of functioning peripheral capillaries before transplantation, due to the high blood viscosity induced by RBC rigidity, as well as the increased number of these functioning microvessels after the normalization of bilirubinemia following liver transplantation.

Further studies of transplanted patients are needed to improve the microcirculation conditions following organ transplantation, and especially liver transplantation.

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