

Melatonin Protective Effects against Liver Ischemia/Reperfusion Injury

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Abstract

Hepatic ischemia-reperfusion (I/R) is a common phenomenon during liver surgery, transplantation, infection and trauma which results in damage and necrosis of the hepatic tissue through different pathways. Mechanisms involved in I/R damage are very intricate and cover several aspects. Several factors are involved in I/R-induced damages; briefly, decrease in sinusoidal perfusion and ATP generation because of low or no O₂ supply, increase in production of reactive oxygen species (ROS) and inflammatory factors and destruction of parenchymal cells resulted by these molecules are of the main causes of liver tissue injury during reperfusion. Melatonin's antioxidant effect, and regulatory roles in the expression of different genes in the I/R insulted liver have been investigated by several studies. Melatonin and its metabolites are of the powerful direct scavengers of free radicals and ROS, so it can directly protect liver cell impairment from oxidative stress following I/R. In addition, this bioactive molecule up-regulates anti-oxidant enzyme genes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). Tumor necrosis factors (TNF- α) and interleukin-1 (IL-1), as potent pro-inflammatory factors, are generated in huge amounts during reperfusion. Melatonin is able to alleviate TNF- α generation and has hepatoprotective effect during I/R. It reduces the production of pro-inflammatory cytokines and chemokines via reducing the binding of NF- κ B to DNA. Imbalance between vasodilators (nitric oxide, NO) and vasoconstrictors (endothelin, ET) during I/R was shown to be the primary cause of liver microcirculation disturbance. Melatonin helps maintaining the stability of liver circulation and reduces hepatic injury during I/R through preventing alteration of the normal balance between ET and NO. The aim of this review was to explore the mechanisms of liver I/R injuries and the protective effects of melatonin against them.

Keywords: Liver; Melatonin; Ischemia; Reperfusion

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Introduction

Hepatic ischemia/reperfusion injury (I/RI) is commonplace in liver transplantation, resection, trauma, shock and the damage mechanisms are still remain unsolved. A large number of factors and intermediate substances are involved in I/RI. Ischemia activates Kupffer and other hepatic cells to release ROS, proinflammatory cytokines such as TNF- α , IL-1 and eicosanoids during reperfusion which all of these promote I/RI (1). On the other hand, there is strong evidence to suggest that, the integrity of microscopic blood flow is an important

indicator of survival after ischemia-reperfusion in different tissues (2). Communication between these pathways are quite complex, since the events of I/R between the onset of reperfusion and final results of poor performance or non-performance of the liver, are not described in detail.

Melatonin, the main product of the pineal gland, and its metabolites have been studied for their antioxidative and other physiological roles. The aim of this review was to describe the beneficial effects of melatonin on reducing I/RI.

Melatonin

Melatonin, N-acetyl-5-methoxytryptamine was first isolated and identified from the bovine pineal by Aaron Lerner in the 1950s (3, 4). In 1960, Axelrod and Weissbach explained melatonin synthetic pathway in the pineal gland (5). Although the pineal gland is the main fountain of the blood melatonin, several other tissues are capable of producing imperceptible amounts of the hormone which predominantly affects locally. Melatonin secretion by pineal gland has a rhythmicity that is regulated via diurnal light/dark cycles in which light inhibits the synthesis and secretion of melatonin (6). Numerous physiological roles of melatonin as a multitasking molecule have been found in various studies (7, 8).

Melatonin synthesis, secretion and catabolism

Biosynthesis of melatonin in pinealocytes consists of four enzymatic reactions after uptaking L-tryptophan from the blood: 1) 5-hydroxytryptophan is produced from L-tryptophan by the action of tryptophan-5-hydroxylase (TPH), 2) decarboxylation of 5-hydroxytryptophan by L-aromatic amino acid decarboxylase (AAAD) forms 5-hydroxytryptamine (serotonin), 3) serotonin is converted to N-acetylserotonin by arylalkylamine-N-acetyltransferase (AA-NAT) and finally, 4) the enzyme hydroxyindole-O-methyltransferase (HIOMT) converts N-acetylserotonin to melatonin (Figure 1). Melatonin synthesis is under a fine regulation and shows a remarkable circadian rhythm in which light to the retina prevents its production. The two key enzymes AA-NAT and HIOMT have the most important role in the pathway of melatonin synthesis, but in most cases AANAT is the rate-limiting enzyme (9, 10).

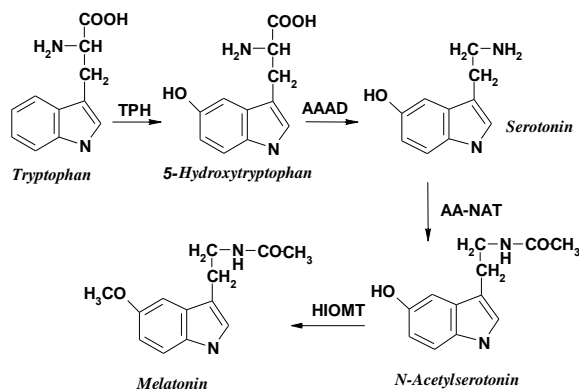


Figure 1. Melatonin synthetic pathway in pinealocyte. TPH, tryptophan hydroxylase; AAAD, aromatic amino acid decarboxylase; AA-NAT, arylalkylamide-N-acetyltransferase; HIOMT, hydroxyindole -O-methyltransferase.

Immediately after synthesizing, melatonin releases into the circulation, thus its blood concentration exhibits the pineal activity since there is no storage of

the hormone in the gland. Secreted melatonin in the bloodstream can easily access to different body fluids because of its lipophilicity (11).

Physiological roles of melatonin

Melatonin is one of the most bioactive molecules which affects cellular physiology via receptor dependent and receptor-independent pathways and has various biologic roles; the latter mainly includes directly free radical scavenging reactions (12). On the other hand, melatonin controls several important physiological and neuroendocrine actions by two membranes bound receptors, MT1 and MT2 (13), which belong to G-protein coupled receptors (GPCRs) superfamily with seven trans-membrane domains (14). Activation of MT1 receptors inhibits adenylate cyclase and lowers cyclic adenosine monophosphate (cAMP) levels, followed by protein kinase A (PKA) activity (15, 16). MT2 stimulation inhibits adenylate cyclase and soluble guanylyl cyclase pathway (17). MT1 stimulation results in vasoconstriction whereas vasodilation occurs during MT2 activation (12). A third type of melatonin receptor, MT3, is the enzyme quinone reductase 2 (QR2), that prevents electron transfer of quinones and takes part in anti-oxidative stress defense (18, 19). Melatonin also acts as a natural ligand for nuclear receptors of the retinoic acid receptor family; ROR α , ROR α 2 and RZR β . Activation of this receptor seems to play a role in some aspects of immune regulation (20, 21).

Melatonin, circadian rhythmicity and seasonality

The main role of melatonin in mammals is to mediate dark signals, circadian rhythmicity and seasonal functions such as the seasonal cycles of reproduction, hibernation, thermomodulation and fasting (22-25). Nocturnal melatonin secretion reduces core body temperature via peripheral vasodilation, enhances cortisol secretion, sleep tendency and conducts to sleep. Thus, it seems reasonable to accept melatonin as an endogenous coordinator (26-28).

Melatonin as an antioxidant

Melatonin antioxidant activity was demonstrated in numerous investigations. Direct free radical scavenging action of melatonin has been considered since 1993. Different types of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be neutralized by the hormone (29-33). Melatonin is shown to be a more potent antioxidant than vitamin E and glutathione; vitamin E is the reference in this field (30, 34, 35). The ability of melatonin to counteract ROS arises from its unique cyclic structure to exchange electron, and its easy penetrability across various biological membranes

(31, 36, 37). Besides its direct action to scavenge ROS and RNS, melatonin reduces oxidative stress by stimulation of antioxidative enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) (38-42). Also, melatonin induces production of glutathione as a major intracellular antioxidant and diminishes mitochondrial electron leakage (43).

Melatonin as an immunomodulator

Evidence in different animal models has suggested direct relationships between nocturnal peak of melatonin and the number of blood lymphocytes (44, 45). In young voles, increased periods of darkness enlarges the size of the thymus (46). Melatonin regulating effects on the immune system are carried out through enhancing the generation of interleukin-1 (IL-1), IL-2 and tumor necrosis factors (TNF), increasing the activity of T-helper and natural killer (NK) cells. Melatonin also suppresses the expression of 5-lipoxygenase by B cells (44, 47). In addition, survival of B lymphocytes can be prolonged by the anti-apoptotic effect of melatonin (48). On the other hand, some studies have suggested that melatonin can inhibit immune responses in some cases indicating its anti-inflammatory properties (49). Taken together, melatonin has immune-enhancing and immune-suppressing effects (50).

Melatonin as an oncostatic agent

Melatonin protective effects against cancer are now well established. The oncostatic action of melatonin is attributed to its antiproliferative, antioxidant, immunomodulatory and proapoptotic properties (6, 50, 51). Physiological and pharmacological concentration of melatonin suppresses growth of estrogen-positive human breast cancer cells via enhancing glutathione levels and by regulating estrogen signaling pathway (51-53). It was shown that melatonin blocks estrogen receptor and inhibits genes related to estrogen-responsive cancer (54, 55). In hepatomas, melatonin antitumor effect is exerted by inhibition of linoleic acid uptake; 13-hydroxyoctadecadienoic acid resulted from oxidation of linoleic acid, serves as an energy source for tumor cells (56). Another possible mechanism for the melatonin's oncostatic effect is the inhibition of telomerase activity because the elevated enzyme activity was seen in many tumor cells (57). In addition, melatonin antioxidant properties protect genome against oxidative damage. Melatonin can also help DNA repair once it has been injured (58). Generally, in different types of tumor, (such as breast cancer, endometrial cancer, colorectal cancer, prostate cancer, stomach cancer, cervical cancer and lung cancer) nocturnal levels of melatonin and its

main secretory metabolite, 6-sulfatoxymelatonin, are reduced (59).

Other biological functions of melatonin

In addition to above mentioned properties, melatonin has other various physiological activities. Melatonin helps bone formation by stimulating osteoblast proliferation and suppressing osteoclast differentiation. Additionally, it enhances the expression of osteoprotegerin in osteoblast and scavenges free radicals by which reduces osteoclast activity on bone. These show melatonin protective effects on bone (6, 60). Studies have demonstrated a possible role of melatonin in energy expenditure and body mass regulation (61). Melatonin as a vasodilatory hormone reduces systolic, diastolic and mean blood pressure (62). Autonomic cardiovascular modulation is affected by melatonin (63). Patients with coronary heart disease or cardiac failure have a lower nocturnal melatonin levels compared to healthy individuals (64). Melatonin antioxidant activity can protect myocardium against oxidative damages during myocardial ischemia-reperfusion (65). In mitochondria, melatonin not only diminishes free radical production, but also stimulates complex I and complex IV activities by which enhances ATP synthesis (66). Alzheimer's disease (67), Parkinson's disease (68), Huntington's chorea (69) and amyotrophic lateral sclerosis (70), are some examples of neurodegenerative diseases which can be retarded by melatonin (61). Melatonin administration as a hypnotic compound induces sleep in healthy individuals and can improve sleep quality and duration in insomnia patients (71-73).

Melatonin and liver I/RI

Liver I/R is a complicated process causing tissue injury during liver transplantation, trauma, sepsis, hemorrhage and hepatic surgery. In these pathophysiologic situations a period of ischemia followed by reperfusion stimulates a series of reactions which finally damage liver tissue and cause hepatic dysfunction. This impairment is called ischemia reperfusion injury (I/RI) (Fig. 2) (74-76). Liver, brain, heart, kidney and intestine tissues which are O₂-dependent and obtain their energy exclusively by mitochondrial oxidative phosphorylation, are influenced by I/RI (77-80). During ischemic phase energy deficiency resulted from insufficient O₂, increases cell membrane permeability, interferes in ion homeostasis and stimulates hydrolases. In addition, decreased cytosolic pH directed by ATP degradation and elevated glycolytic rate, activates Na⁺/H⁺ exchange pump and elevates intracellular sodium (81, 82). High intracellular concentrations of Na⁺ as a result of Na⁺/K⁺-ATPase inhibition

activate $\text{Na}^+/\text{Ca}^{2+}$ exchange and elevate cytosolic Ca^{2+} (2, 83, 84).

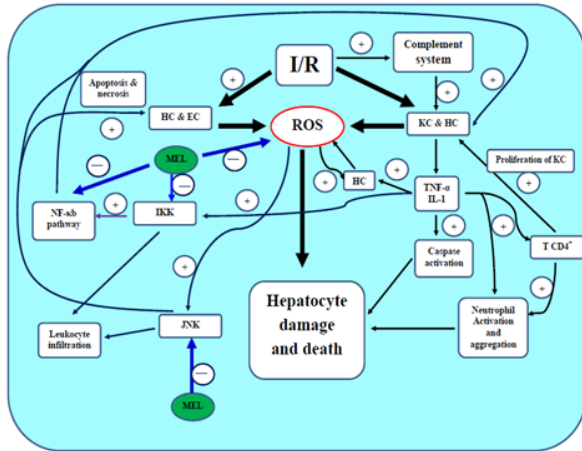


Figure 2. Mechanism of I/R injury and the effect of melatonin to decrease liver tissue damages. This complicated diagram shows the variety of factors affecting liver pending I/R. It is obvious that ROS have the central role and most of pathways damage hepatocytes through ROS production. Melatonin reduces HC damage and death by inhibition of IKK, NF- κ B, JNK pathways and ROS production. EC, endothelial cell; HC, hepatocyte; IKK, I κ B kinase; IL-1, interleukin-1; JNK, c-Jun N-terminal kinases; NF- κ B, Nuclear factor κ B; TNF, tumor necrosis factors.

This stimulates proteases and hydrolases which finally can cause cell death. Increased osmotic pressure due to high intracellular Na^+ can lead to cell swelling and membrane rupture (85). Building up mitochondrial matrix Ca^{2+} results in making transition pores in the membrane and degradation of cellular ATP by the reverse action of mitochondrial ATPase. All of these processes can cause necrotic cell death (82, 86). On the other hand, ATP-deficiency and resultant failure of active transmembrane transport leads to Kupffer (KC) and endothelial cell swelling, narrowing of the sinusoidal space and reduction of hepatic microcirculation (85, 87, 88). Furthermore, an imbalance in the generation of endothelin (ET) (vasoconstrictor) and nitric oxide (NO) (vasodilator), as the main modulators of vascular tone, intensifies narrowing of the sinusoidal lumen (89, 90). Decreased microcirculation leads to KC, and neutrophil activation and accumulation, and stimulates them to produce ROS and proteases (77, 91). Activated KC not only release ROS but also produce proinflammatory cytokines, including IL-1 and TNF- α (69, 91, 92). Stimulated CD4⁺ T-lymphocytes by IL-1 and TNF- α produce interferon gamma (INF- γ), TNF- β and granulocyte-macrophage colony-stimulating factor (GM-CSF) (93). These stimulator factors trigger KC to release TNF- α and IL-1 which finally lead to neutrophil accumulation in sinusoids (68, 77, 94). Although activated neutrophil and KC are the main origin of ROS (95, 96), xanthine

dehydrogenase and xanthine oxidase from stimulated hepatocytes and endothelial cells produce parts of ROS (77, 91, 97-99). ROS not only directly damage membranes and intracellular organelles, but it can also impair liver microcirculation. Within 0.5 to 4 hours after reperfusion, large amounts of ROS are produced by activated KC. Later, more ROS are generated by activated neutrophils, hepatocytes and sinusoidal endothelial cells (95, 100, 101). As a potent endogenous antioxidant, melatonin plays a protective role against ROS (70). Studies have shown that increased oxidative stress elevates the production of melatonin and urinary excretion of its metabolites (32). Melatonin is a strong scavenger of highly reactive and toxic $\cdot\text{OH}$ (102). In addition, melatonin neutralizes and deactivates many damaging radicals such as $\text{NO}\cdot$, $\text{LOO}\cdot$, $\text{O}_2\cdot^-$, H_2O_2 , HClO , ONOO - through exchange of electrons (103-105). These strong radical detoxifying effects of melatonin are also exerted by its metabolites; for example, cyclic 3-hydroxymelatonin generated through the reaction of melatonin with free radicals is itself able to neutralize further free radicals. This is also possible for the product of this reaction (32, 106). Accordingly, melatonin and some of its metabolites may directly react with various cell-damaging radicals and neutralize them (67), but this is not the only function of melatonin (107). Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are up-regulated by melatonin (13). Expression of pro-oxidant enzyme gene such as 5- and 12-lipo-oxygenase is down-regulated by melatonin (61). Pinealectomy increases lipid peroxidation in brain. Reduction of melatonin and its metabolite antioxidant effects increases oxidative stress damages, because lipid peroxidation is a known mechanism of cell injury by ROS (108, 109). Atalla et al demonstrated that elevated levels of ROS injured liver tissue during I/R via lipid peroxidation (110). Melatonin reduces the levels of malondialdehyde and hepatic injury indices during I/R through decreasing oxidative stress in rats (111, 112). Following I/R, levels of oxidized proteins in the mitochondria of liver cells and in liver homogenates are increased as associated with increased lipid peroxidation (113). Concurrent reduction of antioxidant capacity and increase of ROS production results in oxidative stress in fatty livers (114). Activities of CAT and GPx are increased during I/R. The mitochondrial damage indicator, glutamate dehydrogenase, is also increased in such conditions (115). Melatonin inhibits oxidative stress and protects liver through reduction of ROS and increment of antioxidant enzymes (112, 113, 116). Cytokines play a prominent role in hepatic injury during reperfusion; these compounds could launch

and sustain inflammatory responses (117). KC activated during ischemia are capable of producing large amounts of TNF- α and IL-1 during reperfusion, which both are considered as potent pro-inflammatory factors. It seems that reperfusion-induced injuries are caused by TNF- α and IL-1 along with complement factors (C5a) and platelet activating factor (PAF) through activation of neutrophils. Furthermore, TNF- α and C5a increase the expression of adhesion molecules which are necessary for neutrophil extravasation. TNF- α and IL-1 produced by KC activate T CD4+ lymphocytes. In early stages of hepatic reperfusion, these stimulated lymphocytes enhance proliferation of KC and increase accumulation of neutrophils in the liver. Thus, these two types of cells reciprocally activate each other. Meanwhile, TNF- α can induce cell death through stimulation of caspase 8 (77, 117, 118). Thus, inhibition or reduction of TNF- α production, can protect liver from I/RI. Melatonin is able to alleviate TNF- α generation (119, 120) and so has hepatoprotective effect during I/R (121).

Nuclear factor κ B (NF- κ B) is a key factor in expression path of a large number of pro-inflammatory cytokines and adhesion molecules which play an important role in the immune responses (122). Studies show that NF- κ B is particularly important in the activation of KC (123), and ROS produced by them stimulate the binding of NF- κ B, as a transcription factor, to DNA in Kupffer, endothelial (EC) and hepatic cells (124-126). Therefore, deactivation of NF- κ B in KC can play an important role in the reduction of I/RI.

Melatonin reduces the production of pro-inflammatory cytokines and chemokines via reducing the binding of NF- κ B to DNA (69, 121, 127, 128). Inhibition of adhesion molecules synthesis following blockade of NF- κ B effect, inhibits the adhesion of leukocytes to endothelium and their extravasation. In addition, melatonin prevents migration of neutrophils to the inflamed area and exacerbation of the inflammatory response (129). During I/R, KC produce excess amounts of ROS as the key molecules in the production TNF- α , IL-1, and various chemokines (leukocyte infiltration factors into the tissues) through activation of c-Jun N-terminal kinases (JNK). TNF- α stimulates the hepatocytes via its specific receptors to produce ROS, which in turn activate JNK in hepatocytes and results in necrosis and apoptosis. Moreover, stimulation of TNF- α receptor activates the I κ B kinase (IKK) pathway, which causes the infiltration of leukocytes of the liver along with JNK. All these alterations lead to I/R-induced liver damage (130, 131).

As previously stated, melatonin and its metabolites react directly with ROS and neutralize them. It also

reduces ROS production by increasing the expression of antioxidative enzymes and decreasing the prooxidative enzymes. Furthermore, melatonin inhibits the IKK and thus NF- κ B pathways which occurs simultaneously with inhibition of the JNK pathway (132). Inhibition of JNK pathway inhibits the production of cytokines and infiltration of leukocytes into the liver tissue (131).

Microcirculatory disturbance is an inevitable phenomenon during I/R accepted as one of the most important damaging components during reperfusion. As mentioned earlier, extensive molecular changes occur during ischemia and damage the liver tissue directly and indirectly. In fact, factors affecting liver blood flow exert indirect effects.

Considering the central role of liver in body metabolism, an especial perfusion system consisting of a proprietary system of capillaries called hepatic sinusoids, supplies blood to the hepatocytes. Sinusoids walls are covered by sinusoidal endothelial cells (SEC), KC, and hepatic stellate cells. SECs are located in the walls of the sinusoids to form a porous wall with a selective permeability. Also these cells exert endocytotic and antigen-presenting activity and can produce a large number of biologically active substances, such as cytokines, eicosanoids, endothelin-1, and nitric oxide (133).

There are three reasons for liver microcirculation failure during I/R:

1- Lack of oxygen during ischemia leads to depletion of ATP inside Kupffer and sinusoidal endothelial cells. Reduction or absence of ATP disables many energy requiring processes such as energy consuming membrane pumps and thus, alters the homeostasis of Na⁺, Ca²⁺, and H⁺ ions. Alongside activation of a variety of hydrolytic enzymes, these pathological conditions lead to the swelling of KC and sinusoidal endothelial cells thus narrow sinusoids, which eventually result in liver circulation failure (130, 134). It is obvious that mitochondria are affected by ischemia because of their main role in oxygen consumption and energy (ATP) generating. Melatonin is a lipophilic molecule, so, it easily diffuses into cells and intracellular organelles such as mitochondria. Due to its antioxidative properties, melatonin protects the mitochondrial proteins responsible for the production of ATP (in respiratory chain), mitochondrial DNA (135), and the inner mitochondrial membrane (136) against ROS and RNS. In addition, melatonin increases the expression of the mitochondrial genes responsible for encoding of the proteins of complex IV in the respiratory chain (137). Okatani Y, et al showed that melatonin reduces respiratory and ATP synthesis disturbances, lipid peroxidation, and mitochondrial edema during hepatic I/R and exerts a protective effect on

mitochondria (138). Therefore, melatonin prevents swelling of SEC and narrowing of sinusoids and protects liver against a decrease of microcirculation during I/R through protection of mitochondria against an ATP deficiency and prevention of harmful changes in homeostasis of ions such as Na⁺ and Ca²⁺ which can affect the cell volume.

2- As mentioned previously, immune and inflammatory responses are activated during ischemia in the liver. Activation of the complement system from C5a stimulates KC, and neutrophils and platelet aggregation in the sinusoids. Production of large amounts of ROS, interleukins and TNF- α causes synthesis of adhesion molecules by the SEC and further stimulation of neutrophils. Formation of neutrophil-neutrophil and neutrophil-platelet complexes, as well as the presence of a large number of active KC, although not resulting in complete blockage, disrupts sinusoidal microcirculation (139). Melatonin reduces activation and accumulation of inflammatory cells such as neutrophils and KC through inhibiting the synthesis of pro-inflammatory cytokines and adhesion molecules, and in this way minimizes alterations of liver microcirculation (68, 129, 132).

3- Imbalance between vasodilators and vasoconstrictors during I/R was found to be the primary cause of liver microcirculation disturbance (88). As the most important vasodilator, NO is produced by two types of nitric oxide synthase (NOS) in the liver; endothelial nitric oxide synthase (eNOS) which is expressed in the SEC and inducible nitric oxide synthase (iNOS) as expressed in macrophages, endothelial cells, hepatocytes, etc. and is induced by cytokines and other inflammatory factors (140). Endothelin (ET), the strongest vasoconstrictor which is synthesized and released by the vascular endothelium, counteracts the effects of NO. ET can be induced by low-shear stress, turbulent circulation, hypoxia, cytokines, angiotensin II, and adrenaline (141). During I/R, SEC, KC, and stellate cells secrete ET in response to hypoxia, ROS, bacterial lipopolysaccharides, and pro-inflammatory cytokines (142). Koeppel et al showed that the expression of ET receptors is higher in stellate cells than the SEC, which in turn is higher than the KC (143).

Many studies have demonstrated alterations of ET, iNOS, and eNOS gene expression during I/R as down-regulation of eNOS and up-regulation of the two others (111, 112). In early stages of reperfusion, decrement of hepatic circulation has been attributed to the contraction of sinusoids as the result of ET increase (144, 145). During I/R, ET induces sinusoidal contraction and liver blood flow failure through contraction of stellate cells, since the use of ET receptor antagonists and ET-1 antiserum

improves the hepatic microcirculation during reperfusion (126). Several studies suggest that melatonin decreases ET gene expression during I/R (111, 112, 146), and can improve hepatic circulation during reperfusion through preventing the contraction of stellate cells and sinusoids.

As a major vasodilator, NO plays an important role in the regulation of liver blood flow in normal and pathological conditions. As mentioned earlier, eNOS and iNOS are NO-producing enzymes in the liver; although iNOS gene is induced in pathological conditions such as ischemia (140). eNOS gene is expressed constitutively only in SEC cells, which produces a small amount of NO for adjustment of tonicity of sinusoids in short-term (147). Unlike eNOS, iNOS gene is not expressed in normal circumstances, but it is up-regulated during inflammation in all nucleated cells, such as KC, hepatocytes, neutrophils, and endothelial cells, which produce large amounts of NO in long-term (148). Regardless of the producing enzyme, the synthesized NO exerts its effects through activation of soluble guanylate cyclase (sGC) and then increasing cyclic guanosine monophosphate (cGMP) level (149). It is noteworthy that NO derived from eNOS protects liver against I/RI via regulating dilatation of sinusoids, neutralization of ROS, inhibition of platelets aggregation and neutrophils adhesion, and counteracting the effects of ET (150). Liver damage is higher in eNOS knockout mice compared to wild type. Also in transgenic mice with an over-expressed eNOS, the amount of liver damage during I/R is significantly lower than that in the wild type (151, 152). In addition, eNOS inhibitors aggravate I/R damage, while L-arginine improves microcirculation and reduces I/R damage (153). Whether iNOS-derived NO is beneficial for I/R phenomena is not clear yet. But some studies have indicated that the effect of iNOS can depend on the duration of liver ischemia, for example, in animal models, if ischemia is applied less than 60 min, iNOS will increase liver damage; while comparison of perfusion in the iNOS knockout mice with the wild type showed delays in the 1-h time point with similar perfusion rates at 3 and 6 h. Moreover, hepatic damage was higher in eNOS knockout mice compared to that in wild type at 3 h post-reperfusion (154). It seems that in hepatic ischemia longer than 60 minutes, iNOS-derived NO contributes to the development of I/R through a variety of warm and cold ischemia, because the excessive NO induces the production of pro-inflammatory cytokines and chemokines in neutrophils and KC, and increases the harmful peroxynitrite (152). On the other hand, the excess NO can result in systemic hypotension and shock (155). Liver can be protected against deleterious effects of

I/R by eNOS-derived NO in all stages and by iNOS-produced NO in later stages. According to researches, NO causes this effect through the inhibiting decrease of ATP, preventing the increase of TNF- α , IL-12, and IL- β , reducing oxidative damage induced by ROS and RNS, preventing decrease of glutathione, confronting harmful effects of ET, preventing the increase of adhesion molecule, and improving liver microcirculation during I/R (152). Several mechanisms have been suggested about the protective effect of melatonin on liver through affecting NOS and ET during I/R (Figure 3).

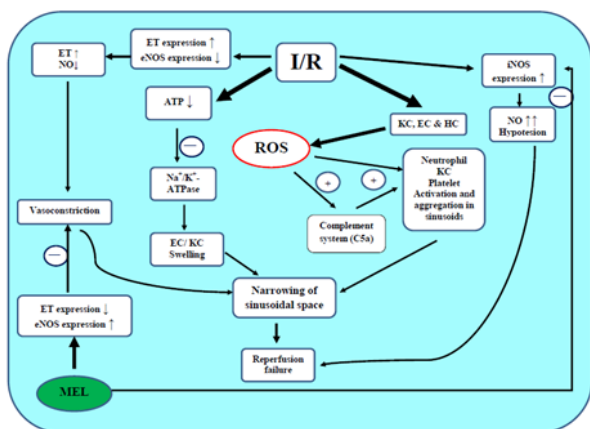


Figure 3. Mechanisms of microcirculation failure during reperfusion after hepatic ischemia and the melatonin protective effects. I/R causes ATP depletion, ET and iNOS gene overexpression, KC, EC and HC activation and generation of large amounts of ROS, which all of these changes conduct liver I/R injury. Melatonin reduces the expression of ET and at the same time increases the availability of NO, therefore, prevents sinusoidal perfusion defeat during the reperfusion stage of I/R. EC, endothelial cell; eNOS, endothelial nitric oxide synthase; ET, endothelin; HC, hepatocyte; iNOS, inducible nitric oxide synthase; KC, Kupffer cell; MEL, melatonin; NO, nitric oxide; ROS, reactive oxygen species.

Melatonin reduces the expression of ET and at the same time increases the availability of NO, therefore, protects function and structure of the liver against harmful and damaging effects during I/R (156). One of the well-known mechanisms of I/R damage is overproduction of ROS, which directly attack, destruct, and deactivate biomolecules including membrane lipids and proteins as well as DNA (157). Endothelial cells are the main target of these radicals. On the other hand, the reaction of NO with ROS reduces usable and available NO. Therefore, the main vasodilator is reduced in liver sinusoids, leading to decreased liver circulation. Pretreatment with melatonin acceptably decreases hepatic injury during I/R. Melatonin prevents decrement of NO through reducing oxidative stress. In addition, melatonin increases eNOS gene expression (68, 156). NO can protect liver via reducing TNF levels and inflammation, thus it can indirectly counteract the

effects of I/R (112, 158). Increased iNOS expression during reperfusion (159) is known as the possible mechanism of liver injury. Melatonin reduces iNOS expression unlike eNOS (68, 105, 112, 160-162). However, the effect of iNOS-derived NO and the expression of iNOS raise conflicting results. Taken together, melatonin helps maintaining the stability of liver circulation and reduces hepatic injury during I/R through preventing alteration of the normal balance between ET and NO.

Conclusion

Interruption of blood flow during ischemia brings about the stoppage of nutrient and oxygen flow, and removal of waste materials from the hepatic tissue. With the start of reperfusion, inflammatory reactions started during ischemia are exacerbated, and along with the production of ROS and RNS, huge amounts of a variety of inflammatory cytokines are produced. By their chemotactic effects, many of them result in the influx of neutrophils and a diversity of immune cells into the hepatic tissue, eventually damaging it. Disruption of normal blood flow during reperfusion is another major problem worsening the above conditions. Previous studies have utilized different methods and compounds to reduce I/R damages, one of which is melatonin. Melatonin is believed to have multiple biological effects. Its powerful antioxidant properties and regulatory effects on the expression of different genes can protect liver against I/R.

Author contributions

Kh-TA and GhK gather data. Kh-TA wrote the manuscript. GhK reviewed and Kh-TA revised the article.

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