Melatonin Salvages Neural Tissue from Ischemia/Reperfusion Injury

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Abstract: The search for effective drugs that will curtail neural damage resulting from transitory interruption of the blood supply to the brain has a long investigative history. Unfortunately, many chemical agents that were shown to be effective in reducing brain ischemia/reperfusion (I/R) injury in animals have been found to be much less beneficial or totally ineffective in humans. In the last 15 years, melatonin has been widely tested in terms of its ability to reduce the morphological damage, biochemical and molecular alterations and behavioral disturbances resulting from I/R injury in animals, usually rodents. These studies have documented that melatonin is highly beneficial in terms of reducing the changes that accompany transitory ischemia of the brain followed by reperfusion with oxygenated blood. Melatonin's protective actions are attributed to its potent direct free radical scavenging activity, its stimulation of antioxidative enzymes, its ability to suppress the inflammatory response, and its actions in preserving cellular homeostasis and preventing molecular events that culminate in apoptosis, etc. In view of its uncommonly low toxicity and high efficacy, it seems appropriate to give melatonin consideration for use in the human with the intent of reducing the numerous negative aspects of stroke.

Keywords: Melatonin, stroke, ischemia/reperfusion injury, brain damage, neural apoptosis, mitochondrial malfunction.

INTRODUCTION

Stroke is a major cause of death and disability throughout the world. The severity of brain damage during the ensuing period of ischemia (hypoxia) is dependent on the thoroughness and duration of the interruption of neural blood flow. Alterations in flow thresholds cause different degrees of physiological disruption and tissue destruction. Modest depressions in the perfusion of neural tissue with oxygenated blood may cause transitory functional impairment of the nervous system with normal physiology eventually being restored. Conversely, near total and/or prolonged reduction in blood flow to an area of the brain often results in permanent morphological tissue loss and severe lingering neurophysiological deficits. The destroyed tissue near the center of the stroke is commonly referred to as the umbra (the core of the lesion) while the tissue at the margin of the umbra that is only functionally compromised is referred to as the penumbra. For any of the damaged tissue to re-establish something reminiscent of normal function, blood flow to the stroke area must be restored to a sufficient level within a short time.

It is obviously essential that if neural function is to be restored and tissue loss is to be held to a minimum, the blood supply to the affected area must be re-established as quickly as possible. When blood flow is restored to a neural area that has been transiently deprived of oxygen (has been hypoxic), however, the arrival of oxygenated blood causes additional morphological tissue loss and neurophysiological disturbances. Nevertheless, without re-instatement of the blood supply, the neural tissue beyond the site of vessel obstruction would have no capability of functional recovery.

The goal of on-going research is to define methods and drugs which will ameliorate neural damage and improve functional recovery resulting from stroke. The current review considers the experimental data obtained from animal studies that melatonin, an endogenously produced indoleamine [1], improves stroke outcome by reducing the amount of neural damage and recouping as closely as possible normal neurophysiology. Several earlier reviews [2-7] have also summarized these data; the current survey will especially summarize the reports that have appeared after the aforementioned review articles.

STROKE MECHANISMS: FREE RADICAL INVOLVEMENT

Stroke, also referred to as ischemia/reperfusion (I/R), is a cerebrovascular accident or brain attack. Certain individuals are more inclined to have a stroke than others. Those who are prone to neurological stroke are individuals suffering with high blood pressure, atherosclerosis or other cardiovascular disorders and especially those who routinely smoke cigarettes. Additionally, obesity, advanced age and diabetes mellitus are predisposing conditions for I/R injury to the central nervous system [7-9].

A stroke is often localized (focal ischemia) when it is a consequence of the interruption of a single blood vessel due to a clot or resulting from the ruptured blood vessel (hemorrhagic stroke). Global ischemia, which involves the entire central nervous system, can also occur, e.g., as a result of cardiac arrest, asphyxia or carbon monoxide poisoning. Regardless of whether the neural lesion is focal or global, the mechanisms of free radical-mediated neurological damage have many common features.

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Much of the tissue loss that results from I/R injury is believed to be a consequence of massive production of both reactive oxygen (ROS) and reactive nitrogen species (RNS). The oxygen-based reactants that mutilate neurons and glia include the superoxide anion radical (O_2•-), hydrogen peroxide (H_2O_2) and especially the hydroxyl radical (•OH) (Fig. **1**); this latter oxygen derivative, because of its very high reactivity, is believed to account for in excess of 50% of the damage that occurs during I/R injury. The O_2•- is also detrimental since it couples with the nitric oxide radical (NO•) to generate the peroxynitrite anion (ONOO-). This latter RNS is essentially equivalent to the •OH in terms of its ability to oxidize essential molecules and cause neuronal and glial cell death [10, 11]. Collectively, the ROS/RNS are often referred to as free radicals, although technically some are not radicals, e.g., ONOO- (which does not contain an unpaired electron in its valence orbital, a requirement to be classified as a radical).

The toxic oxygen and nitrogen-based reactants are generated in ischemic and reperfused neural tissue as a result of a number of processes. Thus, I/R results in the discharge of excessive amounts of the neurotransmitter glutamate which causes excitotoxicity [12, 13], neural acidosis [14], release of "catalytic iron", elevation of intracellular levels of Ca^{2+} [15] and the accumulation of free fatty acids, especially arachidonic acid due to the activation of phospholipase A_2, a reaction aided by the high intracellular levels of Ca^{2+} [16]. The release of "catalytic iron" is detrimental since it is involved in the reduction of H_2O_2 to the highly toxic •OH [17].

There are several features that make ischemia and the subsequent reperfusion in the central nervous system especially devastating. These factors include the following: (a) the brain uses a disproportionately large portion of the

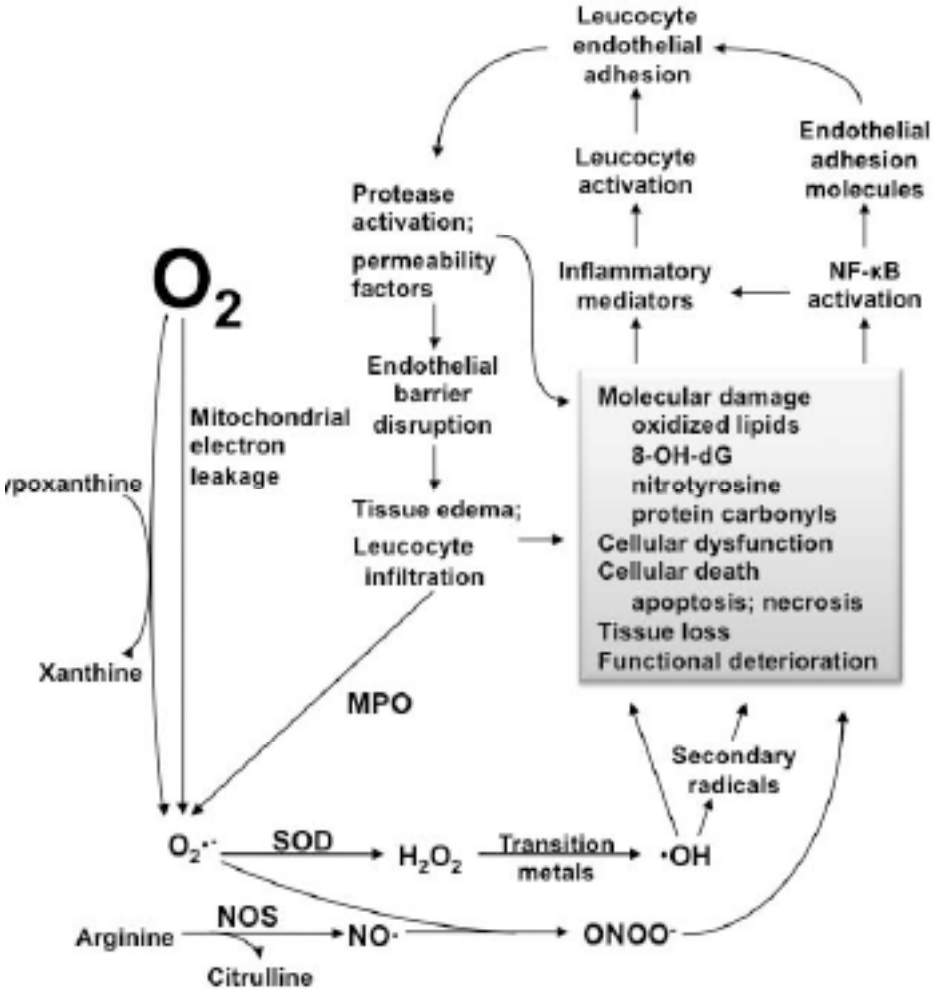

Fig. (1). Some of the multiple processes by which transitory ischemia (hypoxia) and reperfusion (reoxygenation) damages brain tissue. A major contributory factor to the tissue destruction that occurs is the generation of toxic free radicals and related reactants. These toxic reactants cause multiple forms of molecular and physiological disruption (summarized in the box) which causes other changes that destroy tissue. Once ischemia is initiated it is imperative that blood flow to the ischemic region be re-established (reperfusion). Unfortunately, flooding ischemic tissue with oxygenated blood generates additional radical products that extend the damage. Conversely, if blood flow is not restored, the post obstruction tissue permanently deteriorates. O_2, molecular oxygen; O_2·-, superoxide anion radical; H_2O_2, hydrogen peroxide; ·OH hydroxyl radical; NO·, nitric oxide; ONOO-, peroxynitrite anion; SOD, superoxide dismutase; NOS, nitric oxide synthase; MPO, myeloperoxidase; 8-OH-dG, 8-hydroxy-2-deoxy-guanosine (a damaged DNA product).

inhaled O_2. Although it is roughly 2% of the body weight, the central nervous system, at rest, is estimated to utilize 20% of the inhaled O_2. This proportion further rises as the brain activity increases. Since O_2 is the precursor to many free radicals and related derivatives, the CNS exhibits an elevated susceptibility to oxidative and nitrosative damage because of the inordinately large number of ROS/RNS it generates; (b) the brain contains large quantities of polyunsaturated fatty acids (PUFA); these fats are readily oxidized by radicals and once initiated the process is self-propagating due to the high toxicity of the lipid peroxyl radical. (c) The brain is rich in non-heme iron. When this iron is released it becomes "catalytic iron" and promotes the Fenton and Haber-Weiss reactions which lead to the formation of the highly toxic •OH. (d) Vitamin C (ascorbic acid) is locally in high concentrations in the CNS. Although an antioxidant and therefore potentially beneficial in protecting against free radical damage, in the presence of free iron this vitamin becomes a potent pro-oxidant, i.e., it causes the production of free radicals. (e) Finally, the CNS is remarkably poorly equipped with antioxidative enzymes. These enzymes typically metabolize toxic reactants to innocuous species and are used by all cells, but less so by the neurons, to reduce free radical toxicity. Because of this deficiency, radical products in the brain have a greater chance of mutilating essential molecules.

The cellular and molecular damage meted out by free radicals and their derivatives cause a plethora of negative changes in the brain which eventually account for the tissue loss and neurophysiological deficits that appear [18-21]. As summarized in Fig. (**1**), the molecular destruction that occurs involves lipids (which are abundant in all cellular membranes), DNA (often measured as 8-hydroxy-2-deoxyguanosine) and proteins. This damage precipitates cytokine release and other inflammatory responses that lead to changes in the permeability of the endothelial lining which also contributes to increased patency of the blood-brain barrier. One consequence of these changes is the development of tissue edema which, in the enclosed space of the skull, develops pressure on neurons and glial which further compromises their physiology. The inflammatory response is also aggravated by the arrival of leucocytes at the damaged site; these cells, due to the activity of a resident enzyme, myeloperoxidase (MPO), generates numerous O_2•- which then sustain the process of highly toxic free radical generation.

Given the role that excessive radical production plays in neural tissue mutilation related to I/R, it is not surprising that molecules which neutralize these toxic brigands, i.e., antioxidants, have frequently been tested as a means of attenuating tissue loss and the consequential neurophysiological deficits associated with the transitory interruption of the blood supply to the brain [22-25]. The protection afforded by a given exogenously-administered antioxidant against neural I/R injury depends on its ability to get to the intracellular sites where the damage occurs, to its concentration at the site of damage and to its efficiency as a direct free radical scavenger and indirect antioxidant. While free radicals are generated throughout the cell, they are particular abundantly produced within mitochondria due to leakage of electrons from the electron transport chain [26-28]. Given the ease with which mitochondria produce radicals that contribute to molecular damage, it is essential that an antioxidant which is designed to reduce oxidative stress during I/R has ready access to mitochondria and is highly effective in neutralizing radicals and related derivatives. Many synthetic and naturally-occurring molecules that have proven effective in reducing molecular damage in animal models of I/R have been remarkably ineffective in having a similar protective action when used in human clinical trials. Thus, great caution must be employed when interpreting the outcome of an animal study of neural hypoxia and reperfusion injury in terms of what its efficacy may be in human stroke.

MELATONIN AS AN ANTIOXIDANT

The discovery by Tan and co-workers [29] that melatonin, a pineal-derived indoleamine, was an uncommonly effective free radical scavenger launched a new area of research related to this molecule. Prior to this discovery, melatonin was primarily known for its ability to influence circadian rhythms [30, 31], promote sleep [32, 33], and regulate annual changes in reproductive competence in photoperiodic, seasonally-breeding mammals [34-37]. Whereas these subjects remain active areas of investigation, studies further defining melatonin's ability to protect against oxidative stress are expanding especially rapidly [38-43].

The observations related to the high efficacy of melatonin in scavenging the •OH as reported by Tan and colleagues [29] has been repeatedly confirmed [44-50] using techniques that are widely accepted as being the best currently available for such measurements. The original finding was also important given that the radical shown to be scavenged by melatonin was the devastatingly reactive •OH. As mentioned above, this particular O_2 derivative is believed to account for greater than 50% of the oxidative stress that occurs *in vivo*.

Tan *et al.* [29] performed their studies in a pure chemical system in which hydrogen peroxide (H_2O_2) was exposed to 254 nm ultraviolet light to generate the •OH. In this system, melatonin was much more effective in quenching the •OH than was the spin trapping agent dimethyl-pyrroline-N-oxide (DMPO). The quenching efficiency of melatonin was measured using electron spin resonance (ESR) spectroscopy. When compared with the synthetic antioxidant, mannitol, and with the natural antioxidant, glutathione, melatonin again proved superior. Using a combination of gas chromatography, mass spectrometry and nuclear magnetic resonance, Tan *et al.* [29] also identified the product that was formed when melatonin interacts with the •OH; the product was identified as cyclic 3-hydroxymelatonin. It is theoretically generated when melatonin scavenges two •OH. Subsequent publications have shown melatonin to be a scavenger of other toxic reactants including singlet oxygen ($^1O_2$) [51], NO• [52, 53], the ONOO- [54-56] and H_2O_2 [57]. Since each of these reactants is capable of impairing neurons and glia, one might anticipate that melatonin may be beneficial in protecting against I/R damage.

When melatonin functions in the detoxification of ROS/RNS, the products formed, i.e., cyclic 3-hydroxymelatonin and others, are likewise capable of neutralizing radicals and their derivatives [58-60]. In addition to cyclic 3-hydroxymelatonin [61], other

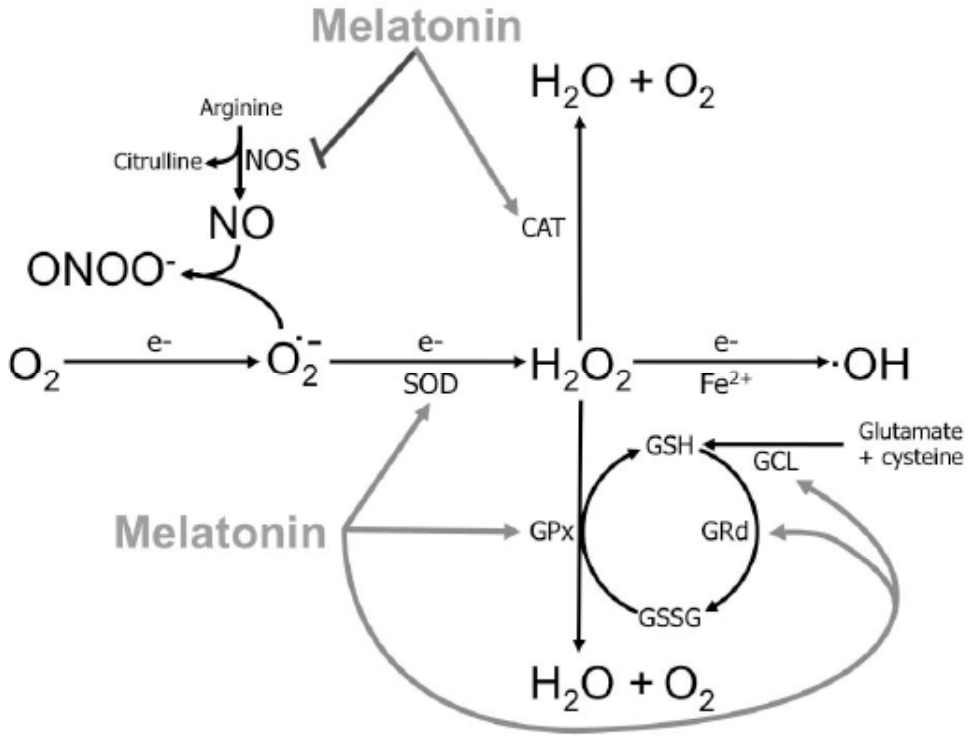

Fig. (2). This figure summarizes the antioxidative enzymes which melatonin upregulates. In a variety of different studies, melatonin has been shown to promote the mRNA levels and/or activities of superoxide dismutase (SOD) (both cytosolic and mitochondrial), glutathione peroxidase (GPx), glutathione reductase (GRd), and catalase (CAT). The stimulation of SOD removes the superoxide anion (O_2•-) and reduces the generation of the peroxynitrite anion (ONOO-). The upregulation of GPx and CAT removes the precursor and reduces the production of the hydroxyl radical (•OH). GRd upregulation ensures that the majority of the glutathione within cells is in the reduced form. Melatonin also inhibits the pro-oxidative enzyme, nitric oxide synthase (NOS) and promotes de novo synthesis of glutathione by stimulating glutamylcysteine ligase (GCL).

metabolites that reportedly actively scavenge toxic reactants include N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) [62, 63] and N1-acetyl-5-methoxykynuramine (AMK) [64, 65]. Additionally, however, there may be other melatonin derivatives that function as scavengers [66]. These by-products of melatonin neutralize radicals in a sequential manner; this sequence is referred to as the scavenging cascade of melatonin [67].

Melatonin's ability to directly scavenge oxygen and nitrogen-centered radicals and their products is only one of the means by which it reduces nitro/oxidative stress. In a process referred to as a radical avoidance [40], melatonin stimulates Complex I and Complex IV of the mitochondrial respiratory chain [68-70]. In so doing, electron leakage from these complexes is attenuated and the adjacent O_2 molecules are not reduced to O_2•-. Given that melatonin has an action at the mitochondrial level confirms that the indoleamine enters cells and subcellular organelles including mitochondria. This is supported by the measurement of melatonin within these organelles [71] and is visualized, using fluorescent techniques, when glial cells are treated with melatonin and mitochondrial free radical generation is reduced [72, 73]. Since mitochondria are a major site for free radical generation, the ability of melatonin to reduce electron leakage and the reduction of O_2 at this location could be a major means by which the indoleamine stymies the damage inflicted by toxic reactants.

The stimulation of antioxidative enzymes by melatonin has been known for more than a decade [74-76]. Normally these catalysts function in the removal of free radicals from the intracellular environment by converting them to non-radical products. The activities of these enzymes have a central role in limiting free radical damage to essential molecules.

The isoforms of superoxide dismutase (SOD), i.e., MnSOD in mitochondria and CuZuSOD in the cytosol, dismutates the O_2•- to H_2O_2. This reduces the intracellular concentration of O_2•- and prevents its coupling with NO• to form the strong oxidizing agent, ONOO-. Melatonin, when given exogenously to experimental animals induces the activities of both MnSOD and CuZuSOD [77, 78]. Also, under situations where the toxic load of cells is increased, melatonin administration prevents the drop of SOD activity allowing the cell to more easily rid itself of excess O_2•- [79-81].

An upregulation of SOD, without a commensurate rise in the enzymes which remove H_2O_2 from the cell, would be detrimental since the hydroperoxide can be readily converted to the •OH. Thus, like melatonin often stimulates SOD, it also upregulates the two enzymes, glutathione peroxidase (GPx) (of which there are several isoforms) and catalase (CAT) which metabolize H_2O_2 to innocuous products [82-85]. In doing so, the number of •OH generated is held in check since its precursor is reduced to low intracellular

concentrations. The association of melatonin with the regulation of enzymes which modulate free radical concentrations are summarized in Fig. (**2**).

NO, once formed, can couple with the O_2•- to generate the strong oxidizing agent, ONOO-. Because of this, the enzyme that catalyzes the formation of NO, i.e., nitric oxide synthase (NOS), is often considered to be pro-oxidant. In a number of studies, melatonin has been shown to reduce the activity of NOS [86, 87], an action that may well contribute to its substantial antioxidative activity. Whether the inhibitory action of melatonin on NOS activity is due to this molecule per se, or to one of its metabolites, i.e., AMK, is being debated [88].

Reduced glutathione (GSH) is an important intracellular antioxidant which is not uncommonly depleted from cells that are under elevated oxidative stress. GSH is utilized in the reaction of GPx with H_2O_2 when it is converted to its oxidized disulfide, GSSG. Usually, greater than 95% of the glutathione within cells is in the reduced form, i.e., as GSH. Hence, when it is converted to GSSG it is quickly re-cycled back to GSH by glutathione reductase (GRd). Interestingly, melatonin also reportedly stimulates the activity of GRd, making GSH available as an antioxidant and as a substrate for GPx [70, 89, 90]. Thus, melatonin availability favors a high GSH:GSSG ratio.

Given that GSH is an important component of the cellular antioxidative defense system, it is essential that its synthesis be maintained at a high level. Melatonin contributes to this by stimulating the activity of the rate-limiting enzyme in GSH synthesis, glutamylcysteine ligase (GCL) (formerly, gamma-glutamylcysteine synthase) [91, 92]. Melatonin's action on GCL is illustrated in Fig. (**2**).

Melatonin clearly has a host of means whereby it could protect the brain from oxidative stress during hypoxia resulting from an ischemic episode followed by reperfusion injury. Reperfusion injury is damage that occurs when an ischemic region is reperfused with oxygenated blood and the tissue re-initiates aerobic metabolism. Regardless of the multiple mechanisms that melatonin employs to protect tissue from oxygen and nitrogen-based reactants, it is unknown which of the factors is most important in preserving neural tissue during hypoxia and reoxygenation.

DAMAGE CONTROL: MELATONIN PROTECTS AGAINST I/R INJURY

While the majority of studies which used melatonin to ameliorate the damage associated with I/R have used experimental models of focal ischemia, in particular middle cerebral artery occlusion (MCAO) [3-6, 93], global ischemia models have been used in a number of reports [94-97]. Regardless of the model employed, melatonin was able to minimize the neural damage to both grey and white matter resulting from transitory hypoxia followed by reoxygenation [98]. Most often the protective actions were attributed to the ability of the indoleamine to incapacitate free radicals that are generated in abundance during I/R [99, 100]. Besides protecting the brain, melatonin is likewise effective at the level of the spinal cord in limiting neural damage resulting from a free radical assault [43].

While scavenging of free radicals has been commonly invoked as a major means by which melatonin stymies neural and glial death during the acute phase of I/R, this is likely not the only option to explain the protective actions of melatonin. Melatonin also suppresses NO• production by reducing nrNOS activity in neurons via a calmodulin-dependent pathway [86, 87, 101] and abolishing iNOS expression in infiltrating macrophages due to melatonin's ability to reduce NF-κB translocation to the nucleus or its binding to DNA [102-104]. It has also been documented that melatonin stimulates NADH-coenzyme Q reductase (Complex I) and cytochrome c oxidase (Complex IV) of the respiratory chain within mitochondria. This results in a decrease in election leakage from these sites with a consequential lowering of free radical generation while maintaining normal ATP production [68-70, 105, 106]. Preservation of ATP levels is critical to preserving functional tissue and reducing apoptosis/necrosis in the area of the brain transiently deprived of blood. Finally, melatonin administered either before ischemia (usual duration is 30-90 minutes) onset or at reperfusion also reduces excitotoxicity and cerebral infiltration of immune cells both of which contribute to neural damage during I/R (Fig. **1**) [107].

As noted above, the most common model in which melatonin has been explored to determine its efficacy in attenuating damage during I/R is MCAO. In these studies, blood pH, pCO_2, pO_2, blood pressure and core temperature were commonly monitored and were found unchanged when melatonin was administered [108, 109].

While the vast majority of studies have used pharmacological amounts of melatonin in an attempt to reduce neural damage due to hypoxia and reoxygenation, melatonin at physiological concentrations also may assist in reducing the damage. Surgical removal of the pineal gland, a procedure that lowers endogenous blood levels of melatonin to a minimum, also causes the development of larger cortical infarctions (relative to those in pineal-intact animals) four to six hours after MCAO [110]. Melatonin also reduces the inflammatory response that accompanies brain damage during ischemia; this aids in limiting tissue destruction in the affected brain region [111]. The lower infiltration of inflammatory cells into the damaged neural tissue may stem from melatonin's ability to suppress mRNA and protein expression of tumor necrosis factor-α (TNF-α) and the intercellular adhesion molecule-1 (ICAM-1) as shown in the colon [112].

The ability of melatonin to reduce infarct size and edema during I/R [3, 6] involves the same processes that the indoleamine utilizes to lessen apoptosis. The exogenous administration of melatonin in advance of ischemia induction or at reperfusion restores the injury-induced loss of phosphorylated Akt (pAkt), p-Bad and Bcl-X_L levels and prevents the dissociation of p-Bad from 14-3-3 as well as attenuating caspase-3 activation [113, 114]. Melatonin is also capable of preventing the opening of the mitochondria transition pore and the discharge of cytochrome c into the cytoplasm [72, 115]. As astrocytes play an important role in preserving neural homeostasis, it is interesting that several studies have shown that melatonin prevents the death of these cells that accompanies their exposure to H_2O_2 or to glutamate [72, 73, 116].

Tissue plasminogen activator, i.e., t-PA, is frequently used clinically to dissolve clots in vessels so the downstream tissue can again be perfused with oxygenated blood. t-PA itself has some neural toxicity when it is used for its intended purpose. Studies by Kilic *et al.* [113] and by Chen *et al.* [116] documented that melatonin, given in conjunction with t-PA, at the onset of MCAO or six hours after reperfusion decreased t-PA mediated iNOS expression and restored pAkt levels.

Koh [117] has further examined the role of melatonin in preventing apoptosis by investigating whether the indoleamine modulates the anti-apoptotic signal through the activation of Akt and its downstream targets, FKHR, AFX and 14-3-3. In rats that underwent MCAO, melatonin prevented the ischemia-mediated reduction in pPDK1, pAkt, pFKHR and pAFX but failed to alter the level of 14-3-3. These findings are consistent with melatonin's protective effects during I/R involving the activation of Akt and the phosphorylation of FKHR. Koh [118] also tested whether melatonin's ability to reduce apoptosis in the brain damage by hypoxia and reoxygenation related to the activation of a mammalian target of rapamycin (mTOR) and p70S6 kinase as well as its downstream target, S6 phosphorylation. Besides documenting once again that melatonin reduces infarct volume and apoptosis that results from I/R (Fig. **3**), this investigator reported that the presence of melatonin during neural I/R prevented the injury-induced reduction in Akt activation and the drop in phosphorylation of mTOR and p70S6 kinase as well as the subsequent decrease in S6 phosphorylation. Again, these results document the involvement of Akt and its downstream targets as a means by which melatonin preserves the viability of cells during I/R.

From the data summarized above, it is obvious that melatonin functions in multiple ways to mitigate neural damage during the acute phase of neural hypoxia and reoxygenation. One of the major processes by which melatonin mediates protection of neurons and glial presumably relates to its ability to function as a direct free radial scavenger and indirect antioxidant. How critical these roles are, however, in holding brain damage to a minimum is difficult to precisely quantify. Other actions of melatonin that may aid in reducing neural damage in I/R include its, a) ability to increase the efficiency of electron flow through the mitochondrial respiratory chain, b) anti-inflammatory actions, c) reducing excitotoxicity and d) limiting apoptosis. Since these processes are all inter-related, it is likely that they are all essentially equally important in resisting neural destruction that accompanies transitory ischemia followed by a re-established flow of oxygenated blood in the acute phase of stroke.

In the days following the acute stroke phase, the interval commonly known as the sub-acute phase (3-14 days post ischemia), melatonin-treated animals also fare better. These animals typically exhibited improved electrophysiological responses relative to those measured in non-melatonin treated animals [109]. Similarly, in spinal cord-injured rats given melatonin immediately subsequent to the induced trauma exhibit improved motor and sensory evoked potentials as well as behavioral performance (motor function score and inclined plane test) more than a week after the traumatic episode [119]. Biochemically, molecular indices of oxidative stress, i.e., levels of lipid peroxidation, amount of protein carbonyls, etc.) are always lower in the injured neural tissue from melatonin-treated animals than from those receiving vehicle.

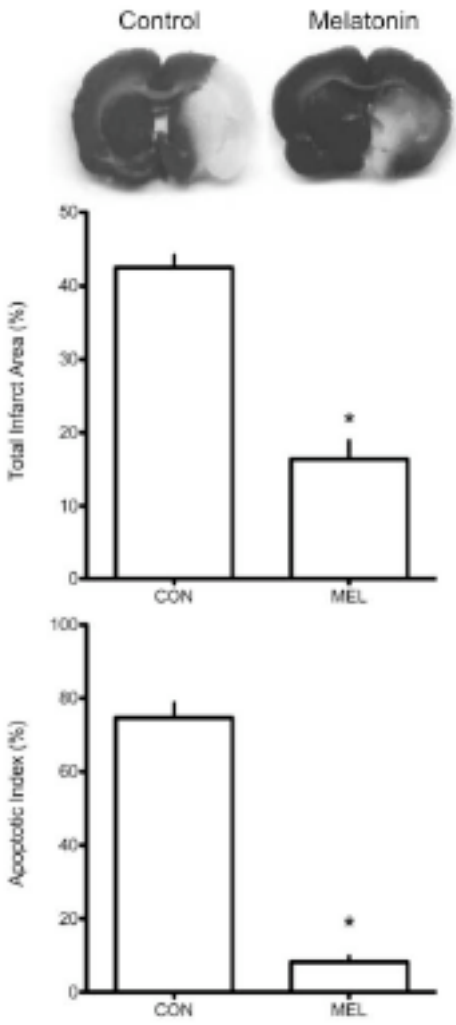

Fig. (3). Infarct volume (light area on front sections of the brain) and apoptosis frequency (estimated by percentage of TUNEL positive cells in the cerebral cortex) at 24 hours after permanent occlusion of the middle cerebral artery in rats. Melatonin treated animals were given 5 mg/kg of the indole 30 minutes prior to initiation of ischemia. Melatonin significantly reduced both infarct volume and the apoptotic index (*p <0.01). Total infarct area is expressed relative to the total area of the frontal brain section. TUNEL stained cells, as an index of apoptosis, were counted in the cerebral cortex on the side of the arterial occlusion. All other parameters measured also indicated the preservation of ischemic brain tissue after melatonin treatment. The amount of brain tissue salvaged by melatonin is representative of many other studies as well. This figure was modified and adopted from Koh [117].

Long-term (after a month) benefits of melatonin are also apparent in models of I/R. When measured 90 days after the induction of global ischemia in rats, place learning (Morris water maze) and working memory (eight-arm Olton radial maze) were improved in the melatonin-treated rats relative to those not given the indoleamine [120]. Behavioral

preservation was associated with fewer pyramidal cells lost in the hippocampus after melatonin treatment; these neurons are considered to be involved with memory. Besides preserving the pyramidal neurons, the long-term benefits of melatonin may also relate to its ability to stimulate neurogenesis [121-123].

CONCLUDING REMARKS

The multifaceted functions of melatonin have proven that this molecule has important protective actions in the central nervous system and it has been shown to be especially beneficial in preserving brain morphology, biochemistry and neurobehavior that are disrupted by I/R. Unfortunately, many methods that have been found to be effective in protecting the brain in experimental I/R models, have failed to be useful in the clinical situation. With regard to melatonin, it has never been examined as to its potential protective actions during ischemia and reperfusion of the brain of humans. It has, however, been widely tested in humans where it possesses potent antioxidant activity (as in animals) [42, 84, 124] and, furthermore, it has never been found to be toxic or to have significant side effects. In view of this, it should be given consideration not only for protection of the brain from I/R injury but other organs as well where the consequences of hypoxia and reoxygenation are highly detrimental.

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