Summary. Cerebral edema/brain edema refers to the accumulation of fluid in the brain and is one of the fatal conditions that requires immediate medical attention. Cerebral edema develops as a consequence of cerebral trauma, cerebral infarction, hemorrhages, abscess, tumor, hypoxia, and other toxic or metabolic factors. Based on the causative factors cerebral edema is differentiated into cytotoxic, vasogenic, osmotic and interstitial cerebral edema. Treatment of cerebral edema depends on timely diagnosis and medical assistance. Pragmatic treatment strategies such as antihypertensive medications, nonsteroidal anti-inflammatory drugs, barbiturates, steroids, glutamate and N-methyl-D-aspartate receptor antagonists and trometamol are used in clinical practice. Although the above mentioned treatment approaches are being used, owing to the complexity of the mechanisms involved in cerebral edema, a single therapeutic strategy which could ameliorate cerebral edema is yet to be identified. However, recent experimental studies have suggested that melatonin, a neurohormone produced by the pineal gland, could be an effective alternative for treating cerebral edema. In animal models of stroke, melatonin was not only shown to reduce cerebral edema but also preserved the blood brain barrier (BBB) integrity. Melatonin’s beneficial effects were attributed to its properties, such as being a potent anti-oxidant, and its ability to cross the BBB within minutes after its administration. This review summarizes the beneficial effects of melatonin when used for treating cerebral edema.

Key words: Cerebral edema, Melatonin, Cytotoxic cerebral edema, Vasogenic cerebral edema, BBB disruption, Vascular endothelial growth factor, Aquaporin, Matrix metalloproteinases, Nitric oxide

Introduction

Cerebral edema or brain edema refers to excess accumulation of fluid in the brain parenchyma resulting in increased brain volume. Any injury to the brain could result in cerebral edema, including conditions such as traumatic brain injury (TBI), ischemic stroke, brain hemorrhages, infections and tumors. In the above mentioned conditions, although there is a primary injury to the brain parenchyma, the subsequent occurrence of edema has been reported to contribute further to neural tissue damage, resulting in neurological dysfunction. Furthermore, cerebral edema is also reported as a secondary complication to unrelated diseases such as diabetic ketoacidosis, acute liver failure, high altitude sickness and salicylate poisoning (Adeva et al., 2012).

Cerebral edema may occur either due to the intracellular accumulation of water or due to the disruption of the BBB which results in extracellular accumulation of fluid and protein in the brain. Although cerebral edema is classified into cytotoxic, vasogenic, interstitial and osmotic edema, the common forms observed clinically are cytotoxic and vasogenic cerebral edema. Symptoms of cerebral edema are non-specific and vary depending on the cause and severity of injury. Milder forms of edema are generally reversible and the brain volume is compensated with a decrease in cerebrospinal fluid (CSF) and blood volume. However, these cerebral autoregulatory mechanisms fail as the brain fluid volume rapidly increases in severe edema.
resulting in structural alterations such as thinning of white matter, brain midline shift, cerebral ischemia and, ultimately, fatal cerebral herniation through the foramen magnum (Bosma et al., 2002; Ho et al., 2012).

Despite investigations being underway for decades an effective treatment for cerebral edema is still lacking. However, several investigations have proposed pragmatic medical treatments such as hyperventilation, osmotherapy (mannitol and hypertonic saline), diuretics, hypothermia and sedation (propofol, barbiturates) to alleviate cerebral edema (Ho et al., 2012). Corticosteroids, which are known to reduce the BBB permeability, were also proposed to be a therapeutic alternative for treating vasogenic edema. For cases that are obstinate, a decompressive craniectomy is preferred as a last resort. Recently, the use of melatonin (5-methoxy-N-acetyltryptamine), a hormone produced by the pineal gland, to reduce the BBB permeability, were also proposed to be a therapeutic alternative for treating vasogenic edema. It is highly lipophilic and has the ability to cross the BBB, which was evident with its increased concentration in the brain minutes after its administration (Le Bars et al., 1991). Although growing experimental evidence points to the fact that melatonin is effective in reducing cerebral edema arising from different etiologies, clinical studies are warranted to better understand its therapeutic abilities. In this article, we have reviewed the pathophysiology of different forms of cerebral edema and have summarized the current knowledge available on the beneficial effect of melatonin in alleviating the damage caused by cerebral edema.

**Types of cerebral edema and the mechanisms involved**

As mentioned above, there are four different types of cerebral edema. However, the cytotoxic and vasogenic forms of cerebral edema are well appreciated clinically. Several mediators have been implicated in the pathogenesis of cerebral edema (Fig 1). In this section, the role played by the different mediators such as aquaporin-4 (AQP4), vascular endotelial growth factor (VEGF), nitric oxide (NO), adenosine triphosphate (ATP) etc. in causing various forms of cerebral edema is discussed.

**Cytotoxic edema**

Cytotoxic edema or cellular edema occurs as a result of impairment in the energy-dependant mechanisms, a common observation documented in several forms of brain injury. It affects the grey matter more than the white matter, resulting in excess accumulation of fluid within the neurons, glial cells, axons and myelin sheaths. Being involved in the clearance of K⁺ and glutamate, astrocytes are more prone to accumulate water when compared to other brain parenchymal cells (Nag et al., 2009). Cytotoxic edema is more prevalent in conditions where the brain cells undergo a hypoxic-ischemic insult, excitotoxic insult or are exposed to metabolic poisoning. Other causes of cytotoxic edema include TBI, infections and metabolic disorders of kidney and liver. In hypoxic-ischemic conditions, the cells use their stored reserves of ATP to abrogate the oxidative phosphorylation mechanisms (Heo et al., 2005). Owing to the resultant deficiency of ATP, the affected cells fail to carry out the energy dependent mechanisms and fail to maintain homeostasis. Furthermore, the lack of ATP results in superfluous entry of cations, primarily Na⁺, into the cells. This inability of the cells to control the excess influx of cations is mostly attributed to the formation of cytotoxic edema. In this type of cerebral edema, while there is an excess influx of cations such as Na⁺ into the neurons and glial cells, the efflux of these cations from the cells fails. The extrusion of Na⁺ from the cell is essential to maintain cellular volume. Furthermore, the influx of Na⁺ creates a driving force for the influx of anions such as Cl⁻ through the chloride channels to neutralize the ionic shift formed within the cell. The subsequent increase in osmolarity within the cell favours the inflow of water into the glial cells or neurons through the AQP channels, a water channel present on the plasma membrane, (Go, 1997; Kempski, 2001) resulting

**Fig. 1.** An illustration summarizing the various mediators involved in causing different types of cerebral edema such as cytotoxic, vasogenic, osmotic and interstitial cerebral edema.
in cytotoxic edema. The cells further undergo morphological changes such as swelling and rupture of plasma membrane. The swelling of glial cells, especially the astrocytes, is a hallmark of cytotoxic edema. Astrocytes are known to outnumber the neurons and have the capacity to expand five times more than their original volume. Also, the presence of AQP4 on the astrocytic foot processes has been implicated in this (Manley et al., 2000). This was also evident with findings from AQP4 null mice which demonstrated reduced astrocytic swelling and increased survival in mice subjected to water intoxication (Manley et al., 2000) and ischemic stroke (Manley et al., 2000). Despite having normal levels of AQP4, reduced astrocytic swelling was reported in dystrophin null mdx-βgeo transgenic mouse and the α-syntrophin null mouse subjected to ischemia. Since AQP4 requires dystrophin and α-syntrophin for its membrane localization, the lack of these molecules in the above mentioned mice models meant that AQP4 is less expressed on astrocytic foot processes. Furthermore, there was reportedly reduced cerebral edema in the above mentioned experimental models, which implies that AQP4 could have a role in causing cytotoxic edema (Frigeri et al., 2001; Vajda et al., 2002; Amiry-Moghaddam et al., 2003). In addition to hypoxia and ischemia, cellular poisoning could also lead to the development of cytotoxic edema. For example, cellular intoxications with exposure to chemicals such as methionine sulfoximine and cuprizone isoniazid were reported to cause swelling of astrocytes. Triethyltin and hexachlorophene intoxications cause accumulation of water in intramyelinc clefts and produce striking white matter edema, whereas axonal swelling is a hallmark of exposure to hydrogen cyanide (Nag et al., 2009). Cytotoxic edema is itself not fatal, but the ionic gradient created in the extracellular space due to the excess intracellular accumulation of water leads to transport of molecules across the BBB. During the initial stages of cytotoxic edema the BBB remains intact. Subsequently, during the chronic phase the BBB disrupts resulting in vasogenic edema.

Vasogenic edema

BBB is crucial for maintaining homeostasis in the brain microenvironment. Any damage to the BBB could result in increased vascular permeability, leading to vasogenic edema. Contrary to cytotoxic edema, in vasogenic edema the white matter is more prone to accumulate fluid than the grey matter owing to the presence of loose extracellular spaces (ECS). Vasogenic edema is known to occur as a consequence of disorders such as stroke, cardiac arrest, respiratory distress and carbon monoxide poisoning. Unlike the capillaries in the rest of the body the brain microvasculature lacks fenestrations and contains very few endocytic vesicles, suggesting limited transendothelial transport (Cornford and Cornford, 2002). Studies using molecular trackers have shown that the BBB is only permeable to small lipophilic molecules while the rest are generally actively transported across the BBB via specialized mechanisms/pathways (Nau et al., 2010). Thus the presence of vasogenic edema has been demonstrated clinically and experimentally by immunostaining for whole serum proteins, albumin, fibrinogen or fibronecin. Furthermore, in experimental animals the breakdown of the BBB is also assessed with the help of tracers such as Gadolinium DPTA, 125iodine-labeled serum albumin, Evans blue, horseradish peroxidase (HRP), Rhodamine isothiocyanate (RHiC) and dextrans (Nag, 2003).

The BBB is formed by tight junctions between the capillary endothelial cells, which rest on a basal lamina. The basal lamina is surrounded by astrocytic end feet, which have specialized channels and transporters known to be essential in maintaining BBB function (Zador et al., 2007), and pericytes. Based on the fact that vasogenic cerebral edema is due to the disruption of BBB followed by a leakage of intravascular fluid into the brain and lack of mechanisms to clear the excess fluid, the hypothesis that water channels of astrocytes, especially the AQP4 channels, might have a role in the formation of vasogenic edema was put forth. Furthermore, this hypothesis was supported with findings from AQP4 null mice which reported an increased intracranial pressure associated with increased brain water and subsequent mortality (Papadopoulos et al., 2004; Bloch et al., 2005). Models in which vasogenic edema was a predominant form of edema, including cortical冷 injury, tumor implantation and brain abscess models, ascertained a role for AQP4 in clearing the fluid accumulated in the extracellular space (Papadopoulos et al., 2004; Bloch et al., 2005), as AQP4-null mice used for the above mentioned conditions were shown to accumulate more water in the brain when compared to wild-type mice. Irrespective of the way the water enters the brain the main route of exit from the brain to the blood has been suggested to be via the AQP4 channels expressed on the astrocytic foot process (Nag et al., 2009).

Additionally, factors such as VEGF, NO, inflammatory mediators and reactive oxygen species (ROS) have been reported to be responsible for BBB disruption. VEGF is known to increase BBB permeability (Hermann and Zechariah, 2009); however, the mechanism involved in increasing vascular permeability has not been completely elucidated. In conditions such as hypoxia and cerebral ischemia, VEGF has been documented to increase cerebral vascular permeability and thereby induce vasogenic cerebral edema (Zhang et al., 2000; Harrigan et al., 2002; Kaur et al., 2006a; Kaur and Ling, 2008). Consistent with this is the finding that either systemic or intracerebroventricular administration of VEGF during the early hours of post ischemic phase could increase BBB leakage and tissue damage (Zhang et al., 2000; Abumiya et al., 2005; Kaya et al., 2005). However, efforts to inhibit VEGF had remarkable neuroprotective effects, which included...
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Reduced BBB permeability, reduced risk of hemorrhagic transformation following focal cerebral ischemia, decreased infarct volume and cerebral edema (van Bruggen et al., 1999; Harrigan et al., 2003; Kaya et al., 2005; Kimura et al., 2005; Chi et al., 2007; Kumai et al., 2007; Chiba et al., 2008; Koyama et al., 2010). VEGF is known to act via its two receptors VEGF receptor 1 (VEGFR1) also known as fms-like tyrosine kinase 1 (Flt-1) and VEGF receptor 2 (VEGFR2) also known as fetal liver kinase 1 (Flk-1). Both the receptors for VEGF; VEGFR1 and VEGFR2 have been shown to have a role in maintaining BBB permeability (Kilic et al., 2006; Vogel et al., 2007; Abdul Muneer et al., 2012; Mendonca et al., 2013). Schreurs et al (2012) demonstrated that VEGF requires both VEGFR1 and VEGFR2 to enhance the permeability of blood vessels. In the blood samples collected from women suffering from pre-eclampsia, levels of VEGFR1, a soluble VEGF receptor, was found to be elevated and it was suggested to increase BBB permeability in these women (Amburgey et al., 2010). In the postmortem brains of patients with multiple sclerosis, the increased expression of VEGF by astrocytes and VEGFR2 by endothelial cells was correlated to increased BBB permeability (Argaw et al., 2006). In animal models of CD8 T-cell induced BBB disruption, inhibition of neuropilin, a co-receptor for VEGFR2/Flk-1 activation, was demonstrated to decrease BBB disruption (Suidan et al., 2012). In brain microvessel endothelial cell (BMVEC) cultures, inhibition of Flt-1 effectively decreased the increased permeability (Argaw et al., 2009). Although the mechanism by which VEGF exerts destructive effects on the BBB still remains elusive, studies have proposed a role for VEGF in diminishing tight junction proteins in the brain vascular endothelium (Argaw et al., 2009; Morin-Brureau et al., 2011). In cultured BMVEC, VEGF was found to decrease the transendothelial electrical resistance and the expression of proteins such as zona occludens-1 and occludin, which are components of tight junctions between vascular endothelial cells. Argaw et al (2009) demonstrated that VEGF could reduce the expression of tight junction component claudin-5 leading to BBB breakdown. Additionally, VEGF is known to influence the phosphorylation of proteins including those containing receptor phosphotyrosine Src homology 2 domains (Guo et al., 1995; Wang et al., 2001). As phosphorylation of proteins is essential for assembly and maintenance of tight junctions, excess VEGF could be speculated to interfere with tight junction assembly and disruption. These findings provide evidence that VEGF reportedly up-regulated in conditions such as cerebral ischemia (Lennmyr et al., 1998), TBI (Shore et al., 2004) and tumors (Senger et al., 1994), could contribute significantly to the disruption of the BBB and in the initiation of vasogenic edema. Furthermore, VEGF has been found to increase the permeability of the BBB by inducing NO production (Mayhan, 1999). In vitro studies have also shown that it causes enhanced vacuolations of the endothelial cells (Julien et al., 2008) resulting in enhanced transendothelial transport of serum derived substances (Kaur et al., 2006a).

NO, which is known to modulate the transport of ions, nutrients, and other molecules, is also known to regulate the function of the BBB (Janigro et al., 1994). However, excess production of NO has been shown to increase the permeability of the BBB (Shukla et al., 1996; Thiel and Audus, 2001). Administration of NO donors and nitric oxide synthase (NOS) inhibitors was helpful in understanding the contribution of NO in causing BBB disruption and subsequent vasogenic edema (Boje, 1995, 1996; Koedel et al., 1995). In mice administered with lipo-polysaccharide, NO produced from inducible NOS (iNOS) was shown to enhance the permeability of BBB (Minami et al., 1998). The permeability of cerebrovascular endothelium cultures increased when they were treated with NO donors (Uteberggenov et al., 1998). Factors such as histamine, VEGF and glutamate, which are known to enhance the permeability of the BBB mediate their action through NOS and NO (Mayhan, 1996, 1999; Mayhan and Didion, 1996). In addition, NO is believed to mediate bradykinin and tumour necrosis factor-α (TNF-α) induced BBB disruption (Nakano et al., 1996; Worrall et al., 1997). These findings support the notion that synthesis/release of NO could contribute to increased BBB permeability and thereby vasogenic edema.

Apart from the above factors, matrix metalloproteinases (MMPs), zinc- and calcium-dependent endopeptidases, are known to degrade all kinds of extracellular matrix proteins, including fibronectin, laminin, proteoglycans and type IV collagen (Sternlicht and Werb, 2001; Rosenberg, 2002). Growing evidence from both experimental and clinical studies suggests that MMPs are involved in weakening and rupturing the brain vasculature, thereby increasing the risk of cerebral haemorrhage/vascular leakage (Rosell et al., 2008; Rosell and Lo, 2008). The type IV collagenase which belongs to the MMP proteolytic family is capable of degrading the basal lamina of blood vessels and is implicated in the development of vasogenic edema in conditions including multiple sclerosis, bacterial meningitis and ischemic stroke (Leppert et al., 2001; Nag et al., 2009). Different forms of MMPs are reported to be expressed in different cell types; for example MMP-9 is known to be expressed on endothelial cells whereas astrocytes express MMP-2 (Candelario-Jalil et al., 2007). Several studies have demonstrated that the expression of MMP-9 is increased following a brain injury. Furthermore, MMP-9 is known to be activated by ROS and its expression is likely to be regulated by oxidative stress (Rajagopalan et al., 1996; Mori et al., 2004; Pustovrh et al., 2005). In patients suffering from ischemic stroke the levels of MMP-9 remained elevated even after months following the episode (Clark et al., 1997). In animal models of cerebral ischemia, MMP-9 levels were found to be elevated, and associated with this there was BBB disruption (Aoki et al., 2002; Tejima et al., 2009). In mice subjected to white matter damage
Seo et al. (2013) demonstrated the increased synthesis of MMP-9 by oligodendrocyte progenitor cells and the subsequent reduction in BBB integrity. In spontaneously hypertensive rats, both permanent and temporary middle cerebral artery occlusion resulted in increased production of MMP-9 and MMP-2 (Rosenberg et al., 1996; Yang et al., 2007). Associated with this, the expression of tight junction proteins claudin-5 and occludin was reduced (Yang et al., 2007) suggesting disruption of the BBB and the subsequent development of vasogenic cerebral edema. However, treatment using inhibitors for MMPs, MMP neutralizing antibodies and knockout studies have shown a significant reduction in BBB damage (Asahi et al., 2000, 2001; Yang et al., 2007). Asahi et al (2000) reported that in animal models of focal cerebral ischemia, the BBB was preserved when the gene for MMP-9 was knocked out. Taken together, these findings add support to the point that MMPs have a role in the development of vasogenic edema.

Hypoxic-ischemic conditions are also associated with an inflammatory response in the central nervous system (CNS) (Bona et al., 1999; Cowell et al., 2002) resulting in disruption of the BBB (Tu et al., 2011). Chemokines and cytokines, produced by glial and endothelial cells in hypoxic-ischemic conditions, enhance lymphocyte migration across the BBB (Bona et al., 1999) and also increase the permeability of the blood vessels (Glabinski and Ransohoff, 1999). TNF-α, which is synthesized in the brain in response to middle cerebral artery occlusion, hypoxia, TBI, bacterial meningitis etc. has been reported to cause disruption of the BBB (Yang et al., 1999). In rats, intracisternal injections of TNF-α resulted in increased transport of radiolabelled albumin across the BBB suggesting disruption of the BBB (Kim et al., 1992). In bovine BMVEC, TNF-α was found to enhance permeability by inducing the production of cyclooxygenase-2 (COX-2) and prostaglandins (Mark et al., 2001). In rats, prostaglandin E2, which is produced by COX-2 when injected intracerebrally enhances the permeability of the BBB (Schmidley et al., 1992). In a rat cranial window model, selective inhibition of COX-2 reduced TNF-α mediated BBB breakdown (Trickler et al., 2005). Besides TNF-α, interleukin (IL)-1β has also been reported to alter BBB permeability (Argaw et al., 2006). In autopsy brain samples from patients suffering from multiple sclerosis Argaw et al (2006) demonstrated the increased concentration of IL-1β, hypoxia inducible factor -1α (HIF-1α) and VEGF. They speculated that IL-1β, by activating HIF-1α and its target VEGF, could increase BBB permeability. The IL-1β induced BBB breakdown is likely to be mediated by cytokine-induced neutrophil chemoattractant -1, which is known to attract neutrophils (Anthony et al., 1998). Similar to TNF-α, IL-1β was also reported to favour BBB breakdown by inducing COX-2 and prostaglandin synthesis (Moore et al., 2004). Furthermore, studies have suggested that TNF-α and/or IL-1β could favour BBB breakdown by inducing MMP-9 expression via the nuclear factor-κB (NF-κB) signalling pathway in different types of cells (Esteve et al., 2002; Xie et al., 2004; Liang et al., 2007). COX induced by both TNF-α/IL-1β has been shown to increase the expression of MMP-9 (Candelario-Jalil et al., 2007).

**Osmotic edema**

In this type of edema there exists an osmotic gradient between the blood and the brain tissue while the BBB remains intact. Osmotic edema is more common in subjects having diabetic ketoacidosis, inappropriate secretion of anti-diuretic hormone, acute dilutional hyponatremia and in patients undergoing excessive hemodialysis (Sterns et al., 1986; Glaser, 2001). In the above mentioned conditions, the serum Na+ ions drop below normal values and when the serum Na+ is less than 120 mmol/L, the intravascular fluid enters into the brain (Arieff et al., 1976). Development of osmotic edema might lead to an increase in the CSF volume in the ventricles by seepage of fluid from the brain (DiMattio et al., 1975). Osmotic edema is also possible when the brain tissue osmolality is high and the plasma osmolality is normal, which is prevalent following brain hemorrhage, infarction and contusions (Nag et al., 2009).

**Interstitial edema**

Interstitial edema is another rare form of edema and is best characterized in noncommunicating hydrocephalus and hepatic encephalopathy (Kale et al., 2006). Although the pathogenesis of interstitial cerebral edema is still unknown, studies on animal models of hydrocephalus have thrown light on the molecular mechanisms involved. In kaolin-induced hydrocephalus, although the cerebral blood flow was above the ischemic levels, the development of interstitial edema impaired the oxidative metabolism at a very early stage (Kawamata et al., 2003). The rise in the intraventricular pressure results in the migration of CSF into the white matter and thereby increase the extracellular fluid volume (Milhorat et al., 1970). The reduction in the expression of AQP4 in the brain is implicated in the reduced clearance of the excess extracellular fluid, ultimately leading to cerebral edema (Bloch et al., 2006). In more advanced stages, there is destruction of myelin and axons followed by activation of microglia in the white matter, leading to a thinning of white matter (Del Bigio, 1993). Associated with this there might be disruption of ependyma and distortion of cerebral vessels (Del Bigio, 1993).

**Melatonin**

Melatonin (5-methoxy-N-acetyltryptamine) is a derivative of amino acid tryptophan and was first discovered by Lerner and his co-workers (Lerner et al., 1958). Melatonin was, however reported to be synthesized by other tissues such as retina (Dubocovich,
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Melatonin and ischemic injury

One of the early symptoms following ischemic injury is the formation of cerebral edema. It is essential to abate the cerebral edema to avoid subsequent chronic neural damage (Kondoh et al., 2002). Several studies have shown the effectiveness of melatonin in reducing cerebral edema following ischemic insults (Lee et al., 2004, 2005). Administration of melatonin to animal models of ischemia has been proven beneficial as melatonin inhibited the activation of microglia and prevented the transmigration of leukocytes into the ischemic brain (Lee et al., 2007). Melatonin was also shown to maintain the integrity of the BBB following ischemic stroke (Chen et al., 2006). In Mongolian gerbils, melatonin was demonstrated to reduce the brain water content which was increased following ischemia (Cuzzocrea et al., 2000). Since cytotoxic cerebral edema and vasogenic cerebral edema are the most common forms of edema found in a clinical set up, the beneficial effect of melatonin in treating these types of edema are detailed below.

Melatonin and cytotoxic edema

Melatonin has been proven effective in reducing the cellular changes associated with cytotoxic edema. For example, in rats subjected to cold-induced brain injury melatonin significantly reduced the swelling of neurons and axoplasm, and prevented the disruption of the plasma membrane (Gorgulu et al., 2001). In addition, the cellular organelles such as nucleus and mitochondria were protected from degenerative changes (Gorgulu et al., 2001). In hippocampus of neonatal rats, following hypoxic exposure, there were swollen dendrites, axons and astrocyte processes (Kaur et al., 2008). However, these changes were reported to have subsided following melatonin administration to hypoxic rats (Kaur et al., 2008). A similar finding was reported by Kaur et al. (2006b) in the cerebellum of hypoxic rats, wherein melatonin reduced hypoxia-induced swelling of astrocytes and AQP4 expression on them, suggesting a reduced water uptake by these cells, and hence, decreased edema formation. Melatonin was shown to facilitate the down-regulation of AQP4 by activating protein kinase C (Bhattacharya et al., 2014). In animal models of middle cerebral artery occlusion, melatonin was shown to remarkably reduce the death of glial cells at the ischemic core (Borlongan et al., 2000). In hippocampal slice cultures treated with amyloid beta (Aβ), melatonin was shown to prevent structural...
changes, including edema and necrotic profiles (Clapp-Lilly et al., 2001). Furthermore, in the brains of Anabas testudineus, melatonin has been reported to have an influential effect on the Na⁺/K⁺ ATPases (Divya et al., 2006) which is essential for preventing cytotoxic edema. In rats subjected to cerebral ischemia, administration of melatonin restored down-regulated Na⁺/K⁺ ATPases activity in the brain (Toklu et al., 2009). The remarkable ability of melatonin to interact with mitochondrial complexes and to enhance electron flow (Martin et al., 2002) could also be attributed to the potential of melatonin in inhibiting cytotoxic edema. Taking all of the above into consideration, it could be speculated that melatonin could inhibit/prevent cytotoxic edema by enhancing energy flow or by influencing Na⁺/K⁺ ATPase.

**Melatonin and vasogenic edema**

Melatonin, as mentioned above, is a potent antioxidant and because of this property it has been shown to be effective in reducing vascular permeability and thereby the vasogenic edema (Cuzzocrea et al., 1997; Bilici et al., 2002). Moreover, melatonin was shown to reduce the increased vascular permeability by interacting with endothelial cells (Lotufo et al., 2006) and inhibiting the binding of neutrophils to endothelial cells (Lotufo et al., 2001). Alternatively, melatonin is also reported to exert its suppressive effects on the tissue concentration of VEGF (Kaur et al., 2006b, 2007; Sivakumar et al., 2008) resulting in reduced vascular permeability and edema in many parts of the CNS (Kaur et al., 2006b). Studies on different kinds of cancers have revealed that melatonin has the potential to decrease the serum concentrations of VEGF (Lissoni et al., 2001; Pandi-Perumal, 2006). In rats subjected to hypoxia, increased expression of VEGF and NO was a hallmark finding suggesting edema. Melatonin has been shown to suppress these factors and thereby the vasogenic edema in different regions of the brain including periventricular white matter, hippocampus, cerebellum, ventrolateral medulla, nucleus tractus solitarius and choroid plexus (Kaur et al., 2006b, 2008, 2010, 2011; Sivakumar et al., 2008). In hypoxic brains, melatonin not only reduced the production of VEGF and NO, but also reduced the vascular leakage which was evident with reduced uptake of rhodium isothiocyanate (RhIC) and horseradish peroxidase (HRP) injected intraperitoneally or intravenously (Kaur et al., 2006b, 2008). In addition, in hypoxic neonatal rats melatonin effectively reduced the expression of Flt-1 and Flk-1 expression on the retinal vasculature (Kaur et al., 2013) and also in the brain (unpublished data). This could be attributed to the efficacy of melatonin in preserving the integrity of the BBB.

In animal models of ischemia, melatonin was demonstrated to decrease the expression of iNOS and neural NOS (nNOS) (Koh, 2008). In the brains of Mongolian gerbils melatonin was shown to prevent the increase in NO concentration and peroxynitrite concentration which is implicated in causing damage to the BBB (Guerrero et al., 1997). Melatonin prevented N-methyl-D-aspartate induced excitotoxicity by inhibiting nNOS (Escames et al., 2004) and subsequent NO production. N1-acetyl-5-methoxykynuramine (AMK), a metabolite of melatonin in the brain, was reported to be the active compound against nNOS and NO production (Leon et al., 2006). Melatonin was speculated to either directly scavenge NO or to inhibit nNOS activity by interacting with calmodulin, a molecule required for NO production from nNOS (Bettahi et al., 1996; Pozo et al., 1997). The inhibition of NO production is of major significance as it can hinder the generation of free radicals and peroxynitrite (Pandi-Perumal et al., 2006) which are associated with BBB breakdown.

Melatonin was shown to be beneficial in cerebellar edema by down-regulating AQP4 expression in the astrocytes (Kaur et al., 2006b) following hypoxic injury. In rat models of subarachnoid haemorrhage (SAH) administration of melatonin was found to significantly reduce mortality. This was accompanied with a reduction in brain water content (Ayer et al., 2008). This could be attributed to the ability of melatonin to influence the expression of proteins such as VEGF and AQP4, which are up-regulated in SAH (Badaut et al., 2003; Josko, 2003) and which are known to play a role in causing edema. These findings imply that melatonin protects the integrity of BBB.

Besides VEGF and AQP4, melatonin has the remarkable ability in ischemic brains to reduce MMP-9 levels, which are known to play a role in BBB breakdown. The mechanism by which melatonin reduces the MMP-9 levels remains to be completely elucidated. However, the ability of melatonin to inhibit the activation of NF-κB (Beni et al., 2004; Qin et al., 2012) and increase the expression of tissue inhibitors of metalloproteinase (TIMP)-1 (Swarnakar et al., 2007; Paul et al., 2008) has been implicated in melatonin mediated reduction of MMP-9. In human umbilical vein endothelial cells (HUVEC) treated with IL-1β, melatonin protected the integrity and permeability of the HUVEC monolayer by down regulating the NF-κB pathway and subsequent production of MMP-9 (Qin et al., 2012). In mouse models of focal cerebral ischemia, following reperfusion, melatonin was reported to increase TIMP-1 and plasminogen activator inhibitor-1 which was associated with the concomitant reduction in MMP-9 levels (Tai et al., 2010). In addition, melatonin is known to exert its protective property by activating the extracellular signal-regulated kinases (ERK1/2) pathway. Melatonin mediated ERK1/2 activation has also been implicated in TIMP-1 up-regulation, which is essential for MMP-9 down regulation (Hung et al., 2008). Furthermore, a recent study has pointed out that melatonin inhibits the catalytic activity of MMP-9 by binding to its active site (Rudra et al., 2013).

Besides the above, melatonin prevented BBB damage by inhibiting oxidative stress and inflammation.
The excess free radicals formed in any pathological condition could cause a detrimental effect on the highly lipid enriched brain by oxidatively degrading the lipids and also disrupting the BBB. However, administration of melatonin has been reported to neutralize the deleterious effects of free radicals and protect the brain. Clinically, in newborns affected by sepsis, melatonin reduced serum malondialdehyde (MDA) levels (an end product of lipid peroxidation) (Gitto et al., 2001). Similarly, melatonin, when administered prior to in-utero asphyxia, prevented the formation of highly toxic hydroxyl radicals in the brains of late gestation sheep fetus (Miller et al., 2005). In rat models of hypoxic periventricular white matter damage, melatonin administration rendered protection to the white matter by significantly reducing the MDA levels, which were elevated due to hypoxia (Kaur et al., 2010). A similar finding was reported by Cuzzocrea et al. (2000) in the brains of melatonin treated post-ischemic Mongolian gerbils. Furthermore, accumulating evidence, both clinically and experimentally, has shown the efficacy of melatonin in reducing inflammation in the brain (Gitto et al., 2001; Welin et al., 2007; Hutton et al., 2009). In asphyxiated neonatal rats, melatonin treatment was demonstrated to reduce the number of activated microglia, which have a pivotal role in the inflammatory cascade (Welin et al., 2007). Melatonin was found to inhibit the inflammatory process by preventing cytokine production in activated microglia (Min et al., 2012). In LPS-stimulated Raw264.7 cells, melatonin inhibited the excess production of TNF-α, IL-1β, IL-6, IL-8 and IL-10 production (Xia et al., 2012). Melatonin was reported to impede NF-κB translocation (Min et al., 2012) which is essential for cytokine production. Melatonin when provided in combination with electroacupuncture, effectively decreased the expression to two primary inflammatory mediators TNF-α and COX-2 (Liu and Cheung, 2013).

Taken together, it could be stated that melatonin, either by influencing the expression of factors such as VEGF, NO and AQP4 or by directly scavenging the free radicals and reducing inflammatory mediators could protect the integrity of the BBB and prevent vasogenic cerebral edema.

**Conclusion**

Molecular mechanisms leading to cerebral edema are multifaceted and require further research to understand the complexity involved. Although research in the past decades led to the identification of molecules involved in the pathogenesis of cerebral edema, an effective treatment remains to be fully explored owing to the contributions from multiple pathways. A single therapeutic agent which could ameliorate all the pathologies associated with cerebral edema is clearly desirable. Recent findings have suggested that melatonin could be a therapeutic molecule of interest as it has been proven beneficial in reducing cerebral edema and associated pathologies. Although melatonin has been proven beneficial in experimental studies involving animals, clinical studies are warranted to better understand the efficacy of melatonin. In view of the beneficial effects of melatonin it is justified to suggest that melatonin might be a potential therapeutic agent for treatment of cerebral edema arising from different etiologies.

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