The role of Circadian rhythm in blood-brain barrier permeability

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Abstract. The blood-brain barrier (BBB) is a crucial structure that regulates the exchange of molecules between the brain and the bloodstream. Recent studies have shown that the BBB permeability exhibits a rhythmic pattern regulated by the circadian rhythm (CR). The CR is regulated by core clock genes that form transcriptional-translational feedback loops, which control the expression of proteins involved in BBB permeability regulation. The diurnal variation in BBB permeability is controlled by tight junction proteins, aquaporin-4, and ion transporters, which exhibit rhythmical expression patterns. Therapeutic approaches targeting rhythmical BBB permeability have important implications for drug delivery and clinical potential in treating disease such as Alzheimer's disease and multiple sclerosis which are two neurological disorders associated with disrupted circadian rhythms and BBB dysfunction. This review aims to introduce the role of CR in BBB permeability regulation, highlighting recent findings on the molecular mechanisms underlying CR regulation of BBB permeability and discussing the potential implications for drug delivery.

Keywords: Blood-brain barrier, molecular clock, circadian rhythm, permeability, drug delivery.

1. Introduction

The blood-brain barrier (BBB) is a protective network of large, tightly connected cells that separate the blood and brain. These cells are called "endothelial cells" (EEC). The endothelium lines most capillaries in the body, and the BBB is a subset of this lining. The tight junctions are responsible for the selective permeability of the BBB, allowing only small lipid-soluble molecules to go in past. The tight junctions also prevent the passage of large hydrophilic molecules, including most drugs and therapeutic agents. The role of the BBB is to act as a guard to the brain from harmful toxins and pathogens while maintaining brain homeostasis [1]. Through this, the BBB ensures that the brain is protected from the entry of harmful pathogens for instance bacteria, viruses and fungi. In addition, the BBB maintains a stable environment for neuronal signalling by regulating the concentration of ions and neurotransmitters.

Recent studies have shown that the CR regulates BBB permeability. The BBB permeability varies over the day, with the highest permeability occurring during the night [2]. The molecular clock regulates this diurnal variation in BBB permeability. Clock and Bmal1 are expressed in endothelial cells of the BBB and regulate the expression of tight junction proteins, for instance claudin-5 and occluding [2]. These tight junction proteins are essential for maintaining BBB integrity and preventing the entry of harmful substances into the brain. The Clock and Bmal1 proteins also regulate the expression of aquaporin-4 (AQP4) [2]. AQP4 is a water channel protein that facilitates the movement of water across the BBB. In addition, Cry and Per proteins regulate other ion transporters involved in BBB permeability regulation. These findings suggest that CR plays an important role in regulating BBB permeability [2].

Both Bmal1 knockout mice and wild-type mice exposed to light at night exhibited a massive increase in BBB permeability. This increase in BBB permeability happened irrespective of the fact that serum levels of ghrelin, a hormone involved in food intake, were lower in both groups [2]. This suggests that BBB permeability can be regulated by the central circadian clock and its interaction with peripheral glands [2]. In addition to CR regulation of BBB permeability through Clock-Bmal1 complex, another pathway may regulate diurnal variation of BBB permeability. Bmal1 knockout mice exhibited higher BBB permeability than their wild-type counterparts. This result suggests that Bmal1

knockout mice have an altered central and peripheral CR [3]. These results indicate that multiple pathways in the brain, including the central circadian pathway and peripheral circadian-controlled glands, regulate BBB permeability [2].

CR affects drug clearance by regulating BBB permeability. CR also influences the timing of drug delivery to a target organ or tissue. Genetic mutations that lead to defects in the core clock gene Bmal1 or Cry1 produce impaired responses to nocturnal hypoxia [3]. These findings suggest that murine diurnal rhythms are influenced by both the intrinsic Bmal1-Cry1 oscillator as well as extrinsic changes in oxygen levels [2, 4]. Circadian-regulated BBB permeability could regulate the delivery of drugs to the brain. When the brain is exposed to nocturnal hypoxia and normal serum levels of ghrelin are present, Bmal1-Lck KO mice display increased peripheral ghrelin levels. This suggests that BBB permeability may play a role in determining drug clearance from the blood. CR controls BBB status on an hourly basis [2, 4] Through this mechanism, BBB permeability can maintain the normal level of a drug in the blood.

2. The influence of CR on drug delivery

Circadian oscillations may also regulate BBB permeability to enable proper clearance of other drugs, including morphine and ethanol. In these mice lacking TGR5, circadian oscillations in serum levels of cho and cyclophilin A are disrupted. This disruption results in an impairment of peripheral ghrelin signaling [2]. Disturbed BBB permeability may weaken some drugs outside the brain and make them more susceptible to leakage into the blood [3]. A deficiency of the clock protein, Bmal1 (brain and muscle Arnt-like protein 1), leads to disrupted sleep/wake cycles, hyperactivity and cognitive deficits. As a result of defective clock proteins, many Bmal1 mutant mice exhibit increased BBB permeability. If a drug were administered at night or in conditions of nocturnal hypoxia, these mutant mice would exhibit increased serum levels of the drug. This could lead to adverse drug interactions with the brain and impair the effectiveness of the drug [2].

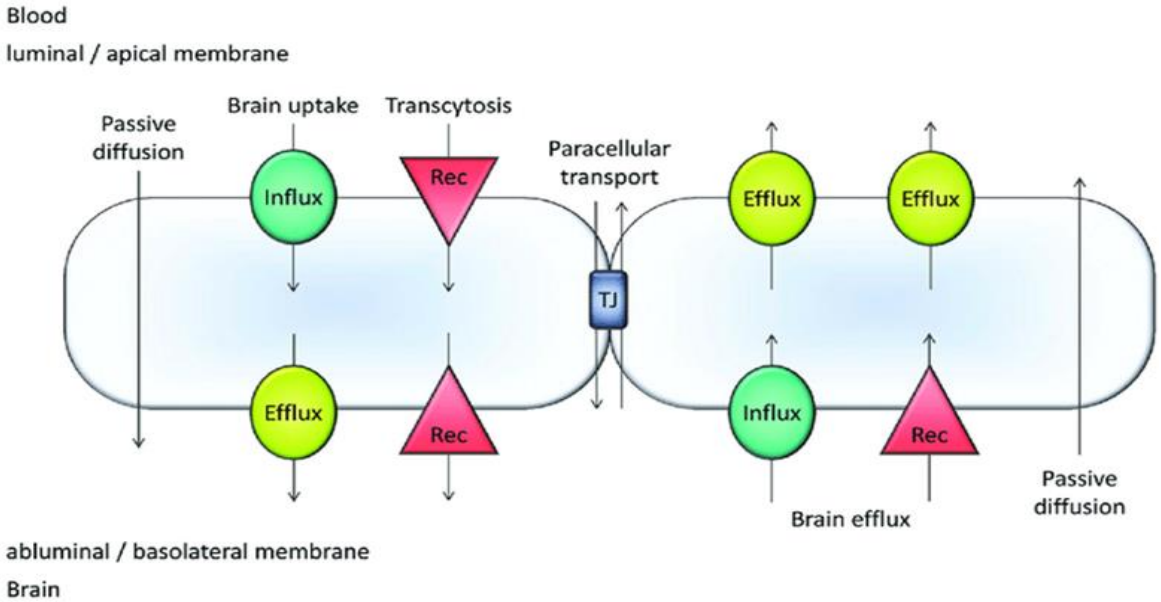

Figure 1. Major pathways at the BBB (Source; Somonte 2020 DOI:10.3389/fnagi.2019.00196).

Clock-Bmal1 regulates the expression of tight junction proteins through the transcriptional activation of Kruppel-like factor 4 (Klf4). Klf4 regulates tight junction protein expression and is required to maintain BBB integrity. The molecular clock regulates the expression of tight junction proteins in the BBB [4]. The expression of these proteins oscillates with a 24-hour rhythm, with the highest expression occurring during the night when BBB permeability is. Several transporters, like P-glycoprotein and multidrug resistance-associated protein 1, are rhythmically expressed in the BBB. These transporters are crucial in protecting the brain from toxic substances and drugs.

3. Therapeutic approaches targeting rhythmical BBB permeability

The rhythmical expression of BBB transporter proteins has important implications for drug delivery. Drugs that require high BBB permeability for effective delivery may be administered at night when BBB permeability is highest. Conversely, drugs that are substrates for transporters highly expressed during the daytime may have reduced efficacy if administered at night for instance [5]. A study by [6] demonstrated that the permeability of the BBB exhibits diurnal fluctuations in mice. The study showed that the expression of certain BBB transporter proteins, such as P-glycoprotein and breast cancer resistance protein, is higher during the daytime than at night, resulting in lower BBB permeability at night. This pattern was found to be disrupted in mice with disrupted circadian rhythms, such as those with mutations in core clock genes. Another study by [7] investigated the effects of administering a chemotherapy drug, temozolomide, at different times of the day on its efficacy in mice with brain tumors. The study found that administering temozolomide at the time of peak BBB permeability resulted in higher drug concentrations in the brain and improved tumor control. In humans, a clinical trial by [8] investigated the effects of administering a chemotherapy drug, irinotecan, at different times of the day on its efficacy in patients with brain tumors. The study found that administering irinotecan at the time of peak BBB permeability resulted in higher drug concentrations in the brain and improved treatment outcomes. In terms of neurological disorders, a study [6, 7] showed that disruption of circadian rhythms in a mouse model of Alzheimer's disease led to increased BBB permeability and accelerated disease progression. This suggests that targeting the rhythmical expression of BBB transporter proteins may be a promising therapeutic approach for treating Alzheimer's disease. Another study [9] investigated the effects of targeting the circadian clock in a mouse model of multiple sclerosis. The study found that modulating the expression of clock genes improved disease outcomes by reducing inflammation and BBB dysfunction. For example, targeting the higher expression of neurotoxic substances during the daytime may help reduce their accumulation in the brain and slow the progression of neurological disorders. There have been studies on specific ion transporters and their regulation by the circadian rhythm. For example, the expression of the glucose transporter GLUT1 in the BBB has been shown to be regulated by the core clock gene Bmal1, with higher expression during the dark phase [10]. Similarly, the expression of the amino acid transporter LAT1 has been shown to be regulated by the circadian clock in the BBB, with higher expression during the light phase [11]. Regarding targeted drugs, there are some drugs that target specific ion transporters in the BBB, such as monocarboxylate transporter 1 (MCT1) inhibitors for treating brain tumors [12]. The efficacy of these drugs in stopping tumor growth depends heavily on the time-of-day administration. For instance, the efficacy of MCT1 inhibitors in primary glioblastoma is higher at night [13].

Alzheimer's disease and multiple sclerosis are two most dominant disorders associated with disrupted circadian rhythms and BBB dysfunction. Growing evidence suggests that disruptions in circadian rhythms can contribute to the development and progression of Alzheimer's disease and multiple sclerosis. Another study found that CR disruptions can exacerbate the symptoms of multiple sclerosis and that treatments aimed at restoring circadian rhythms can improve the clinical outcomes of patients with multiple sclerosis [14]. Therapeutic approaches targeting the rhythmical expression of BBB transporter proteins may effectively treat these disorders. For example, targeting the higher expression of neurotoxic substances during the daytime may help reduce their accumulation in the brain and slow the progression of neurological disorders. In addition, drugs that require high BBB permeability for effective delivery may be administered at night when BBB permeability is highest. In contrast, drugs that are substrates for transporters highly expressed during the daytime may have reduced efficacy if administered at night [15]. One promising area of research is the application of chronotherapy in treatment of neurological disorders. Chronotherapy involves administering drugs at specific times of day to take advantage of the body's natural rhythms and improve treatment efficacy. For example, a recent clinical trial found that administering the drug interferon-beta at night, when BBB permeability is highest, resulted in significantly greater reductions in brain lesions in patients with multiple sclerosis compared to administration during the day [16].

In terms of Alzheimer's disease, a study [17] investigated the effects of administering the drug memantine, which is a substrate for the glutamate transporter excitatory amino acid transporter 1 (EAAT1), at different times of the day on its efficacy in an animal model of Alzheimer's disease. The study found that administering memantine at the time of peak BBB permeability resulted in higher drug concentrations in the brain and improved outcomes. Another recent study [18] investigated the effects of administering the drug ubenimex at different times of the day in a mouse model of Alzheimer's disease. They found that administering ubenimex at the time of peak BBB permeability resulted in higher drug concentrations in the brain, reduced amyloid plaque formation, and slowed disease progression. These studies suggest that targeting memory and learning may be a promising area for applications of chronotherapy for Alzheimer's disease. Furthermore, studies using animal models show that blocking circadian clock genes may improve outcomes in neurological disorders. For example, an experiment [19] found that a clock gene, Bmal1, is upregulated in the brains of Alzheimer's disease patients and that knocking down this gene improves cognitive function in rodent models. Another study by [20] found that deleting the key enzyme Bmal1 from mouse embryos significantly reduced the development of Alzheimer's disease-like symptoms in adult mice.

4. Benefits of Chronotherapy

Chronotherapy represents an important area of research because it has a variety of potential benefits for humans. One advantage of chronotherapy is that drugs that require high BBB permeability to be effective can be taken at the time of day when BBB permeability is highest [21]. For example, if a drug is highly sensitive to blood-brain barrier transport, it would need to be taken during the early circadian phases when BBB permeability is high. If a drug requires a transporter that has low expression during the circadian phases, it should also be taken at this time to ensure maximum effectiveness [21, 22]. A common example of this involves timing treatments for epilepsy based on the epileptic seizure cycle. In addition, chronotherapy could be used to improve treatment outcomes in diseases with circadian rhythms or cyclical behavior [23]. For example, a recent study [24] found that the age of onset of bipolar disorder is affected by circadian rhythms and that patients with bipolar disorder have various circadian abnormalities. Thus, chronotherapy may be a useful way to treat bipolar disorder in many patients. Chronotherapy is also useful for targeting specific neurological dysfunctions that may have circadian or cyclical components [25]. For example, Chronic pain is a common adverse effect of many drugs and chronic pain can be treated effectively with the aid of chronotherapy. Chronic pain occurs when neurons in the spinal cord are damaged, resulting in abnormal signals being sent to the brain. Chronotherapy may be useful in treating this condition because it allows high blood-brain barrier permeability at night when BBB permeability is highest and target therapeutics at this time to improve outcomes [26]. Additionally, traditional electroconvulsive therapy (ECT) still remains the gold standard for the treatment of depression and is effective at a much higher percent in patients with bipolar disorder and other CR disturbances [27]. Chronotherapy may be useful for some patients because it allows high BBB permeability during the day when ECT is most effective. Generally, chronotherapy may have several benefits for humans including increased efficacy of drugs that require high BBB permeability to be effective, improved treatment outcomes in diseases with circadian or cyclical components, and easier treatment of chronic pain [28]. These potential benefits suggest that chronotherapy should be used more extensively as a research tool to improve our understanding of how drug actions work in the human body.

Typical Chronotherapy Protocols Chronotherapy protocols vary widely, but many involve administering drugs at certain times of the day for a specific amount of time. A common protocol is to administer drugs at the time of high BBB permeability in the early circadian phases and then gradually extend it further into the circadian phase until it reaches peak BBB permeability [29]. A more detailed protocol involves administering the drugs at a constant time of the day, but with a variable amount of time over the course of several weeks to months. This type of protocol occurs when multiple drugs are administered at different times of the day to match their BBB permeability

at each time. This allows for a gradual increase in drug concentrations in the brain leading to improved treatment outcomes for some diseases [30]. The onset of chronotherapy protocols is also variable, with most initiating very early in the circadian phase or late in the circadian phase depending on how long they should last and how much gradual increase in drug concentrations may be needed. A typical chronotherapy protocol involves administering a drug at the time of peak BBB permeability during the early circadian phase, such as during de-etiolation, followed by administering it at a constant time for a certain amount of time [31]. Most protocols involve a gradual increase in drug concentrations in the brain; however, some start with high concentrations. This is because timing treatments based on peak BBB permeability is difficult in people with Alzheimer's disease, multiple sclerosis, and dementia [31]. Therefore, these patients require drug administration at a constant time of the day for an extended period of time to achieve improved treatment outcomes.

5. Potential implications for drug delivery

The CR regulation of BBB permeability has potential implications for drug delivery. The diurnal variation in BBB permeability suggests that drug delivery may be optimized by timing drug administration to coincide with the peak BBB permeability. The CR regulation of drug transporters and receptors suggests that the efficacy of drug delivery may vary over the day. Drugs that are substrates for transporters and receptors that vary daily may have different pharmacokinetic profiles depending on the administration time [32]. Dysregulation of the CR can result in sleep disorders and metabolic imbalances, which the adverse effects of drug treatment can exacerbate. CR regulation of BBB permeability may be a therapeutic target for sleep disorders and metabolic imbalances associated with drug therapy. Furthermore, the circadian regulation of BBB permeability suggests that chronotherapy may be useful for targeting specific neuropsychiatric disorders such as Alzheimer's disease and episode-specific treatment of bipolar disorder [33].

6. Conclusion

Ion transport across the BBB is crucial in maintaining neural system function. The CR regulates BBB permeability, which may vary over the day. This variation in permeability suggests that drug delivery may be optimized by timing drug administration to coincide with the peak BBB permeability. Therapeutic approaches targeting the CR regulation of BBB permeability may help address neurological disorders associated with disrupted circadian rhythms and impaired BBB function.

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