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Review

Circadian rhythms in thrombosis and atherothrombotic events

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Abstract

Biological circadian rhythms in living organisms are regulated by molecular clocks. Several of these clocks are present in blood vessels, peripheral tissues, and immune cells. There is strong evidence linking dysregulation of circadian rhythms to the development of cardiovascular disease. Dysregulation of circadian rhythms is believed to activate inflammatory processes at specific times of day, leading to an increased risk of thrombosis and atherosclerosis progression. Research into circadian clock genes and molecular networks has the potential to identify therapeutic targets to reduce cardiovascular risk. In this review, we summarize the evidence linking circadian rhythms to thrombosis and atherothrombotic events and discuss potential therapeutic implications.

Keywords: circadian clock; autonomous; shift workers; thrombosis; atherosclerosis; acute coronary syndrome; stroke

1. Introduction

The word circadian (from the Latin *circa diem*, meaning *approximately a day*) describes endogenous biorhythms with a periodicity of 24 hours created by the rotation of the Earth [1]. The light-dark cycle regulates the self-sustained central circadian clock in the hypothalamic suprachiasmatic nucleus (SCN), and the central clock then resets peripheral clocks in other tissues. This clock network generates biorhythms in physiological functions and behaviors [2]. A negative feedback loop allows the circadian clock to self-regulate. Organisms possessing circadian clocks can synchronize physiological and behavioral processes to cyclic environmental factors, called Zeitgebers (from the German for *time giver*), showing that they have extrinsic adaptive value; moreover, circadian clocks can also coordinate with internal metabolic processes, suggesting that they have intrinsic adaptive value [3].

Circadian rhythms are maintained by inputs from a number of structures. A prominent example is the retina, which captures photons and transmits information through neurons to the SCN. Once incoming stimuli are integrated by the central clock, hormonal and neuronal stimuli regulate circadian gene expression in peripheral tissues [4]. Peripheral tissues are not passive in this process; non-SCN cells in peripheral tissues are equipped with their own endogenous circadian oscillators that direct rhythmic expression of clock genes. These clock genes exert a broad-ranging control over many biological processes, including fundamental metabolic pathways, such as glucose homeostasis and lipogenesis, or macrophage activity in the kidneys, liver, adipose tissue, and the vascular system [2,5].

Changes during the day are observed in a host of physiological parameters, including cardiac contractility; blood pressure; body temperature; heart rate; and electrophysiological parameters such as PR, QRS, and QTc-interval [6–8]. Diurnal increases in blood pressure occur twice per day, and the evening rise is mediated by the endogenous circadian clock [9]. Initially, the main regulator of circadian variations in physiological parameters was thought to be the autonomic nervous system. However, knock-out studies of clock genes in animal models and solid organ transplantation studies in humans have shown that while blockade of the autonomous nervous system diminishes rhythmicity, alterations to the molecular circadian clock induces a more general disruption of the 24-hour variation of physiological parameters. Thus, after autonomic denervation (occurring as a result of solid organ transplantation), 24-hour rhythmicity is maintained for some physiological parameters, such as heart rate, whereas whole-body genetic disruption of the molecular circadian clock blunts the diurnal variation of multiple physiological parameters [10,11].

There is evidence linking altered daily variations in these parameters to thromboembolic and cardiovascular events [12,13]. Thus, the disruption of circadian

rhythms seen in shift workers and sleep-disorder patients is associated with higher cardiovascular risk and adverse health outcomes [14]. Potential mechanisms include the dysregulation of circadian rhythms that regulate immune function, including those affecting lipid metabolism, pro-inflammatory cytokines, and immune-cell dynamics; these changes can lead to endothelial dysfunction, dyslipidemia, increased blood vessel stiffening, and loss of the nocturnal dip in blood pressure, resulting in hypertension and atherosclerosis-mediated coronary artery or cerebrovascular disease [15,16]. Understanding the dysregulation of circadian rhythms and how this leads to atherothrombotic events is crucial for the development of new treatments. In this review, we highlight evidence supporting a role for circadian rhythms in thrombosis and atherothrombotic events and outline potential therapeutic implications.

2. Atherosclerosis, thrombosis, and circadian rhythms

Atherosclerosis is a common mechanism underlying several manifestations of cardiovascular disease, including myocardial infarction, ischemic stroke, and cardiac arrest [17]. Endothelial dysfunction and high blood cholesterol induce the expression of membrane molecules that recruit leukocytes such as monocytes and other inflammatory cells. Once activated and adhered to the endothelium, monocytes differentiate into macrophages that drive chronic inflammation, proliferation, and apoptosis and lead to the formation of an atherosclerotic plaque [18].

The molecular clock regulates diurnal production of cytokines and oscillations in the numbers of leukocyte populations, including neutrophils, short-lived cells which show an optimal adaptation to the circadian rhythms of mammals [19,20]. Rhythmic recruitment of neutrophils may also be responsible for the circadian manifestation of several inflammatory diseases [19]. Studies in myocardial ischemia mouse models have established that neutrophil infiltration increases at night (zeitgeber time [ZT] 13, or 1 hour after lights off) through a process mediated by the chemokine receptor CXCR2, accounting for the more severe cardiac damage resulting from myocardial infarction at this time [21].

Plaque rupture can produce thrombosis, in which a blood clot forms and occludes the blood vessel. Oscillating circadian patterns have been detected for some of the hemostasis molecules involved in thrombosis, including procoagulation factors such as von Willebrand factor; factors VII, VIII, IX, and X; and anticoagulation factors such as antithrombin (AT), protein C, and protein S. Fibrinolysis molecules (D-dimers and factor X) [22] and the activity of plasminogen activator inhibitor-1 (PAI-1) show morning peaks [23]. Platelet production and aggregation is regulated by the central clock (via thrombopoietin) [24,25], with morning peaks in numbers and afternoon peaks in the activity and expression of activation molecules such as platelet factor 4 (CXCL4) and B-thromboglobulin (B-TG) [26].

Under physiological conditions, nitric oxide (NO) synthesis decreases during daylight hours, with a consequent reduction in vasodilation, and an opposite mechanism operates during the night [27]. Endothelial dysfunction linked to atherosclerosis is associated with further daytime reductions in NO synthesis, resulting in increased vasoconstriction and hypercoagulability, likely contributing to the higher incidence of cardiovascular events such as acute myocardial infarction (AMI) and stroke during morning hours [28–31]. The amplitude of the diurnal variation in blood pressure is increased in patients with hypertension, and the oscillation coincides with the temporal variability in their incidence of acute vascular events, such as AMI, sudden cardiac death, and stroke [32,33]. The independent effect of chronic circadian misalignment on cardiovascular disease risk factors still needs to be studied, but mounting evidence suggests that environmental factors that disrupt physiological rhythms might contribute to cardiovascular events, as well as increasing other risk factors typically associated with cardiovascular disease [34,35]. An imbalance in the circadian patterns of these molecules, inflammatory cells, the endothelium, and platelet function increases the probability of thrombosis and atherothrombotic events (Fig. 1).

3. Circadian rhythm: clock components and vascular involvement

The circadian clock is a cell-autonomous molecular mechanism through which clock proteins regulate circadian rhythms. The main clock proteins include the transcription factors brain-muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (BMAL1 or ARNTL) and circadian locomotor output cycles kaput (CLOCK) and the transcriptional modulators period 1/2/3 (PER1/2/3) and cryptochrome 1/2 (CRY1/2) (Fig. 2, Ref. [2,33,34,36–53]).

BMAL1 expression follows a diurnal pattern, but the phase and amplitude vary depending on the tissue and cell type. In vascular tissues such as in vascular smooth muscle cells and the mouse aorta coincides with the transition from the dark to the light phase [36], a very similar expression pattern occurs in liver [54]. In the mouse SCN and in the lateral part of the central nucleus of the amygdala, BMAL1 expression peaks at around ZT18, and in contrast, the dentate gyrus of the hippocampus BMAL1 expression peaks at around ZT2.5 (9 hours later) [55]. BMAL1 forms a heterodimer complex with CLOCK that induces the transcription of the circadian proteins PER1/2/3 and CRY1/2, especially during the daylight phase [54]. The BMAL1-CLOCK heterodimer induces transcription by binding E-box sequences in the PER1/2/3 and CRY1/2 promoter regions [56]. During the evening, PER1/2 and CRY1/2 associate and translocate to the nucleus [37,54]. During the night, the PER and CRY proteins are progressively phosphorylated, and the phosphorylated proteins are ubiquitinated by specific E3 ligases and eventually degraded by the proteasome

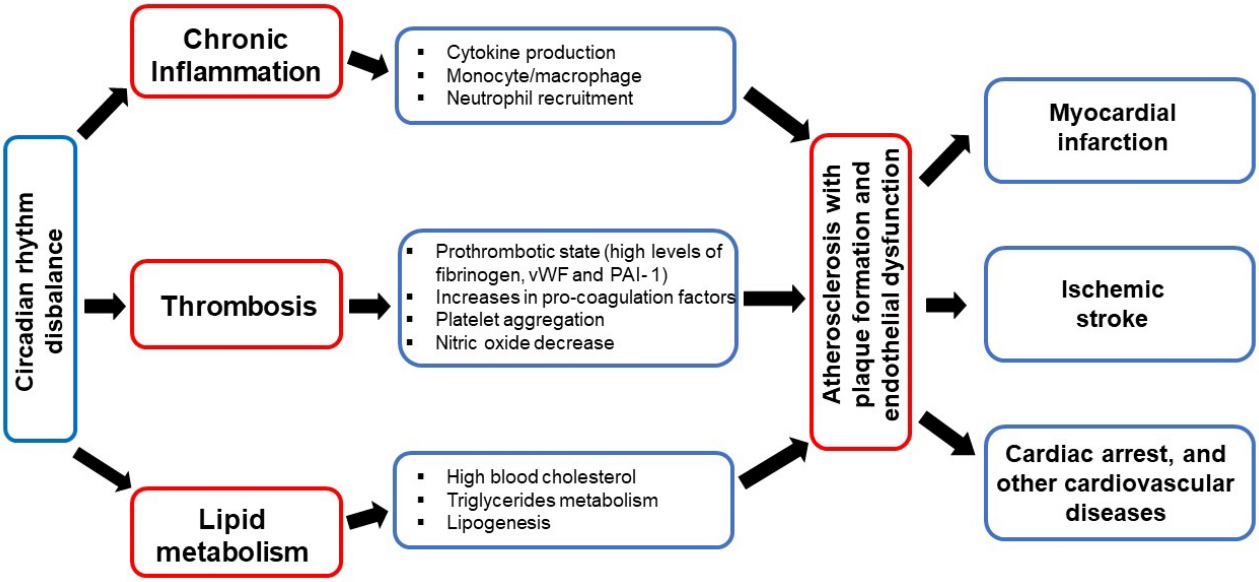

Fig. 1. Atherosclerosis, thrombosis, and circadian rhythms. Atherosclerosis is a common pathophysiological mechanism underlying cardiovascular disease. Under physiological conditions, the molecular clock regulates the diurnal production of cytokines and the recruitment of leukocytes and other inflammatory cells. The molecular clock also regulates critical lipid homeostasis mechanisms controlling cholesterol and triglyceride metabolism. Hemostasis molecules involved in thrombosis and that show a circadian rhythm include pro-coagulation factors, platelet aggregation factors, and vasorelaxation molecules. Disruption of the circadian patterns governing these molecules or controlling inflammatory cells and platelet function may contribute to endothelial dysfunction, leading to increases in thrombosis and atherothrombotic events, including myocardial infarction, ischemic stroke, and cardiac arrest.

[38]. PER1/2 and CRY1/2 repress their own transcription via a negative feedback loop after physically binding to heterodimers formed by BMAL1 with CLOCK or its paralog NPAS2 [54,57]. BMAL1 and CLOCK are basic helix-loop-helix/Per-ARNT-SIM (bHLH-PAS) proteins [58], and their transcription rate and rhythmicity are subsequently regulated by feedback loops involving two classes of nuclear receptor: the retinoic acid receptor-related orphan receptors (ROR$\alpha/\beta/\gamma$), which positively regulate the expression of BMAL1 and CLOCK, and reverse ERB (REV-ERB α/β), which negatively regulates their expression [2]. BMAL1-CLOCK heterodimers also control the expression of other loop components, such as proline and acidic amino acid-rich basic leucine zipper (PAR-bZIP), thyrotroph embryonic factor (TEF), D-box binding protein (DBP), and hepatic leukemia factor (HLF) [39]. The expression of genes containing D-box-cis elements, such as DBP, is controlled by the repressor E4BP4 and the PAR-bZIP transcription factors [39]. Only 22% of cycling mRNA transcripts in mammals are driven by de novo transcription, suggesting that both transcriptional and post-transcriptional mechanisms contribute to the circadian regulation of cycling mRNA levels in mammals [54]. The circadian clock regulates the transcription of around 10–15% of the genes expressed in the heart and cardiovascular system [40].

Mice lacking BMAL1-CLOCK, PER1/2/3, or CRY1/2 are prone to atherosclerosis, and several mechanisms contribute to this increased susceptibility. One involves the circadian regulation of the expression of chemokines and cytokines such as CCL2, monocyte chemoattractant protein-1 (MCP-1), IL-1, IL-6, and tumor necrosis factor-α (TNF-α) [41,42]. TNF-α-induced activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is involved in monocyte-macrophage (type Ly6chi) activation and migration to the atherosclerotic plaque in BMAL1-deficient animals [59,60]. Macrophages eliminate the exacerbated endotoxin-induced cytokine response observed at night. This activity is altered when the expression of REV-ERBα is compromised [19,61].

Liver metabolism, including lipid and glucose metabolic pathways and especially cholesterol and triglyceride metabolism, loses its circadian variation when BMAL1, CLOCK, or REV-ERB α/β are absent [43,44]. This can also occur upon PER2 loss of function, since PER2 controls peroxisome proliferating activated receptor (PPAR-γ), a critical regulator of lipid homeostasis [45]. In mice with diabetes, liver-specific overexpression of Cry1 can lower blood glucose and increase insulin sensitivity, whereas Cry1/2-deficient mice become obese more rapidly on a high-fat diet than control mice, showing upregulation of adipocyte genes associated with lipogenesis and lipid metabolism [46]. Cry1/2- and REV-ERB α/β-deficient mice have higher than normal blood glucose and impaired glucose metabolism [47,62].

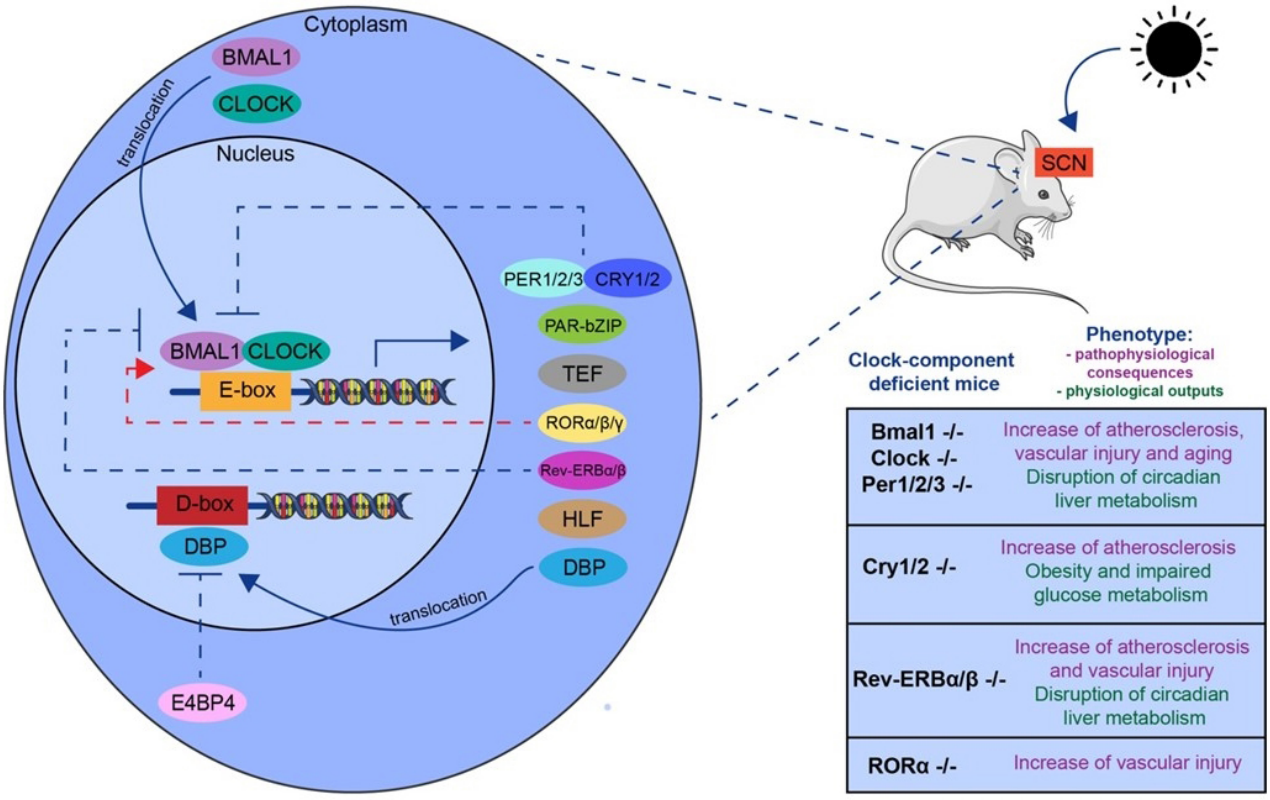

Fig. 2. The mammalian circadian clock in the SCN and its involvement in vascular diseases. CLOCK-BMAL1 heterodimer binding to E-box activates the transcription of different clock genes: PER1/2/3, CRY1/2, Rev-ERVa/b, RORa, HLF, TEF, PIR-bZIP and DBP [33,34,39]. PER and CRY proteins dimerize and block their own transcription by inhibiting CLOCK-BMAL1 activity in the nucleus [36]. Rev-ERB also inhibit CLOCK and BMAL1 expression [2]. This constitutes the negative limb of this transcriptional-translational feedback loop. In the opposite site, ROR proteins positively regulate the expression of CLOCK and BMAL1, constituting the positive limb of the transcriptional-translational feedback loop [2]. Finally, the nuclear factor E4BP4 repress DBP binding to D-box, inhibiting the expression of CCGs, forming the third stabilizing loop [39]. Deficiency or alteration of any of the components of the core circadian clock has an enormous impact at the vascular level, causing atherosclerosis [37–39] and risk of thrombosis [53], alteration of blood hemodynamics [43–47], promoting vascular injury [48], and contributes to aging [49–52]. It also alters liver function [40–42].

Other mechanisms involve hemodynamic and vascular functions. Lack of BMAL1, CLOCK, PER1/2, ROR α, or REV-ERB α/β in hematopoietic, endothelial, or smooth muscle cells leads to vascular injury, reduced blood flow, and an impaired ability to control blood pressure oscillations [48–50,63,64]. Deficiency in BMAL1 or PER2 is characterized by an increased vasoconstrictor response mediated by cyclooxygenase-1 (COX1) and reduced vasorelaxation mediated by decreases in NO and prostaglandin production [51,52]. All these factors increase the risk of hypertension in animal models [53]. The effects of BMAL1 deficiency are cell-type dependent; blood pressure rhythmicity is unaffected by deletion of Bmal1 in mouse cardiomyocytes, but is disrupted by deletion in vascular smooth muscle cells or endothelial cells [65].

The circadian machinery also regulates aging, with deletion of BMAL1 and CLOCK in mice leading to loss of the response to vasopressor stress [66,67]. Similarly, Per1-Per2 mutant mice show accelerated aging in association with altered circadian rhythms [68]. A significant number of CLOCK-regulated genes are associated with the cell cycle and cell proliferation. Wall thickening in CLOCK- and PER-deficient mice may be a consequence of collagen accumulation in the medial vessel layer [66,69].

Clock genes and proteins are linked to thrombosis and fibrinolysis factors [70]. For instance, both CLOCK and BMAL regulate PAI-1 gene transcription [71], and deficiency in BMAL1 may be related to high levels of fibrinogen, vWF, and PAI-1, creating a prothrombotic state with a increased risk in thrombosis [72]. The identification of circadian-clock genes that regulate thrombosis could clarify the role of circadian rhythms in the pathophysiology of thrombosis.

Most evidence for the effects of BMAL1-CLOCK, PER1/2/3, and CRY1/2 deficiency comes from studies in mice. Recently, the European Society of Cardiology Work-

ing Group on Cellular Biology of the Heart published a position paper on the key requirements for preclinical and translational research on circadian rhythms [1], with a view to future trials in humans.

4. Circadian rhythms and cardiovascular disease

4.1 Ischemic heart disease and acute coronary syndrome

Cardiovascular events occur more frequently in the early morning, with morning rates higher for both AMI and sudden cardiac death. The circadian rhythms of inflammatory cells play a role in AMI, and circulating neutrophils may migrate more avidly to the myocardium in the morning, triggering a stronger proinflammatory response due to increased expression of C-X-C Motif chemokine receptor type 2 (CXCR2) [21]. Some of these findings still need to be validated in humans [1].

In an animal model, morning onset AMI resulted in adverse remodeling with fibrosis and a larger infarct than seen after afternoon onset AMI [73], and morning onset AMI predicted atherosclerosis progression and AMI recurrence [74]. Gene disruption of BMAL-CLOCK (positive circadian component) appears to induce diurnal oscillation of fatty-acid oxidation through the transcriptional activity of the clock protein KLF15 [39]. Specific deletion of BMAL-CLOCK in cardiomyocytes increases infarct size in mice and causes sudden cardiac death, whereas disruption of other clock components, such as PER2 and REV-ERB α/β (negative components) reduces infarct size [73,75]. PER2, through the adenosine Adora2b receptor, enhances glycolysis and reduces fatty-acid oxidation in a mechanism dependent on hypoxia-inducible factor (HIF)-1α, leading to reduced infarct size [76].

Similar findings were reported upon deletion of clock genes in fibroblasts, which are involved in post-AMI remodeling. Deletion of fibroblast and cardiomyocyte clock genes led to a decrease in left ventricular ejection fraction and increased left ventricular dimensions [77].

The sleep-wake cycle in mammals is regulated by melatonin, a hormone released by the pineal gland at night. Melatonin has anti-inflammatory and antioxidant properties and this diurnal hormonal regulation is associated with cardiac protection, with melatonin levels correlating with a decreased risk of AMI [78]. Melatonin protects cardiac microvascular endothelial cells by inhibiting autophagy after ischemia–reperfusion injury via the AMP activated protein kinase/mammalian target of rapamycin (AMPK/mTOR) system [79].

4.2 Cerebrovascular disease and stroke

The circadian clock also regulates multiple metabolic functions of the central nervous system (CNS), including the brain and cerebrovascular circulation. As seen with ischemic heart disease, the onset of stroke symptoms shows a diurnal variation [31], with ischemic stroke and transient ischemic attack both predominantly occurring between 6 am and noon. Intracerebral hemorrhage (ICH) and subarachnoid hemorrhage onset shows two peaks (albeit less pronounced than ischemic stroke), with a main peak in the morning and a second in the evening [80]. A diurnal variation in the severity of ICH has been reported, with higher 30-day mortality for patients with morning onset [81].

Circadian rhythms in the brain affect molecular mechanisms, mitochondrial function, and adenosine triphosphate (ATP) levels. At the tissue level, the response of the brain to ischemia is dependent on the time of stroke onset. HIF-1-α (the primary mediator of hypoxia) interacts with the core circadian genes CLOCK and PER2 [82]. Excitotoxicity and oxidative stress affect neurons, and both processes are influenced by circadian rhythms. In mouse models of brain damage, levels of the NMDA receptor and glutamate (responsible for neurotoxicity) differed depending on what time of day the injury occurred [83]. The diurnal variations in glutamate concentration may be related to daily changes in the endogenous production of melatonin, which has neuroprotective and antioxidant properties [84].

In animal models, astrocytes deficient in BMAL1, CLOCK, or PER2 are more vulnerable to reactive oxygen species (ROS) [85–87]. This effect is thought to involve alterations to regulatory processes such as ATP release from astrocytes and oligodendrocytes, reflecting an influence of circadian signaling on glial cells [88]. Circadian genes also regulate endothelial nitric oxide synthase (eNOS), influencing oscillations in the vascular tone of the cerebral arteries, with important implications for ischemic and hemorrhagic stroke [51]. Bmal1 deficiency in mice also affects blood-brain barrier function and permeability [89]. In a mouse model of subarachnoid hemorrhage (SAH), PER2 expression was significantly higher in cells isolated from cerebrospinal fluid after ruptured aneurysm than in cells from unruptured aneurysms, reflecting that clock gene expression might regulates, in part, the severity of SAH, this disturbed molecular circadian rhythms may influence the severity of neuronal injury measured by changes in vasoreactivity, neuronal apoptosis, and myeloid-driven neuroinflammation [90]. Circadian rhythms may also affect stroke recovery, since clock genes are essential for neural stem cell differentiation, and disruption of circadian genes in mice leads to altered neurogenesis [91].

5. Therapeutic implications of circadian rhythms

One of the simplest ways to apply knowledge of circadian rhythms is to perform surgical and other procedures at specific times during the day cycle, which has the potential to enhance treatment success for diseases such as cancer [92]. Correcting circadian rhythm imbalances may also provide a route to reducing cardiovascular risk. For example, night-time administration of antihypertensive drugs improves overall 24-hour blood pressure profiles more ef-

fectively than daytime administration [93]. Moreover, low-dose aspirin is more effective at reducing morning platelet reactivity via cyclooxygenase 1 when administered in the evening rather than in the morning [94]; this may reflect circadian differences in the pharmacokinetics and pharmacodynamics or in the specific target response [1].

Chronopharmacology-based approaches could also be used to synchronize the targeting of inflammatory cell populations with their circadian patterns. For example, this strategy could increase the benefit of treatment with the beta-blocker metoprolol, which reduces infarct size after AMI by interfering with neutrophil and platelet interactions and neutrophil recruitment [95]. Similarly, inhibition of CXCR2 in mice represses leukocyte recruitment to plaques when the treatment is administered at night (ZT17), whereas daytime treatment (ZT5) has no therapeutic benefit [96].

Mouse studies have explored the possibility of modulating circadian rhythms by targeting clock genes. One example is the synthetic REV/ERB α ligand GSK4112, which modulates inflammation by attenuating macrophage cytokine production [61]. The REV/ERB agonist SR9009 is able to suppress atherosclerosis in mice [97], and an anti-inflammatory effect was detected upon targeting of CRY1/2 with the activator KL001 *in vitro* [98].

Another potential therapeutic target is the endovascular protective factor STIR1, a nicotinamide adenine dinucleotide (NAD+)-dependent protein capable of deacetylating transcription factors implicated in inflammatory processes, such as NF/kB [99]. In cell models, reduced levels of STIR1 are associated with altered lipid metabolism and increased foam cell formation [100]. Mice with STIR1 deficiency have altered expression of PER1/2, BMAL1–CLOCK, and CRY1/2, suggesting an involvement in circadian rhythms [101]. STIR1 may affect the circadian levels of metabolites involved in plaque formation, such as acetyl-CoA and NAD+ [102]. In general, STIR1 appears to function as an important link between circadian clock genes and lipid metabolism, suggesting that STIR1 activators could be used to prevent atherosclerosis [16]. This potential has been demonstrated for the STIR1 activator resveratrol, a polyphenol (a plant secondary metabolite). Resveratrol has antioxidant properties and has been shown to reduce obesity rates in fat-fed mice by reducing the circadian expression of the clock genes BMAL1, CLOCK, and PER2, as well as clock-controlled genes related to lipid metabolism, such as Sirt1, Srebp-1c, Pparα, Acc1, and Fas [103]. Other polyphenols that interact with circadian genes, such as equol, have been shown to improve lipid profile by increasing HDL-cholesterol and reducing LDL-cholesterol, total cholesterol, and serum triglycerides, resulting in a reduction in plaque formation and atherosclerosis [104].

Krüppel-like factors (KLFs) are transcription factors implicated in cell proliferation and differentiation. When overexpressed specifically in endothelial cells, KLFs can reduce atherosclerosis by interfering with vascular smooth cell differentiation genes [105]. BMAL1-CLOCK can directly regulate the KLF transcription rate, reflecting the importance of circadian rhythms in this process [106]. Besides its anti-inflammatory effects, KLF2 shows antithrombotic activity, increasing the expression of anti-thrombotic molecules such as thrombomodulin-1 and preventing the binding of thrombin to its receptor, protease-activated receptor 1 (PAR-1) [107].

6. Conclusions

Emerging evidence from experimental and clinical studies suggests that circadian rhythms driven by the molecular clock play a prominent role in the pathophysiology of thrombosis and atherothrombotic events, influencing or precipitating a state of pathological vascular remodeling, endothelial dysfunction, and thrombosis when dysregulated. Targeting circadian mechanisms and molecules may provide therapeutic benefit by interfering with pathological processes and decreasing cardiovascular morbidity and mortality. Further research is warranted to fully define circadian mechanisms and their relationship with cardiovascular disease.

Abbreviations

SCN, hypothalamic suprachiasmatic nucleus; AT, antithrombin; PAI-1, plasminogen activator inhibitor-1; B-TG, B-thromboglobulin; ON, nitric oxide; AMI, acute myocardial infarction; BMAL1 or ARNTL, brain-muscle aryl hydrocarbon receptor nuclear translocator-like protein 1; CLOCK, circadian locomotor output cycles kaput; ROR, receptor-related orphan receptors; PAR-bZIP, proline and acidic amino acid-rich basic leucine zipper; TEF, thyrotroph embryonic factor; DBP, D-box binding protein; HLF, hepatic leukemia factor; MCP-1, monocyte chemoattractant protein-1; TFN-α, tumor necrosis factor-α; PPAR, peroxisome proliferating activated receptor; COX1, cyclooxygenase-1; CXCR2, C-X-C motif chemokine receptor type 2; HIF, hypoxia-inducible factor; AMPK/mTOR, AMP activated protein kinase/mammalian target of rapamycin; ICH, intracerebral hemorrhage; ATP, adenosine triphosphate; ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; KLF, Krüppel-like factor; PAR-1, protease-activated receptor 1.

Author contributions

HB and EPM designed the review. EPM performed the research. EPM wrote the manuscript. AAC and AH provided help and advice on the research topic, figures and references. GM, SH, LV and NR provided help and advice on references and editing. All authors contributed to editorial changes to the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

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