Implications of disturbances in circadian rhythms for cardiovascular health: A new frontier in free radical biology

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ABSTRACT

Cell autonomous circadian “clock” mechanisms are present in virtually every organ, and generate daily rhythms that are important for normal physiology. This is especially relevant to the cardiovascular system, for example the circadian mechanism orchestrates rhythms in heart rate, blood pressure, cardiac contractility, metabolism, gene and protein abundance over the 24-h day and night cycles. Conversely, disturbing circadian rhythms (e.g. via shift work, sleep disorders) increases cardiovascular disease risk, and exacerbates cardiac remodelling and worsens outcome.

Notably, reactive oxygen species (ROS) are important contributors to heart disease, especially the pathophysiologic damage that occurs after myocardial infarction (MI, heart attack). However, little is known about how the circadian mechanism, or rhythm desynchrony, is involved in these key pathologic stress responses. This review summarizes the current knowledge on circadian rhythms in the cardiovascular system, and the implications of rhythm disturbances for cardiovascular health. Furthermore, we highlight how free radical biology coincides with the pathogenesis of myocardial repair and remodelling after MI, and indicate a role for the circadian system in the oxidative stress pathways in the heart and brain after MI. This fusion of circadian biology with cardiac oxidative stress pathways is novel, and offers enormous potential for improving our understanding and treatment of heart disease.

1. Introduction

Circadian rhythms are our internal day/night cycles of behaviour and physiology. They enable us to coordinate our biology with the external environment - to adapt to light and dark, activity and rest, and wake and sleep (reviewed in [1,2]). Circadian rhythms are endogenously driven by a cellular mechanism, which is a transcription-translation feedback loop with an approximately 24-h duration that cycles in all our cells including those of the cardiovascular system [3–7]. A region of the hypothalamus, termed the suprachiasmatic nucleus (SCN), orchestrates the cellular circadian mechanism in the tissues throughout our body, and helps to maintain healthy body physiology (reviewed in [8,9]). Indeed, it is increasingly recognized that intact circadian rhythms play an essential role in maintaining cardiovascular health, well-being, and recovery from heart disease (reviewed in [9–15]). Conversely, disturbing rhythms is associated with an increased risk of heart disease, adverse cardiovascular events, and worse outcomes (reviewed in [9–13,15–17]). Circadian rhythm disturbance is especially relevant in modern society, where we often experience desynchrony due to shift work, sleep disorders, jet-lag, school and work schedules, and the ubiquitous presence of 24/7 electrical lighting.

In this review we discuss circadian rhythms relevant to the cardiovascular system. We provide an overview of the current literature with regard to healthy cardiovascular physiology, and what happens when we disturb rhythms. This includes clinical studies relevant to humans, as well as experimental rodent models that help to define the underlying mechanisms. We highlight especially how disturbing rhythms may pertain to the free radical biology pathways, contributing to the pathophysiology or treatment of heart disease. This is an entirely new frontier for investigation, and increased understanding of such mechanisms will lead to new avenues for applying circadian biology to benefit patients clinically.
2. Circadian rhythms in normal cardiovascular physiology

Life on earth is subjected to 24 h day and night cycles. The circadian system has evolved to allow our physiology to be synchronous with this cycle – humans adapt to be awake during the day and sleep at night. As shown in Fig. 1, the circadian mechanism undergoes rhythmic daily cycling in all our cells, including the cardiomyocytes [3,18]. This regulates the rhythmic output of cardiac genes [3,7] and proteins [19–22], cardiac contractility [19,22–24], and metabolism [9,25]. Ultimately, we observe the output of this cellular mechanism as daily physiologic and behavioural rhythms, many of which are crucial to the cardiovascular system [9,10]. For example, our heart rate [26] and blood pressure [27,28] increase in the day and decrease at night. These normal cardiovascular rhythms help to prepare individuals for the physiological demands of rigorous activity during the day, and are associated with rest and renewal at night (reviewed in [10]). Our endocrine hormones cycle as well, including many relevant to the cardiovascular system. For example, the catecholamines epinephrine and norepinephrine peak in the day and trough at night in healthy individuals [29,30], paralleling the sympathetic and parasympathetic biases of our autonomic nervous system [31,32]. Moreover, circulating levels of innate immune cells and cytokines [30,33–38], clotting factors [30,39], and other classic biomarkers of normal cardiovascular physiology show a similar diurnal pattern [3,19,21,24,25,40]. There is also a morning rise in cortisol and nighttime peak in melatonin; these biomarkers are indicative of central circadian efficiency and diurnal homeostasis [41–43]. Collectively these daily rhythms are beneficial for helping to maintain normal cardiovascular function in healthy people.

3. Circadian rhythms and timing of onset of acute cardiovascular events

Daily rhythmicity is important for a healthy cardiovascular system, yet it also creates a circadian-regulated window of vulnerability that underlies heart disease. The risks for myocardial infarction (MI), sudden cardiac death, ventricular arrhythmias and stroke are greater in the early morning hours as compared to any other time of day or night. There are excellent reviews on the diurnal timing of acute cardiovascular events in humans [9,44,45]. The basic notion is that acute cardiovascular events do not occur randomly throughout the day, but are precipitated in part by circadian regulated factors that create a prothrombotic and/or pro-ischemic state in the early daytime hours [10,11,46,47]. For example, our morning rise in blood pressure is concurrent with increased cardiac output, which has implications for the rupturing of plaques. Similarly, catecholamines surge around wake time and act on the vasculature to increase vasoconstriction and intra-arterial pressure, further precipitating the likelihood of plaque erosion or rupture. There is also greater platelet reactivity (and thus aggregability) and decreased fibrinolysis in the morning, which increases the risk of more aggressive thrombosis or clot formation. Collectively, these circadian regulated factors conspire to increase the likelihood of adverse cardiovascular events during the vulnerable early morning period.

4. Implications of disturbances of circadian rhythms for heart disease in humans

Many individuals experience disturbances in circadian rhythms as a consequence of working in modern society, and this can have profound implications for cardiovascular disease in humans. Approximately 28% of the western workforce operates outside of conventional daytime hours, according to Statistics Canada [48]. Moreover, shift work is considered a risk factor for coronary heart disease, sudden cardiac death, as well as other clinical pathologies, according to the World Health Organization [49]. Mechanistically, frequent shifting of wake and sleep times causes our circadian-controlled central clock (e.g. cortisol and melatonin) [50,51] and peripheral physiology (e.g. blood pressure [52], endocrine hormones [42], catecholamines and other measures of sympathetic tone [43,53], and genes and proteins [23,40,54,55]) to fall out of sync. Many of these factors are crucial for the cardiovascular system such that our internal circadian physiology becomes misaligned with the external environment, and has implications for our health [17]. For instance, shift workers will eat during night shifts, which is not the conventional time for postprandial processes and normal energy metabolism and expenditure [56]. Atypical work shifts leading to fatigue are an obvious factor contributing to reduced performance effects of shift work, chronic desynchrony via long term atypical work schedules is believed to directly and adversely impact our health, and especially our cardiovascular system (reviewed in [9–13]). Thus collectively, these studies demonstrate the crucial relationship between circadian rhythm disturbance and implications of disturbances on the cardiovascular system.

5. Implications of disturbances of circadian rhythms in experimental murine models of heart disease

Rhythm disturbance has profound implications for the cardiovascular system, as noted above. Experimental studies then help to define a direct link between the circadian mechanism and heart health and
disease. For example, we have previously demonstrated that desynchrony between the external environment and the internal circadian mechanism is a direct etiologic cause of cardiomyopathy in +/tau hamsters [58]. The +/tau animals have a mutation in casein kinase 1, a core component of the cellular circadian mechanism, resulting in a 22-h circadian period [59], and reduced longevity [60]. Daily desynchrony between their internal 22-h internal circadian mechanism, and the external 24-h environment, leads to the development of profound cardiomyopathy [58]. In contrast, if the animals are maintained on a 22-h light-dark cycle, consistent with their internal 22-h circadian mechanism, they are protected from developing heart disease [58]. Collectively, these experimental studies show that prolonged circadian misalignment is an etiologic cause of heart disease.

Additional experimental studies in rodents also support the notion that circadian rhythm disturbance is associated with worse cardiovascular outcomes. For example, C57BL/6 mice with a normal 24-h circadian period, when subjected to pressure overload induced cardiac hypertrophy and maintained on an altered light-dark “shift work” cycle, have worse outcomes than those maintained on a normal 24-h light-dark cycle [40]. Desynchrony between their internal 24-h circadian mechanism and the external shift-working environment exacerbates cardiac remodelling and the animals rapidly progress to heart failure [40]. In another study using the MI model in mice, we demonstrate that MI induced by coronary artery occlusion in the daytime triggers different molecular pathways as compared to MI that occur at night [24]. Moreover, disturbing cardiomyocyte-specific CLOCK [19,61] or BMAL1 [25] adversely impacts the regulation of cardiac metabolism. Recent experimental studies also demonstrate that disturbing the circadian mechanism factor CLOCK exacerbates cardiac aging in male mice [55]; surprisingly, female mice are protected from heart disease even when CLOCK is disturbed, due in part to interactions with ovarian hormones [62]. In summary, a wide variety of experimental studies demonstrate the importance of circadian rhythms for healthy cardiovascular function, and how rhythm disturbance adversely affects heart health.

6. Circadian medicine and free radical biology

Intriguingly, targeting the hearts circadian rhythms offers enormous potential to benefit clinical cardiology (reviewed in [15]). Yet despite these contemporary advances, heart disease remains a leading cause of death worldwide. Thus better understanding of the underlying mechanisms in heart disease is warranted, and one area that holds special promise is free radical biology. Here we describe the role of the circadian mechanism in the most prevalent clinical condition, namely MI, and highlight for the first time how free radical biology might coincide with circadian medicine to improve our understanding and treatment of heart disease.

6.1. Circadian rhythm disturbance and MI

During hospitalization for acute illness, patients are often exposed to light stimuli, loud noise, and frequent patient-staff interactions – especially at night [63–66]. Diurnal disruption in intensive and coronary care units affects circadian rhythms and sleep, and is inadvertently harmful to patients [67,68]. Following an acute MI, there is a systematic temporal sequence of inflammatory responses and local events critical to the healing process [69,70]. In the murine MI model we showed that disturbing circadian rhythms and sleep, even for just the first few days after MI, interferes with that orderly cascade of humoral and cellular healing, and worsens long term outcome [23]. Myocardial remodelling occurs both in the day and at night, and thus maintaining rhythms in acute critical care settings can benefit patients clinically.

6.2. Reperfusion injury after MI

It is worth noting that the studies above focused on healing after acute MI; however, many patients reaching hospitals in a timely fashion also undergo coronary artery reperfusion [71,72]. Reperfusion post-MI is essential for cardiac salvage as it restores blood flow to ischemic myocardium, leading to reduced mortality [73]. However, it also triggers a “reperfusion injury”. That is, the previously ischemic cardiac tissue becomes susceptible to myocyte hypercontracture, mitochondrial dysfunction, abnormal activation of immune responses, and damage from oxygen free radicals. These pathways exacerbate cardiac remodelling and worsen outcome [74,75]. While there are many studies on the role of the circadian mechanism in regulating immune responses after reperfusion, very little is known about how it regulates damage from oxygen free radicals. The next section will discuss the role of oxidative stress on remodelling after reperfusion, and novel areas for investigation, and are summarized in Fig. 2.

6.3. Effects of reperfusion injury and oxidative stress on heart and brain

A key area of growing recognition is that oxidative stress is important in the pathogenesis of myocardial repair and remodelling after MI [75]. Cardiac remodelling post-MI involves changes in the structure and function of cardiac myocytes and extracellular matrix (ECM) leading to cardiac hypertrophy, fibrosis, apoptosis or necrosis that may progress to ventricular dilatation eventually resulting in heart failure. Cardiac hypertrophy is an initial compensatory response to stress such as MI, which can then transition to maladaptive hypertrophy and increase risk of heart failure. Similar to cardiomyocytes, other cells of the myocardial tissue such as fibroblasts are also activated in the I/R setting, resulting in fibrosis. The underlying mechanism of cardiac and matrix remodelling is multifactorial; however, substantial evidence both from animal studies and clinical data support the role of increased oxidative stress in cardiac and matrix remodelling [76–80]. In addition to post-translational modification of ECM proteins, redox signaling is an important regulator of ECM gene expression and matrix remodelling. Oxidative stress caused by I/R injury or administration of peroxynitrite increases MMP-2 activity which contributes to contractile dysfunction by degradation of contractile protein (reviewed in [81]). Low concentrations of peroxynitrite may also alter the structural or impaired
regulation of MMP-2 activity [82]. Consequently, MMP-2 degrades denatured collagen as well as other ECM proteins and growth factors required for ECM maintenance and repair [83].

ROS can also act in other ways to cause remodelling after MI. For example, they stimulate the production of inflammatory mediators and these inflammatory cytokines in turn cause further ROS production thereby creating a “vicious cycle” (reviewed in [78,79]). In this regard, hydrogen peroxide can directly induce TNF-alpha via the p38MAPK pathway and in turn mediate cardiac dysfunction and apoptosis [84]. Moreover, proinflammatory cytokines such as TNF-α, IL-1β and IFN-γ increased production of superoxide anion, which reacts with nitric oxide to form peroxynitrite, and in turn alters calcium binding capacity of myofilaments leading to contractile failure [85]. Furthermore, in ischemia/reperfusion settings, these inflammatory mediators trigger cellular differentiation towards the myofibroblast phenotype characterized by cell proliferation, migration and increased production of ECM remodelling proteins (reviewed in [79,86]). The major source of nitroxy radical, superoxide anion and peroxynitrite in reperfused myocardium is mitochondria, xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase isoforms NOX1 and NOX2. Excessive ROS generation triggers lipid peroxidation, DNA damage and mitochondrial dysfunction leading to irreversible cell death. In this regard, peroxynitrite and hydroxyl radicals which are extremely reactive cause direct cell membrane damage, lipid peroxidation and damage to proteins. Oxidative stress also induces abrupt opening of mitochondrial permeability transition pores (mPTPs) resulting in mitochondrial damage and cell death. Moreover, oxidative stress can activate a broad range of hypertrophy signaling pathways including kinases such as extracellular signal-regulated kinase and Jun-nuclear kinase and transcription factors such as NF-xB and AP-1 resulting in hypertrophy [76,77,80]. Low levels of hydrogen peroxide are associated with ERK1/2 MAPK activation and cell growth whereas high levels of hydrogen peroxide are associated with JNK and p38MAPK activation to induce apoptosis.

Understanding these oxidative stress pathways is important because it can lead to new treatments for heart disease. For example, various studies have reported the beneficial effects of antioxidants to the heart in ischemia/reperfusion settings. Furthermore, studies have reported a deficit in cardiac antioxidant reserve upon reperfusion [76,87]. Thus there is significant potential for regulation of oxidative stress as an effective approach to improve cardiac remodelling and function after MI.

### 6.3.1. New frontiers: ischemia/reperfusion injury and imaging after MI

The pathophysiological alterations and structural remodelling occurring after an MI are complex processes, whose inter-dependence has not been completely understood [88]. Alternations at the cellular level further lead to left ventricular structural remodelling comprising infarct zone thinning, remote zone hypertrophy and global left ventricular dilation. In addition, ischemia/reperfusion injury can further worsen or expand the initial ischemic damage resulting in free radical formation, apoptosis, membrane disruption, stunning and reperfusion arrhythmias [74]. Lethal reperfusion injury is apparent from enhanced intracellular and interstitial edema arising from the body’s inflammatory response [89]. Reperfusion injury is also responsible for a phenomenon called “no-reflow” that arises from vascular injury, distal embolization and platelet aggregation, resulting in microvascular obstruction (MVO) and has been correlated with adverse remodelling and poor patient outcome [90–92]. Greater initial ischemia has also been attributed to greater degrees of intramyocardial haemorrhage post-MI and reperfusion is a prerequisite [93,94]. Intramyocardial haemorrhage can occur in 35–50% of the ST-segment elevation myocardial infarction (STEMI) patient population, and association with MVO is recognized as an independent predictor of adverse left ventricular remodelling [95–98].

Considering these complex alterations occurring simultaneously at the level of the myocardium, Cardiac Magnetic Resonance Imaging (CMR) is an attractive non-invasive tool that has gained clinical importance in evaluating soft tissue properties. CMR is considered the gold standard for the assessment of myocardial viability and function post-MI [99]; infarct size by CMR can predict left ventricular remodelling, long-term improvement, and progression to heart failure [100]. CMR can detect the presence of MVO via a lack of contrast uptake in the myocardium through obstructed microvasculature and this has been linked with poor outcomes [91]. Thus, CMR can offer prognostic value in risk stratification in MI patients beyond routine clinical markers [101]. Elevation of CMR relaxation parameters, termed T1 and T2, can also be used to detect edema or inflammation in the tissue in a quantitative manner [102–105]; this has been evaluated in animal models as well as humans. Shortening of T2 and T2* relaxation times has been used to identify haemorrhage and iron stores [102,106].

A recent mechanistic study has indicated that reperfusion haemorrhage is not simply a bystander but is by itself pro-inflammatory and an active contributor to cellular and microvascular damage beyond the initial ischemic insult [107]. There is speculation that free iron released at reperfusion might induce cytotoxicity via the Fenton chemistry in the presence of superoxide radicals (O2-) and hydrogen peroxide (H2O2). The catalytic iron promotes the formation of potent oxidants such as the hydroxyl OH radical, which is highly reactive and capable of mitochondrial damage, peroxidation of membrane lipids and DNA oxidation in the heart [108,109]. A STEMI patient study by Chan et al., demonstrated that iron reversal strategies using chelators at reperfusion can help reduce serum iron levels as well as oxidative stress as measured by plasma F2-isoprostane [110].

### 6.3.2. New frontiers: oxidative stress and the circadian mechanism

Given the key role that locally produced ROS plays in promoting cardiac remodelling, novel approaches for regulating this process are needed. One avenue for approach might be through modulation of the circadian mechanism. This has not yet been investigated, but is likely to be fruitful as circadian rhythms regulate healing after MI and circadian desynchrony contributes to adverse cardiac remodelling post-MI. Exploring combinatory approaches such as attenuating oxidative stress mediated damage by using antioxidants, as well as identifying key cardiac circadian clock targets, may be beneficial in increasing tolerance to injury and improve outcomes. In this regard, a circadian regulated hormone, namely melatonin, appears to be cardioprotective and several in vitro studies have reported that it possess free radical scavenging properties [111]. More recently, Kohsaka et al. [112] demonstrated that loss of function of the core circadian mechanism factor BMAL1 induced expression of genes related to oxidative stress, cardiac remodelling and inflammation. They reported an upregulation of oxidative stress genes such as glutathione peroxidase and superoxide dismutase [112]. Moreover, Lapenna et al [113] reported that glutathione peroxidase activity exhibited a circadian rhythm. These studies further support the notion that the circadian clock plays an important role in regulating oxidative stress pathways that can potentially contribute to cardioprotection.

### 6.3.3. New frontiers: MI, oxidative stress and the brain

Oxidative stress resulting from MI also has consequences for the central nervous system, which can further exacerbate cardiac dysfunction and may underlie cognitive deficits observed following MI and reperfusion (Fig. 3). Neurons are highly susceptible to oxidative damage because of their high energy requirements for the maintenance of mitochondrial and cell membrane voltage potentials (reviewed in [114]). MI and reperfusion leads to increased markers of oxidative stress in the circulation beyond cardiac tissue, for example by increasing the content of oxidized 2′,7′-dichlorofluorescein diacetate in saliva [115], and by increasing the content or activity of protein carbonyl groups, lipid peroxidation, catalase, and superoxide dismutase in blood [116,117]. This suggests that the brain may also experience a post-MI oxidative insult. Preclinical research in murine models confirms that MI increases the content of superoxide within the subfornical organ
[118], and increases both superoxide and peroxynitrite within the hypothalamus and brainstem [118,119], which is directly linked with increased sympathetic tone, cardiomyocyte cell death and decreased survival [118]. Although mechanisms of pathogenesis are not yet elucidated, MI also impairs corticolimbic-dependent cognitive functions such as spatial memory, working memory and object recognition in mice [120–122], and is linked with decreased corticolimbic grey matter volume [123], cognitive performance [124,125] and emotional regulation in human [126]. Future studies are warranted investigating how circadian rhythms regulate neurotrophin ROS responses after MI, as a promising avenue to better understand neurobiological behaviour in patients with heart disease.

6.3.4. New frontiers: angiotensin II (AngII) post-MI and redox signal pathways

Remodelling after MI is also mediated in part by angiotensin II (AngII), a potent component of the renin-angiotensin-aldosterone system (RAAS). AngII is produced locally in the infarcted heart, in part by activated macrophages, and could drive NADPH oxidase isofrom NOX2 to induce superoxide anion and hydrogen peroxide production [127,128]. Angiotensin converting enzyme inhibitors (ACEI) are effective for reducing adverse remodelling after MI [129], and the mechanisms have been extensively investigated using experimental models of infarction [130,131]. The circadian system also appears involved, as core components of the RAAS system undergo diurnal cycling. For example, cardiac ACE exhibits rhythms in a murine model of pressure overload induced cardiac hypertrophy [132]. Diurnal rhythms have also been reported for circulating levels of RAAS in rats, and humans [133–136]. Moreover, targeting the diurnal rhythms in RAAS may help to reduce cardiac remodelling in heart disease. We showed that administering the ACEI captopril at sleep time benefits outcome in a murine model of pressure overload induced cardiac hypertrophy [132]. Conversely, ACEI given at wake time was not effective, indeed cardiac structure and function was similar to placebo controls [132]. Intriguingly, ACEI chronotherapy not only reduces AngII in a rhythmic manner, but likely also reduces downstream redox signaling pathways. In support of this notion ACEI have been reported to attenuate cardiac oxidative stress and adverse cardiac remodelling and upregulate antioxidant reserve in experimental models of MI [130,131]. These areas would certainly be worthy of future investigation.

7. Conclusion

This review summarizes the importance of circadian rhythms for cardiovascular health, and the profound effects of rhythm disturbances on the pathogenesis and pathophysiology of heart disease. Importantly, despite contemporary understanding, heart disease remains a leading cause of morbidity and mortality. Here we discuss key novel relationships between the circadian mechanism and oxidative stress pathways after MI. In terms of translation, manipulating the circadian mechanism could reduce oxidative stress leading to less reperfusion injury, benefit neural pathways, and downregulate RAAS; these novel approaches could lead to improved outcomes after MI. There are many circadian genetic mouse models, and experimental models of heart disease, and pharmacological approaches that could be used to investigate how directly targeting the circadian mechanism can modulate oxidative stress pathways and heart disease outcome. Thus it would seem important to develop these tools and adopt novel directions for investigation, leading to new understanding and avenues for treating heart disease.

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Conflict of interest statement

None.

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