Of Mice and Men
The Quest to Determine a Circadian Basis for Myocardial Protection in Ischemia/Reperfusion Injury

Jay H. Traverse

Circadian rhythms in humans significantly influence cardiovascular biology. The well-known early morning peak of adverse cardiovascular events, such as acute myocardial infarction and sudden cardiac death, are highly correlated with circadian changes in coagulation factors, platelet activation, and endothelial function that act to increase susceptibility to thrombosis in the morning.

Mechanisms of Myocardial Protection in Murine Models

The circadian infarct size reduction observed in the study of Durgan et al could be attributable to improved tolerance to both the initial ischemic insult or reperfusion injury. They observed diurnal variations in the phosphorylation of the prosurvival kinase v-akt murine thymoma viral oncogene (Akt) and one of its downstream targets glycogen synthase kinase-3β (GSK-3β). Phosphorylation of GSK-3β has been shown to increase the threshold for opening of the mitochondrial permeability transition pore by reactive oxygen species that significantly contributes to lethal reperfusion injury. Durgan demonstrated that infarct size was negatively correlated with the phosphorylation status of Akt and glycogen synthase kinase-3β with the peak phosphorylation around the time of minimal infarction (ZT0) and nadir at the time of greatest infarction.

Ischemia leads to activation of pathways favoring oxygen-efficient utilization of glucose to make ATP. The cardiac myocyte clock gene Period 2 (Per2) was identified by Eckle et al in an open-chest mouse infarction model as a metabolic master switch that directs the heart toward oxygen-efficient carbohydrate-dependent metabolism. Per2 protein was upregulated in the setting of ischemic preconditioning and was associated with an increase in glycolytic enzyme production. In contrast, mice with a mutated form of Per2 had larger infarct sizes and were unable to effectively use glycolysis or restore glycogen after reperfusion.

Adenosine receptor activation also seems to be an important mediator of cardioprotection in the setting of ischemia. As demonstrated by Eckle et al, Per2 is stabilized by activation of the adenosine receptor A2b which in turn leads to stabilization of the circadian-expressed hypoxia inducible factor-1α. As a result, glycolysis is enhanced by a Per2-dependent mechanism during ischemia.

In contrast, Virag et al found that infarct size was reduced in the Per2 mutant mouse compared with wild-type after 4 days of permanent coronary artery occlusion. Because these mice did not undergo reperfusion as in the study of Eckle,

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(60 minutes of ischemia followed by 2 hours of reperfusion), it may suggest that the benefit of Per2 is either dependent on reperfusion, lost after prolonged ischemia, or influenced by the heightened inflammation associated with the open-chest infarct model.7

Clinical Observations in Humans

These observations by Durgan et al9 prompted several investigators to retrospectively examine cohorts of patients with ST-segment–elevation myocardial infarction (STEMI) to determine whether humans exhibit a similar circadian-dependent tolerance to ischemia. Compared with experimental studies in mice, the injury response and accompanying inflammation in humans are significantly enhanced, which could overwhelm any circadian mechanisms of protection seen in murine models. In addition, determination of circadian protection in patients may be influenced by the inability to accurately determine the true onset of vessel closure and the much greater variability of infarct size in man for a similar duration of ischemia. This arises from factors including the presence of collateral blood flow, preinfarction angina,3 variations in the degree of revascularization, or the occurrence of distal embolization after percutaneous coronary intervention (PCI). In addition, a variety of medications, such as β-blockers and angiotensin receptor antagonists, may independently influence infarct size.

Despite these limitations, several groups have observed variability in infarct size over a 24-hour cycle, suggesting that this circadian phenomenon may be clinically relevant in humans. Suárez-Barrientos et al10 identified a subgroup (n=811) of 950 consecutive patients with STEMI from a single center in Spain treated with primary PCI or thrombolysis. Patients were subdivided into four 6-hour time intervals starting at midnight based on time of onset of chest pain. The time-dependence of peak creatine kinase and troponin was analyzed by multivariate regression splines. They confirmed the greater incidence of STEMI in the 6:00 AM till noon group and demonstrated that this time interval was associated with the greatest infarct size. Reiter et al11 studied a much more selective subgroup (n=165) of 1031 consecutive patients with STEMI from a single center in Minnesota who had occluded arteries at the time of their primary PCI with ischemic durations between 1 and 6 hours. Patients with preinfarction angina or visible collateral flow to the infarct vessel were excluded, and ischemic times and angiographic areas-at-risk were similar across the 24-hour period. By periodic sinusoidal regression analysis, they observed that peak infarct size was 82% greater than trough and occurred at 1:00 AM onset of ischemia, a time at which the cardiomyocyte clock gene Per2 is at its nadir in human biopsy samples.12 These findings were supported by a similar circadian distribution of left ventricular function, with peak ejection fraction coinciding with the trough of peak creatine kinase release. A similar time of peak infarction (from midnight till 6:00 AM) to that of Reiter et al11 was observed by Fournier et al13 in a cohort (n=355) of 588 consecutive patients with STEMI from Switzerland. These results11,13 are consistent with the observations that the highest incidence of heart failure results from infarctions that occur around midnight.14

In this issue of Circulation Research, Ammirati et al15 provide a fourth analysis investigating the possible circadian effects on ischemic tolerance in humans in the setting of STEMI. Their study is unique in that its cohort was derived from a geographically and ethnically diverse group of patients from Italy, Scotland, and China who participated in the First Acute Myocardial Infarction Study. They enrolled 1099 patients with STEMI with ischemic times <6 hours who were revascularized with primary PCI or thrombolysis. They again noted the peak incidence of myocardial infarction in the morning for both the entire cohort and the 3 distinct geographical groups but found no circadian variability of infarct size. Importantly, they further refined their cohort to match the entry criteria of Suárez-Barrientos10 (n=613) or more restrictive inclusion criteria of Reiter et al11 (n=171) and performed a periodic regression analysis. Again, they were unable to demonstrate a circadian distribution of ischemic tolerance. Because of justifiable concerns of geographic and ethnic differences in their patient population that can affect circadian regulation,16 they studied a second consecutive cohort of patients with STEMI from several centers in Italy (n=624). Again, they failed to find a significant circadian effect of infarct size in this population. It is interesting to note, however, that although not statistically significant, the peak creatine kinase of their derived circadian analysis was highest around the same time point as observed by Reiter et al11 (1:00 AM).

What conclusions can be drawn from this well done study that failed to confirm previous circadian variability of infarct size in patients with STEMI? Importantly, the failure to demonstrate a circadian relationship does not mean that a circadian relationship does not exist. Patient selection is critical for this determination to be made on a consistent basis. It is noteworthy that the previous positive studies10,11,13 all came from single centers with a uniform ethnic and geographic patient population and standardized treatment of STEMI by primary PCI (Table). In contrast, almost half of the patients in the original cohort of Ammirati et al15 received thrombolysis or were not revascularized.

Undoubtedly, a circadian signal of ischemic tolerance in a clinical population is not as robust as the one describing the peak incidence of myocardial infarction in the early morning hours. This has been consistently demonstrated across multiple patient populations for decades. A brief period of ischemia and reperfusion in the mouse cannot be easily duplicated in humans, given the complexity of variables that affect infarct size. In addition, the genomic response to injury in mouse models may correlate poorly with human biology.6 The onset of infarction in patients is not always easily determined, and unless the artery remains closed at the time of primary PCI, the ischemic duration cannot accurately be measured.

Going forward it will be important to determine whether a circadian variability in infarct size is clinically relevant in humans and how this is influenced by factors, such as age, sex, diabetes mellitus, and other comorbidities. If it is indeed relevant, then investigations should be focused on identifying these circadian mechanisms so they can be exploited in the future therapeutically.
Table.  Clinical Characteristics of Trials Investigating Effects of Circadian Dependence of Infarct Size

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Total No. of Patients (No. Analyzed)</th>
<th>No. of Hospitals</th>
<th>% Primary PCI</th>
<th>Mean Age, y</th>
<th>Mean Peak CK, IU/L</th>
<th>Mean Ischemic Time, min</th>
<th>Time of Peak Infarct Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suárez-Barrientos10</td>
<td>Spain</td>
<td>950 (811)</td>
<td>1</td>
<td>79</td>
<td>62</td>
<td>1600</td>
<td>229</td>
<td>6:00 am till noon</td>
</tr>
<tr>
<td>Reiter11</td>
<td>USA</td>
<td>1031 (165)</td>
<td>1</td>
<td>100</td>
<td>59</td>
<td>2543</td>
<td>168</td>
<td>1:00 AM</td>
</tr>
<tr>
<td>Fournier13</td>
<td>Switzerland</td>
<td>588 (353)</td>
<td>1</td>
<td>100</td>
<td>66</td>
<td>2687</td>
<td>162</td>
<td>Midnight till 6:00 AM</td>
</tr>
<tr>
<td>Ammirati15</td>
<td>China, Scotland, and Italy</td>
<td>1099 (1099)</td>
<td>32</td>
<td>52</td>
<td>61</td>
<td>1606</td>
<td>180</td>
<td>None observed</td>
</tr>
</tbody>
</table>

CK indicates creatine kinase; and PCI, percutaneous coronary intervention.

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References

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