Circadian effects on stroke outcome – Did we not wake up in time for neuroprotection?

Johannes Boltze1, Nadine Didwischus1, Martha Merrow2, Robert Dallmann3 and Nikolaus Plesnila4,5

Abstract
The occurrence of stroke in humans peaks in the morning. A recent study revealed that time of day mitigates the therapeutic impact of neuroprotective paradigms. These findings might not only explain the previous failure of translation of neuroprotective therapies but inspire new paradigms in stroke chronopathophysiology research. Taking chronotype into account may complement the many factors that influence efficacy of experimental therapies in stroke.

Keywords
Stroke, animal models, neuroprotection, chronobiology, translational research

Over the past two decades, numerous reasons have been discussed for the failure of classical neuroprotective paradigms in ischemic stroke to translate successfully to clinical practice. Many of those reasons relate to the predictive value of stroke models as well as preclinical study design limitations such as improper randomization and blinding strategies. Researchers learnt quickly from previous translational setbacks. Major improvements in methodological quality were achieved in the stroke field, which may now have outpaced other areas of cardiovascular research in this regard.1 Moreover, an increasing body of evidence suggests that animal models adequately reflect human stroke pathophysiology and outcome when used appropriately. Despite this, translational research on neuroprotectants remained futile. This refocused our attention from pretended fundamental limitations in preclinical research models and strategies to seemingly minor yet impactful design differences between preclinical and clinical studies.

Recently, a potentially game-changing study revealed a surprising influence of treatment time on stroke outcome,2 adding to translationally relevant study design differences.3 A number of established neuroprotective paradigms (normobaric hyperoxia, radical scavenging, NMDA receptor antagonism) were evaluated for efficacy during the inactive (light phase) versus the active (dark phase) phase in nocturnal rodents after stroke. Major treatment effects were only seen for interventions during the inactive phase. A major reason for the phenomenon was a significantly smaller penumbra during the active phase. These findings are highly relevant and timely given the supposed renaissance of neuroprotective approaches in the reperfusion era.4 Indeed, treatments in clinical trials are predominantly performed during the day-time (human active phase) for logistical reasons. If temporally regulated

1School of Life Sciences, University of Warwick, Coventry, UK
2Faculty of Medicine, Institute of Medical Psychology, LMU Munich, Munich, Germany
3Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, UK
4Institute for Stroke and Dementia Research (ISD), Munich University Hospital and Faculty of Medicine, LMU Munich, Munich, Germany
5Munich Cluster of Systems Neurology (Synergy), Munich, Germany

Corresponding author:
Johannes Boltze, School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK.
Email: johannes.boltze@warwick.ac.uk
physiology plays a role in the observed neuroprotective
effects, this may partially explain the translational
failure of neuroprotective treatments we were
experiencing.

Although novel and striking, these findings should
not come as a major surprise. Many biological func-
tions relevant for stroke pathophysiology are orches-
trated by the circadian clock (Figure 1) and may thus
be of relevance for therapeutic interventions. For
instance, blood pressure shows a clear diurnal profile
reaching its nadir at the end of the resting period. In
turn, susceptibility to antihypertensive interventions
varies throughout the day. Cerebral blood vessels
undergo diurnal changes in vascular function that are
relevant to pathology. Moreover, there is a circadian
rhythm in leukocyte migration and function, including
immune reactions. Leukocytes egress the circulation
and accumulate in tissues with a marked daily, tempo-
ral program. Leukocyte accumulation is mediated by
increased adhesion molecule expression on endothelial
cells during the active phase, and is proposed to con-
tribute to worse outcome after myocardial infarction
occurring in the wake phase versus the resting phase.
This could also be of direct relevance to secondary
neuroinflammatory damage and outcome after ische-
mic stroke. Finally, the circadian clock is believed to
have an influence on redox homeostasis and thereby on
neuronal antioxidative defense that is important for
recanalization therapies and research on reperfusion
injury.

What are the implications of these findings for
future translational research? First, validation of time
of day effects in diurnal model systems should be per-
formed. Large animals may help with this as they are
mostly diurnal and better reflect human brain anatomy
and physiology. Large animal stroke models are
increasingly used for assessing reperfusion therapies
and are becoming more widely available, paving the
way for tandem reperfusion and neuroprotection
studies.

Second, the relevance of circadian regulation for
other experimental treatment strategies should be elu-
cidated. For instance, pre- and postconditioning strat-
egies are currently assessed as powerful supplementary
therapies that may significantly improve stroke out-
come. Since such strategies predominantly utilize
changes in local and systemic metabolic processes
that are in turn modulated by circadian clocks, the
effect of intervention time should be systemically inves-
tigated. Moreover, the impact of circadian rhythms
should be considered when optimizing strategies mea-
suring functional outcome that may reveal different
results in the active versus the inactive phase.

Deciphering time of day effects is time-consuming.
A third, interim suggestion would be to alter current
experimental practices when nocturnal species such as
rodents are used. Reversal of the light dark cycle in
animal facilities will harmonize the sleep/wake behav-
ior of animals and researchers. The data presented in
Esposito et al. suggest that acute treatment of stroke

---

**Figure 1.** Processes relevant to ischemic stroke pathophysiology that are controlled by circadian clocks.
patients with neuroprotective strategies over the day may not be effective. Thus, if only active (night) times were assayed in previous preclinical experiments, many neuroprotective strategies would have never been discovered. They likely have value but must be implemented appropriately with respect to chronobiological aspects.

Fourth, circadian rhythms are important elements adding to the interplay of factors such as age, sex and comorbidities that can affect stroke outcome and therapeutic efficacy. The sheer number of these factors and therapeutically relevant combinations make it increasingly challenging to model them sufficiently in translational studies. A potential solution may be to more closely interlink preclinical and early-stage clinical studies. Selecting patient populations who reflect the preclinical cohorts in which best effects were seen may increase chances for successful therapeutic intervention. Selection of eligible patients might be extended beyond criteria such as stroke size or location and may include chronobiological aspects. Stroke patients presenting at night may be assigned to different trials than those presenting during the day.

In summary, the increasing knowledge on circadian clock regulation in stroke and therapeutic outcomes may open the door for new research directions in stroke research. Chrono(patho)physiology might help us to prevent missing out on neuroprotection just by sleeping away the right time for intervention.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References