RESEARCH HIGHLIGHT

A perfect storm: sleep loss causes systemic inflammation and death

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Complete loss of sleep can be lethal, but a mechanism for the lethality has heretofore proven elusive. In a recent work in *Cell*, Sang et al. present findings that suggest a mechanism whereby sleep loss causes systemic inflammation and multiple organ dysfunction; triggering the cytokine storm is a brain-derived chemical, prostaglandin D2, which is released via dynamic regulation of the blood brain barrier.

Sleep is a conserved behavior widely observed in animals ranging from humans to jellyfish.[1] The importance of sleep is underscored by the detrimental consequences of partial sleep loss on cognitive function, neural activity, immune system function, and metabolism. Strikingly, complete loss of sleep can be lethal in certain species. In 1983, Allan Rechschtaffen and colleagues reported that total sleep deprivation (TSD) is deadly to rats.[2] TSD results in a hypermetabolic state (exhibiting weight loss despite increased food intake), skin breakdown, multi-organ failure and, inevitably, death. These TSD experiments utilized a clever technique that the authors developed called "disc-over-water". A rat is placed on a platform that rotates whenever the rat falls asleep, forcing the animal awake to avoid falling into water surrounding the platform. However, the mechanisms of this lethality have remained largely a mystery for 40 years.

While the laboratory mouse has become the workhorse for mechanistic biology studies, its use in TSD experiments has been limited due to difficulty of efficiently keeping mice awake for days. Sang et al.[3] now report the development of a method to totally deprive mice of sleep, which then allows them to study the mechanisms of this long-held mystery of sleep.

The authors name their sleep deprivation (SD) method "curling prevention by water" (CPW). Mice are housed in a chamber whose floor is covered with a thin layer of water. Similar to humans, who assume particular postures during sleep, mice also prefer a curled body posture during sleep. As the mice begin falling asleep, their body attempts to curl, but this causes their nose to touch the water and they are promptly awakened. Despite its simplicity, this new method efficiently deprives mice of ~96% of their daily sleep, which is considerably more than prior TSD protocols. Using this CPW method, they found that 4 days of TSD induces elevation of several cytokines, multi-organ dysfunction, and death. They refer to the observed elevation in cytokines as a "cytokine storm". Cytokine storms have received increased attention recently due to their roles in organ dysfunction caused by acute COVID-19 infection.[4] Cytokine storms are also associated with the phenomenon of "neutrophilia", where elevated numbers of neutrophil immune cells accumulate in the blood.

The authors showed that the increase in circulating neutrophils plays a causal role in SD-induced mortality, as depletion of neutrophils in mice reduced mortality. Therefore, TSD induces a cytokine storm and neutrophilia, which then cause multi-organ dysfunction and death. But, what is the upstream trigger for immune activation?

The authors observed that treating the mice with the medicine acetaminophen (Tylenol) during TSD attenuates the lethality cause by the sleep loss. Since acetaminophen inhibits the enzyme cyclooxygenase, which catalyzes the formation of a class of chemicals called prostaglandins (PGs), the authors hypothesized that PGs are involved. In particular, PGD_2 is a known sleep-inducing proinflammatory lipid.[5] PGD_2 secreted into the cerebrospinal fluid activates the G-protein-coupled receptor (GPCR) DP1 located in the ventral surface of the brain. DP1 activation leads to activation of sleep-promoting neurons in the pre-optic area (POA) of the brain. The authors showed that activation of DP1, even without SD, is sufficient to cause cytokine elevation and neutrophilia. The authors developed and then used a GPCR activation-based (GRAB) sensor to show that PGD_2 levels increase in the POA after SD.

Identifying a role in TSD-induced inflammation for PGD_2, which is produced in the brain, suggests that the brain can regulate peripheral inflammation. While the effect of the immune system on the brain is well studied, the notion that the brain can also affect the immune system, is fairly new. Relevant to this phenomenon, Koren et al.[6] recently reported that insular cortex neurons can induce an inflammatory response in the gut.

The next question is how PGD_2 leaves the central nervous system to trigger peripheral inflammation and organ damage? Notably, molecular movement between the brain and the body is regulated by the blood brain barrier (BBB). Indeed, the authors found that PGD_2 moves across the BBB at a higher rate during SD. How does this happen?

Molecular flux across the BBB is mediated by ATP-binding cassette (ABC) transporters. The BBB exhibits higher levels of efflux during active periods due to increased activity of ABCB1 transporters.[7] Whether this efflux is affected by SD had been unknown. Sang et al. determined that SD attenuates circadian oscillations and increases efflux of xenobiotics. Blocking this efflux with an antagonist that targets a particular class of ABC transporters or by a mutation in a key transporter called ABCC4 decreases TSD-induced mortality,

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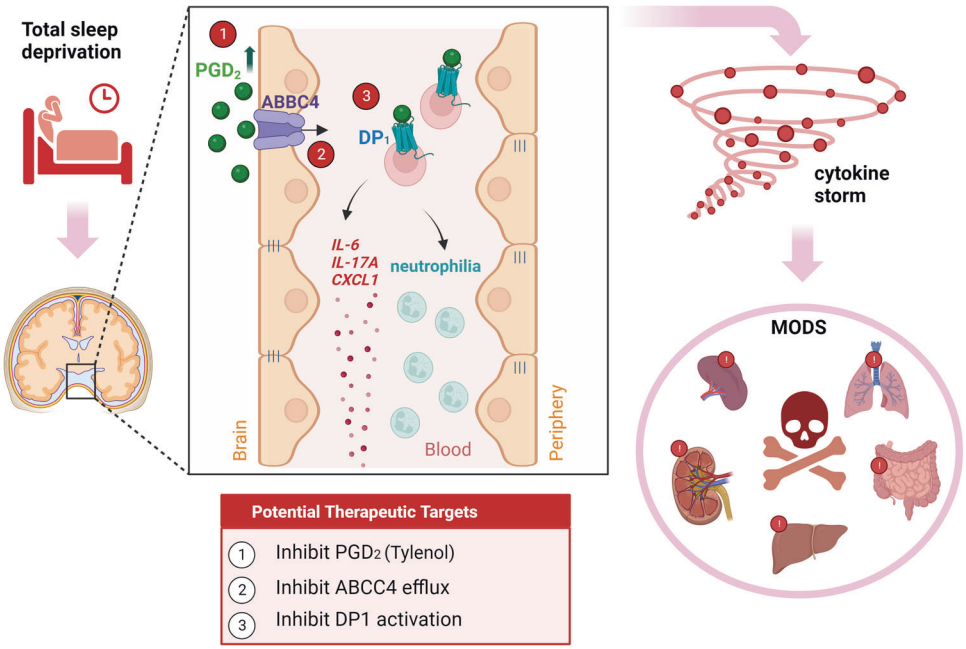

Fig. 1 Potential therapeutic interventions for sleep loss-induced immune dysfunction. TSD triggers a cascade of immune responses that lead to death. During prolonged sleep loss, PGD_2 accumulates in the brain and gets shuttled out of the brain by ABCC4 transporters, and can then bind to DP1 receptor on the ventral surface of the brain, leading to accumulation of proinflammatory cytokines, neutrophilia, and multi-organ failures. Targeting this PGD_2/DP1 axis through inhibiting PGD_2 synthesis, blocking ABCC4 efflux activity, or treating with a DP1 antagonist may alleviate the consequences of TSD. MODS, multiple organ dysfunction syndrome. Created with BioRender.com.

demonstrating the relevance of the BBB in signaling the sleep-deprived state to the periphery.

This study provides a remarkably detailed picture of the mechanism by which TSD activates the immune system (Fig. 1). Moreover, it demonstrates several nodes of potential intervention, by either genetic or pharmacological approaches, to block harmful effects of TSD. While the authors focus on the relationship between TSD and the immune system (which then mediates the organ damage), one wonders whether other adverse effects of SD, such as cognitive and metabolic dysfunction, can be attenuated with the same interventions shown here. It would be important, for example, to investigate whether PGD_2 or ABCC1 inhibitors block the increased homeostatic sleep drive following SD. More provocatively, could these inhibitors be used to mitigate effects of SD on cognitive dysfunction? Can we pull an all-nighter and then simply pop a Tylenol in the morning to improve brain function?

Finally, can this study tell us something about the function of sleep? While one might be tempted to conclude that a core function of sleep is to avoid immune system over-activation, observing the consequences of SD does not necessarily reveal the normal functions of sleep. In particular, the CPW method used by Sang et al. certainly results in emotional stress, elevated muscular activity and changes in temperature and diet associated with SD. As noted previously,[1] physiological processes are closely intertwined such that their disruption can have complex consequences.

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ADDITIONAL INFORMATION

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