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Blue light reduces organ injury from ischemia and reperfusion

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Evidence suggests that light and circadian rhythms profoundly influence the physiologic capacity with which an organism responds to stress. However, the ramifications of light spectrum on the course of critical illness remain to be determined. Here, we show that acute exposure to bright blue spectrum light reduces organ injury by comparison with bright red spectrum or ambient white fluorescent light in two murine models of sterile insult: warm liver ischemia/reperfusion (I/R) and unilateral renal I/R. Exposure to bright blue light before I/R reduced hepatocellular injury and necrosis and reduced acute kidney injury and necrosis. In both models, blue light reduced neutrophil influx, as evidenced by reduced myeloperoxidase (MPO) within each organ, and reduced the release of high-mobility group box 1 (HMGB1), a neutrophil chemotactant and key mediator in the pathogenesis of I/R injury. The protective mechanism appeared to involve an optic pathway and was mediated, in part, by a sympathetic (β_3 adrenergic) pathway that functioned independent of significant alterations in melatonin or corticosterone concentrations to regulate neutrophil recruitment. These data suggest that modifying the spectrum of light may offer therapeutic utility in sterile forms of cellular injury.

blue light | ischemia | reperfusion | organ injury | circadian rhythms

Sunlight, the sole source of energy for all living organisms, has profound effects on life. Prior studies precipitated excitement regarding the therapeutic value of bright light, noting that more light could benefit certain diseases (1–3). But this initial enthusiasm has been dampened by later studies that failed to replicate these findings (4–8). However, light is complex, defined by illuminance (intensity), photoperiod (duration), and wavelength; and yet, few clinical studies have incorporated this complexity into their design. For example, hospitalized patients suffer simultaneously from considerably decreased intensity of daylight, an expanded photoperiod due to continuous or misdirected and thus excessive and obtrusive artificial light called "light pollution," and a change in spectrum between sunlight and artificial light (4, 5, 9). Each of these factors could influence the biology and outcome of disease, and yet have not been rigorously controlled in prior studies. This complexity of light may contribute, in part, to the inability of prior observational trials to identify an association between light exposure and the outcomes studied.

Mammals perceive visible light (400–700 nm), and experimental animal evidence suggests that each dimension of light can modify animal biology (10–14). In rodents, acute bright light exposure induced the release of corticosterone (15, 16). Fish raised under light of blue/green spectrum exhibited reduced oxidative stress by comparison with environmental red light (13, 14). And a novel paradigm posits that seasonal variation in environmental stress (e.g., thermal, starvation) evolutionarily selected animals expressing photoperiodism: the ability to interpret environmental day length (17, 18). Responding to a progressively shorter photoperiod, energetic resources are concentrated in endogenous mechanisms (i.e., immune function) necessary for seasonally appropriate survival strategies. Although these collective data suggest that light may influence adaptive responses to and during illness, the ramifications of acute alterations in light exposure on health and disease remain to be defined (1–4, 7).

In this study we focus on whether the spectrum of light, holding illuminance and photoperiod constant, can favorably modify the biology of critical illness. Using two independent, murine models of ischemia/reperfusion (I/R), we determine that high- illuminance blue spectrum light functioning through an optic pathway mediates adaptive changes in the response to the critical stress of I/R that protects against organ injury.

Results

Blue Light Before Liver I/R Attenuates Liver Injury and Cellular Necrosis. Certain clinical circumstances (e.g., hepatic resection, transplantation) are amenable to pretreatment and characterized by a period of I/R that induces sterile (i.e., absence of infectious etiology) cellular injury. We initially explored whether blue, red, or ambient white fluorescent light altered organ injury when applied before I/R. Pretreatment with blue light protected against liver injury during I/R, as evidenced by reduced serum alanine aminotransferase (ALT) concentration: blue 1,300 IU/L vs. red 2,038 IU/L ($P = 0.01$) and vs. ambient 2,860 IU/L ($P = 0.008$; Fig. 1*A*). Blue light also significantly reduced hepatocellular necrosis (Fig. 1*B*).

Blue Light Before Kidney I/R Attenuates Acute Kidney Injury and Cellular Necrosis. We next explored whether blue light protected against organ injury in an independent model of unilateral renal I/R. Pretreatment with blue light protected against acute kidney injury (AKI), as evidenced by reduced serum cystatin C concentration:

Significance

It is well established that light regulates mammalian biology. And yet, we have been unable to define and thus harness the underlying mechanisms so as to apply them to alter the course of human disease. In this study we determine that the spectrum of light is a critical determinant of its effect on critical illness. We show that an acute and short (24 h) exposure to high-illuminance (1,400 lx) blue spectrum (peak 442 nm) light prior to ischemia/reperfusion (I/R) significantly attenuates the degree of organ injury. Our characterization of the biological mechanisms through which blue light beneficially alters the cellular response to I/R provides an opportunity to develop novel therapeutics for the prevention and treatment of many diseases.

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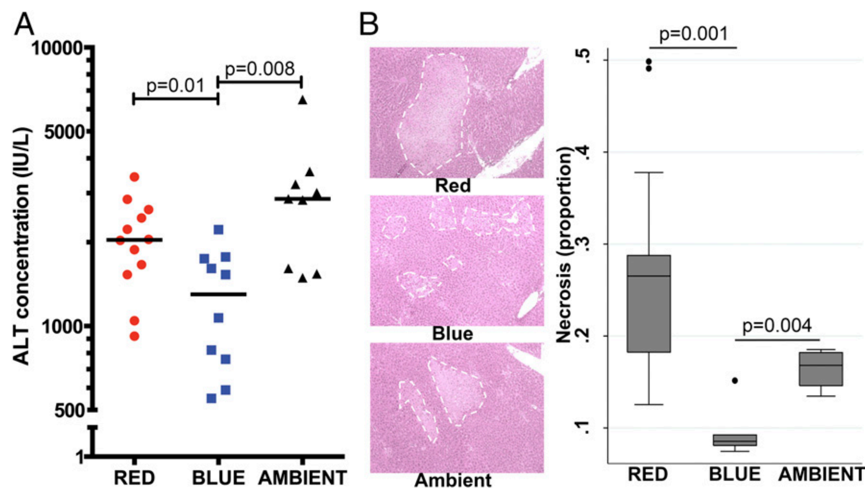

Fig. 1. Blue light before liver I/R attenuates liver injury and cellular necrosis. Mice were exposed to red, blue, or ambient light for 24 h and then subjected to hepatic I/R (10–12 total mice per group for all four experiments combined). (*A*) Serum was assayed for ALT concentration (IU/L). Bar, median. (*B*) Histology (H&E) of liver tissue (200× magnification) was performed to quantify cellular necrosis (representative image of three experiments). White dashed line demarcates regions of necrosis. Corresponding box plots provide summary estimates (bar, median; box, IQR 25–75% range; whiskers, 1.5× IQR) of necrosis. Statistical comparisons were made by nonparametric Mann–Whitney test.

blue 1,069 ng/mL vs. red 1,409 ng/mL ($P = 0.008$) and vs. ambient 1,500 ng/mL ($P = 0.001$; Fig. 2*A*). Blue light also reduced renal tubular cellular necrosis (Fig. 2*B*).

The Effects of Blue Light Are Mediated Through an Optic Pathway. Known pathways through which light may mediate its biological effects are the eyes and skin. We initially interrogated for an optic pathway using Vsx2 KO mice, which undergo optic nerve degeneration (19). Wild-type mice exposed to blue light again exhibited reduced hepatic injury by comparison with red light: ALT 698 IU/L vs. 938 IU/L, $P = 0.03$ (Fig. 3*A*). However, this difference was lost in Vsx2 KO mice: 650 IU/L vs. 645 IU/L, $P = 0.94$.

Blue Light Reduces Liver and Kidney Myeloperoxidase Activity During I/R. The neutrophil is regarded as the ultimate effector of the cellular injury of I/R (20–22). We observed in both models that blue light reduced neutrophil influx into the ischemic organ, as evidenced by reduced liver and kidney myeloperoxidase (MPO) (Fig. 3 *B* and *C*). In addition, the effects of blue light on neutrophil recruitment during liver I/R required an optic pathway (Fig. 3*B*).

Blue Light Attenuates High-Mobility Group Box 1 Release During I/R. Danger-associated molecular pattern (DAMP) molecules are nuclear or cytosolic proteins that are released outside the cell following tissue injury, such as I/R, and can initiate and perpetuate a noninfectious inflammatory/immune response. High-mobility group box 1 (HMGB1), a nuclear DAMP, is a key mediator in the causal pathway of I/R-mediated neutrophil damage (23, 24). We observed that during I/R, mice exposed to blue light exhibited reduced concentrations of serum HMGB1 (Fig. 4). Two bands migrated to the appropriate molecular weight, which has been observed by us and others, and may be due to posttranslational modification, including phosphorylation and acetylation (23, 25, 26).

Blue Light Does Not Alter Serum Melatonin and Corticosterone Concentrations. Many facets of immunity exhibit a circadian pattern and appear under the regulatory influence of melatonin and light (27–29). However, the serum of mice exposed to blue, red, and ambient light demonstrated similar circadian concentrations of melatonin (Fig. 5*A*). Corticosteroid (i.e., cortisol, corticosterone) hormones also exhibit circadian rhythm and may influence immunity (30). However, we observed little difference in systemic corticosterone concentrations in blue-, red-, and ambient-exposed mice (Fig. 5*B*). Finally, acute sleep deprivation has been shown to attenuate neuroinflammation and cell death after ischemia and to attenuate systemic inflammation in murine models of endotoxemia (31, 32). However, we observed similar activities of mice exposed to a 24-h photoperiod of blue and red light (Fig. S1*A*).

Blue Light Functions Through an Adrenergic Pathway. Recently it has been shown that sympathetic signals govern a circadian oscillation

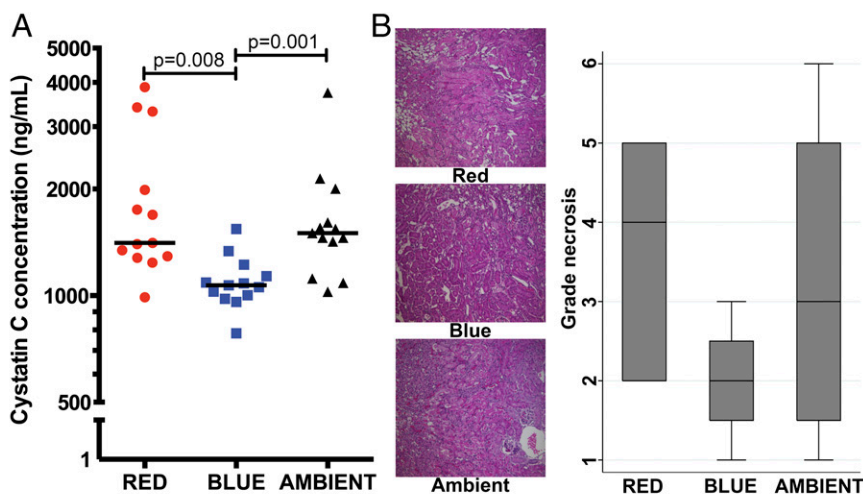

Fig. 2. Blue light before kidney I/R attenuates acute kidney injury and cellular necrosis. Mice were exposed to red, blue, or ambient light for 24 h and then subjected to unilateral kidney I/R (13 total mice per group for all four experiments combined). (*A*) Serum was assayed for cystatin C concentration (ng/mL). Bar, median. (*B*) Histology (H&E) of kidney cortical tissue (200× magnification) was performed to quantify cellular necrosis, blebbing, vacuolization, and cast formation (representative image of four experiments). Corresponding box plots provide summary estimates (bar, median; box, IQR 25–75% range; whiskers, 1.5× IQR) of necrosis. Statistical comparisons were made by nonparametric Mann–Whitney test.

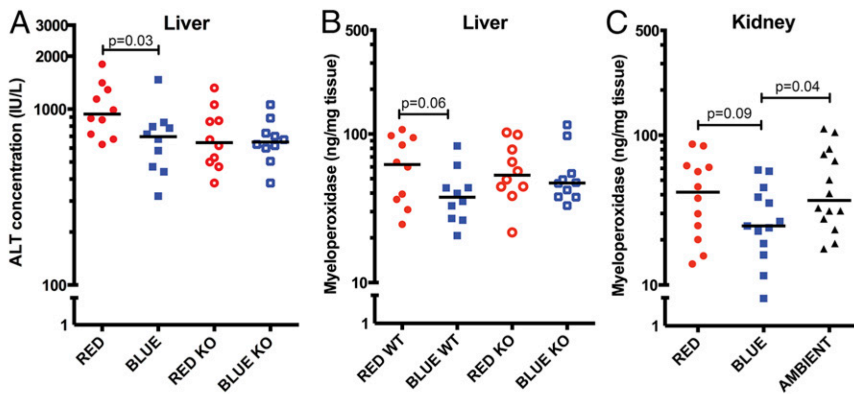

Fig. 3. Blue light functions through an optic pathway and reduces liver and kidney MPO activity during I/R. (*A*) Wild-type 129S1 (closed circles and squares) and Vsx2 Optic KO (open circles and squares) mice were exposed to red or blue light for 24 h and then subjected to hepatic I/R (10 total mice per group for all three experiments combined). Serum was assayed for ALT concentration (IU/L). Bar, median. (*B*) Liver tissue was assayed for MPO (ng/mg tissue protein). Bar, median. (*C*) Mice were exposed to red, blue, or ambient light for 24 h and then subjected to unilateral kidney I/R (12–14 total mice per group for all four experiments combined). Kidney tissue was assayed for MPO (ng/mg tissue protein). Bar, median. Statistical comparisons were made by nonparametric Mann–Whitney test.

in leukocyte emigration into tissues that alters the susceptibility to and outcome from inflammatory diseases (27, 33). Thus, we explored whether blue light regulates sympathetic tone and thereby reduces neutrophil influx and organ injury during I/R. We initially quantified the heart rate variability of mice exposed to blue and red light, focusing on the low frequency/high frequency (LF/HF) ratio as a physiologic parameter of sympathetic activity (34–38). We observed that mice exposed to blue light exhibited lower sympathetic tone, as evidenced by reduced heart rates and LF/HF, in contrast to mice exposed to red light (Fig. S1 *B* and *C*). We next explored whether augmenting adrenergic tone reversed the protective effects of blue light. As shown in Fig. 6*A*, control (saline) mice exposed to blue light exhibited reduced AKI by comparison with control mice exposed to red light: cystatin C concentration: blue + normal saline (NS) 1,068 ng/mL vs. red + NS 1,307 ng/mL ($P = 0.04$). However, when mice were administered the β3 agonist CL-316,243, blue light no longer was protective: cystatin C concentration, blue + CL 1,215 ng/mL vs. red + NS 1,307 ng/mL ($P = 0.25$). Similarly, β_3 agonism partly reversed the reduction in MPO in mice exposed to blue light (Fig. 6*B*). Alternatively, administering the β_3 antagonist SR 59230A (39) to mice before I/R did not reduce AKI: SR 1,154 ng/mL vs. NS 1,019 ng/mL ($P = 0.20$) (Fig. 6*C*).

Discussion

Prior studies precipitated excitement regarding the therapeutic value of bright light or windows in beneficially altering human disease, yet more contemporary studies have failed to replicate these observations (1, 3–8). Thus, whether light can acutely modify the biology of critical illness has never been described. Here we report that a 24-h photoperiod of high-intensity blue spectrum light attenuates organ injury in two clinically relevant, animal models of hepatic and renal I/R. Importantly, the benefits of blue light are translationally relevant to circumstances permitting pretreatment (e.g., transplantation). The mechanism involves an optic pathway that functions, in part, by a permissive withdrawal of β_3 adrenergic tone to reduce neutrophil recruitment. Additionally, blue light inhibits the release of HMGB1, a key mediator of I/R-induced organ injury. Collectively, our data support the potential of blue light as a therapy.

Little data are published on the effects of light spectra on mammalian biology. Fish exposed to green or blue LEDs during starvation exhibited less oxidative stress by comparison with red LED exposure (13, 14). Whether blue and green light were protective or red light was injurious could not be definitively determined. We observed that during I/R, blue light reduced organ injury and cellular necrosis. Our experimental conditions included an ambient fluorescent light exposure to replicate the typical artificial indoor lighting environment (i.e., the hospital). For nearly every outcome parameter assessed, mice exposed to this ambient light exhibited organ injury similar to mice exposed to red light. Thus, our data strongly support that it is blue light that is protective.

One mechanism by which light may alter the response to stress is through changes in immunity: photoimmunomodulation (4, 5, 10, 11, 17, 18, 40). The neutrophil is a fundamental effector of organ injury during I/R, and inhibiting neutrophil influx or function during I/R markedly reduces organ injury (20–22). We observed that the kidneys and livers of mice exposed to blue light had reduced infiltration of active neutrophils (i.e., MPO). Damage-associated molecular patterns (DAMP), such as HMGB1, are released during I/R, and others and we have shown that HMGB1 is a key mediator of inflammation and cellular damage during liver I/R and ischemic AKI (23, 41). Recently it has been shown that HMGB1 is released by necrotic cells and leads to neutrophil recruitment and injury amplification (24). We observed that blue light also reduced the systemic release of HMGB1. Thus, a reduction in the release of HMGB1 and an inhibition of neutrophil

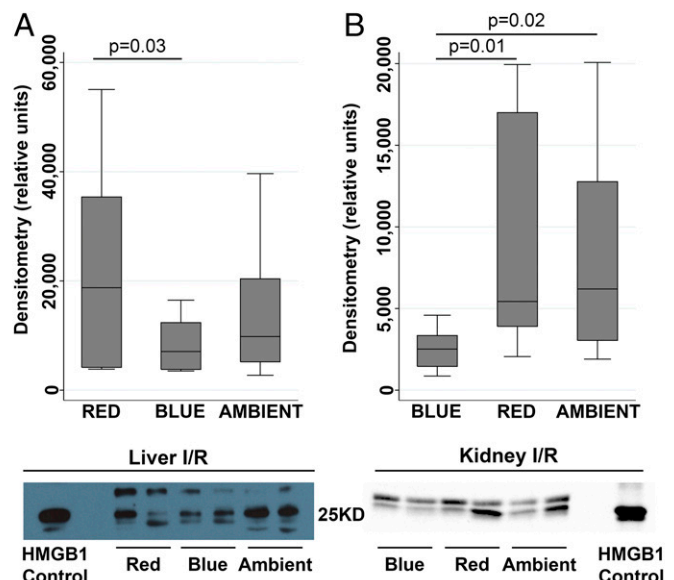

Fig. 4. Blue light attenuates HMGB1 release during hepatic and renal I/R. Mice were exposed to red, blue, or ambient light for 24 h and then subjected to (*A*) hepatic I/R (10–12 total mice per group for all four experiments combined) or (*B*) unilateral kidney I/R (13 total mice per group for all four experiments combined). Serum HMGB1 (29KD) expression (representative immunoblot of four experiments). Corresponding box plots provide summary estimates (bar, median; box, IQR 25–75% range; whiskers, 1.5× IQR) of densitometry of HMGB1 concentration. Statistical comparisons were made by nonparametric Mann–Whitney test.

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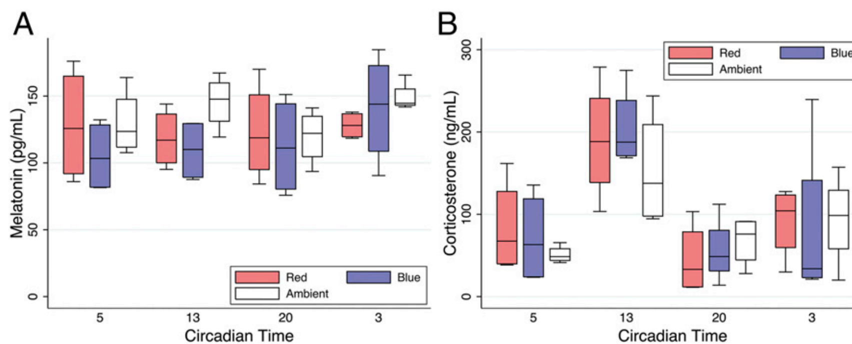

Fig. 5. Blue light does not alter serum melatonin or corticosterone concentration. Mice (C57BL/6) were exposed to red, blue, or ambient light for 24 h (six total mice per group for two experiments combined). Serum was isolated at CT5, CT13, CT20, and CT3 and analyzed for (*A*) melatonin (pg/mL) and (*B*) corticosterone (ng/mL) concentrations. Corresponding box plots provide summary estimates (bar, median; box, IQR 25–75% range; whiskers, 1.5× IQR) of melatonin and corticosterone concentrations. Statistical comparisons were made by nonparametric Mann–Whitney test.

recruitment are two biological mechanisms by which blue light may protect against ischemia.

The physiologic mechanism through which blue light regulated these biological changes required an intact optic pathway. The prototypical mediator of central circadian rhythms and photoimmunomodulation is melatonin (29). Nearly every facet of immunity exhibits a circadian pattern and appears under the regulatory influence of melatonin and light (27, 28, 42, 43). Similarly, corticosteroid stress hormones also exhibit circadian rhythm, are modulated by acute light exposure and may influence immunity (15, 16). However, the effects of blue light occurred without notable differences in systemic melatonin or corticosterone concentrations in comparison with red light. Furthermore, the experimental conditions of blue and red light were characterized by identical photoperiods, illuminances, and temperatures. These observations highlight the importance of the spectrum of light in modifying the biological response to I/R, and that mechanisms distinct from central circadian and adrenocortical axes are operant. Notably, degeneration of the optic nerve as occurs in VsX2 KO mice correlated with reduced AKI during I/R and a phenotype more closely approximating that of mice exposed to blue light. These data may provide sufficient impetus for a clinical investigation of the ramifications of optical blindness on ischemic diseases.

Our analyses of melatonin and corticosteroids were attempts to study the central circadian clock (i.e., SCN, melatonin) and determine whether blue light altered these rhythms relative to red light; our data suggest that it does not. These results do not address whether circadian rhythms were altered, in general, by any or all of the lighting conditions. Although corticosterone concentrations exhibited a circadian pattern of release, melatonin was suppressed by each lighting condition. And, although the capacity for light to suppress melatonin is dependent upon the spectrum, contemporary studies demonstrate that red light (600, 630, 700 nm) of sufficient intensity can also suppress melatonin (44, 45). We speculate that the high illuminance of our intervention (Fig. S2) rendered similar suppression of melatonin for both the red and blue light groups.

Nearly all visceral organs receive direct innervation from the autonomic nervous system (ANS). The two branches of the ANS are key regulators of immune responses (33, 46–48). Acute exposure to bright light has been shown to rapidly induce sympathoexcitation of adrenal, renal, and hepatic tissue (49–51). These data may appear at odds with our overarching paradigm: blue light leads to a withdrawal of sympathetic tone in reducing immune-mediated injury. However, the exposure used in these earlier studies was typically to white fluorescent (49) or incandescent (16, 50, 51) light of brief (10–30 min) duration, the

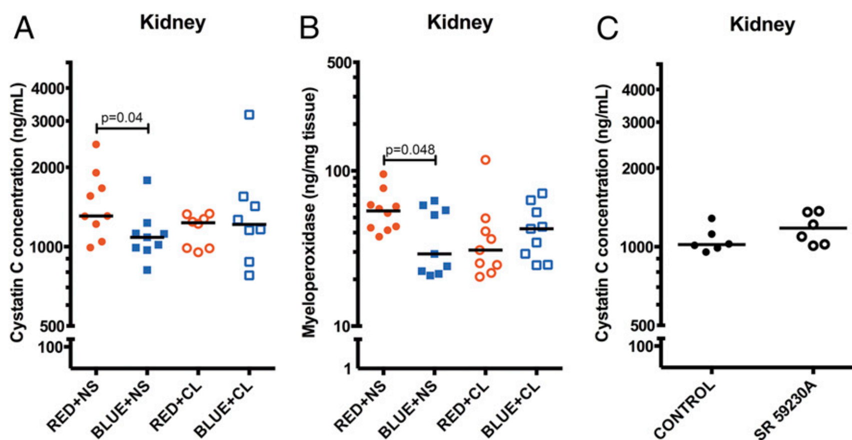

Fig. 6. Blue light functions through an adrenergic pathway. Mice were administered either equivolume saline (0.9%, i.p.) or the β_3 agonist CL316,243 (1 mg/kg, i.p.), and exposed to red, blue, or ambient light for 24 h. Mice were then subjected to unilateral kidney I/R (nine total mice per group for all four experiments combined). (*A*) Serum was analyzed for cystatin C concentration (ng/mL). Bar, median. (*B*) Kidney tissue was analyzed for MPO concentration (ng/mg tissue protein). Bar, median. (*C*) Mice were administered either equivolume saline (0.9%, i.p.) or the β_3 antagonist SR 59230A (5 mg/kg, i.p.) and then subjected to unilateral kidney I/R (six total mice per group for two experiments combined). Serum was analyzed for cystatin C concentration (ng/mL). Bar, median. Statistical comparisons were made by nonparametric Mann–Whitney test.

subsequent assessment of sympathetic activity was performed within minutes, and the duration of sympathoexcitation transient (20–180 min). In contrast, our intervention of a prolonged 24-h photoperiod of rich, blue spectrum that is instituted in mice already exposed to ambient "day" illumination is distinct. In the study of Ishida et al. (16), light exposure did not significantly heighten adrenal sympathetic nerve activity when instituted at daytime. And although Mutoh et al. (49) did observe increased renal sympathetic nerve activity when mice were exposed to light during the day, this sympathoexcitation was minimal and transient (~10 min) with an illuminance less than 2,000 lx; our red and blue lights were 1,400 lx. When we exposed mice to a 24-h photoperiod of blue spectrum light, they exhibited reduced sympathetic tone (i.e., heart rate, LF/HF). Thus, we perceive that our experimental conditions are distinct from the prior literature, our data unique and complementary to those previously published, and that a unifying theme can explain these seemingly disparate results. Although acutely white or fluorescent light may heighten sympathetic activity, during more prolonged exposure, particularly to blue spectrum light, a transition to a withdrawal of adrenergic tone may occur.

Chronic exposure to dim light at night (dLAN) has been shown in rodents to suppress both humoral and cellular immunity (9). Thus, the absence of a true night (<5 lx) could also account for our observations. However, in these prior reports, mice were chronically exposed (3–4 wk) to dLAN before immune challenge. Furthermore, if dLAN were operant in our experiments, its effects would have to have been spectrum specific, because red and ambient light possessed a similar exposure to light at night (LAN) as blue light, yet afforded no protection. Alternatively, a continuous photoperiod may also disrupt sleep, which too has been shown to be immunosuppressive (31, 32). However, mice exhibited similar activity levels with each light condition, and yet blue light was distinctly protective. Thus, we perceive that mechanisms distinct from light pollution or sleep disturbances are at play.

Recently, it has been described that long-range sympathetic signals, modulated by light, govern a circadian oscillation in leukocyte emigration into certain tissues (27, 33). In rodents, sympathetic tone and leukocyte recruitment peak at night, a time of maximal animal activity (27, 33). These circadian rhythms in leukocyte migration alter the outcome from inflammatory diseases; injury is maximal at night when adrenergic tone and neutrophil emigration are highest. Our data suggest that blue light reduces adrenergic tone and thereby, attenuates neutrophil influx. We observed that the administration of a β_3 agonist inhibited the protective effects of blue light and rendered the outcomes with blue light similar to those of red light. By contrast, β_3 antagonism did not afford protection. However, SR 59230A also possesses α_1 antagonism, which potentiates arterial vasodilatation, and thus may induce systemic hypotension and worsen organ ischemia (52). Thus, the lack of protection with SR 59230A may be the consequence of the offsetting effects of β_3 and α_1 antagonism. Alternatively, these results may be interpreted to indicate that a withdrawal of β_3 adrenergic tone is permissive, although not sufficient for attenuating organ injury. This interpretation would also be in agreement with our data demonstrating that β_3 agonism reverses the protection of blue light. Thus, we propose that blue light, functioning through an optic pathway, leads to a withdrawal in β_3 sympathetic tone that permissively reduces neutrophil influx and organ injury. The reduction in cellular necrosis may further lead to a reduction in the release of HMGB1, and thereby "break" an injury amplification loop, characterized by additional neutrophil recruitment and cellular injury.

In conclusion, our data support that light can be harnessed to acutely modify the biology, physiology and outcome of critical illness. Pretreatment with blue light is protective during I/R, and thus represents a possible therapeutic to target and control systemic inflammation and mitigate organ injury in clinical circumstances permitting pretreatment. However, there are distinct differences between the visual, circadian, and immune biology of nocturnal mammals, as studied herein, and that of diurnal *Homo sapiens*. Thus, if light does impart therapeutic value, further investigation to determine the precise characteristics (illuminance, photoperiod, and wavelength) will be critically important to optimize its potential clinical translation.

Materials and Methods

Study Design. Our study was a randomized, controlled laboratory experiment to study the biologic effects of three light spectrums on the organ injury induced by I/R using murine models of kidney and liver I/R. The primary a priori hypothesis is that high-illuminance blue spectrum light reduces kidney and liver organ injury from I/R, by comparison with high-illuminance red and standard illuminance, ambient, white fluorescent light. The primary endpoints are serum cystatin C and ALT concentrations, as validated markers of renal and hepatic cellular injury.

Reagents. Antibodies for HMGB1 (ab79823) and tubulin (ab4074) were obtained from Abcam. The β_3 agonist CL316,243 and β_3 antagonist SR 59230A were obtained from Sigma-Aldrich.

Animal experimentation. We used 8- to 12-wk-old male C57BL/6J, 129S1/Sv-*Vsx2*$^{or-J}$/J (Vsx2, visual system homeobox 2), and their control 129S1/SvImJ mice (The Jackson Laboratory). Homozygous 129S1/Sv-*Vsx2*$^{or-J}$/J mice exhibit abnormal eye development: microphthalmia, small lens, and absence of the optic nerve; otherwise, these mice are fertile and exhibit no other phenotypic abnormalities (19). All experimentation was initiated at CT2 (CT, circadian time set 0 as previous dawn and 12 previous dusk). All animals had ad libitum access to water and LabDiet Prolab IsoPro RMH 3000 diet pellets (LabDiet).

Liver I/R. A segmental (70%) hepatic warm I/R with 60 min of ischemia was used as previously performed (23).

Renal I/R. A unilateral renal I/R model was used (53). A laparotomy was performed, the left kidney was exposed, and the renal pedicle was occluded for 30 min. Reperfusion was then initiated, the contralateral kidney was removed, and the abdomen closed.

The temperature during ischemia was maintained at 31 °C using a warming incubator chamber. At the end of 6 h (liver I/R) or 24 h (renal I/R) of reperfusion, the mice were anesthetized with isoflurane and killed by exsanguination.

For all studies, one investigator allocated each mouse to a single light exposure and a second investigator, blinded to the lighting conditions, performed the surgical experimentation and collected the samples. An investigator blinded to the specific treatment analyzed the data.

Exposure to light. All experiments were conducted in a climatic room maintained on a day–night 12:12 h light:dark cycle (lights on from 0800 to 2000 hours) at an ambient temperature of 23 ± 2 °C and a relative humidity of 60%. Mice were randomly assigned to one of three different spectral illuminances: red (617 nm, 1,400 lx), blue (442 nm, 1,400 lx), and ambient light (fluorescent white light, 400 lx) (*SI Materials and Methods*). For hepatic and renal I/R these lighting environments were administered continuously for the 24-h period preceding experimentation and the respective 6- and 24-h periods of reperfusion. We continuously measured cage temperature and demonstrate that the heat emission modestly affected the cage temperatures (25 ± 2 °C; Fig. S3). Illuminance was measured with a handheld digital lux meter (Digital Light Level Meter LX1330B; Mastech). Spectroradiometric measurements were made with an ISP-80-8-I integrating sphere (Ocean Optics).

Heart rate variability. Mice underwent laparotomy and implantation of a DSI HD-X11 wireless telemetry monitor (DSI) within the peritoneal cavity as previously published (*SI Materials and Methods*) (54). The HD-X11 is a reusable 2.2-g, 1.4-cc wireless telemetry device capable of continuous measurement of one biopotential (e.g., electrocardiogram) and animal activity. After a 24-h period of recovery, mice were exposed to blue and red spectrum light and monitored for 24 h. Data collection and analysis were performed using Ponemah version 5.20 (DSI). Frequency domain parameters of heart rate variability, specifically the LF/HF ratio, were calculated as published (34–38).

Organ physiology. Serum ALT concentration was determined using a DRI-CHEM 4000 Chemistry Analyzer System (Heska) (23). Renal function was determined by assaying serum for cystatin C [interassay coefficient of variability (CV): 2.8%] using an enzyme immunoassay kit (R&D) (55).

Histology. The extent of parenchymal necrosis was graded using H&E-stained histological sections at 200× magnification with evaluation of 10 fields per sample (*SI Materials and Methods*).

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Immunoblot and HMGB1 concentration. Serum was electrophoresed in a 15% SDS/PAGE gel and developed as previously described (23). Densitometry was performed by the NIH Image program (NIH) (56).

Melatonin and corticosterone concentrations. Serum melatonin concentration was quantified using an enzyme-linked immunosorbent assay (interassay CV: 6.5%; IBL). Serum corticosterone concentration was determined by an enzyme immunoassay kit (interassay CV: 2.5%; Enzo Life Science).

Myeloperoxidase. MPO concentration was determined using an ELISA assay kit (interassay CV: 7.6%; Hycult Biotech).

Statistics. Statistical analyses were performed using Stata 12SE software. Values are expressed as medians. Groups are compared by Mann–Whitney rank sum. A $P < 0.05$ was considered statistically significant.

Study approval. We performed all animal experiments in accordance with the NIH Guide for the Care and Use of Laboratory Animals under protocols approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

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