Early Human Development 91 (2015) 381–385

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Phototherapy with blue and green mixed-light is as effective against unconjugated jaundice as blue light and reduces oxidative stress in the Gunn rat model

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ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 16 April 2015
Accepted 20 April 2015

Keywords:
Side effects
Hyperbilirubinemia
Neonate
Light emitting diode
Unbound bilirubin

ABSTRACT

Objective: Phototherapy using blue light-emitting diodes (LED) is effective against neonatal jaundice. However, green light phototherapy also reduces unconjugated jaundice. We aimed to determine whether mixed blue and green light can relieve jaundice with minimal oxidative stress as effectively as either blue or green light alone in a rat model.
Methods: Gunn rats were exposed to phototherapy with blue (420–520 nm), filtered blue (FB; 440–520 nm without < 440-nm wavelengths, FB50 (half the irradiance of filtered blue), mixed (filtered 50% blue and 50% green), and green (490–590 nm) LED irradiation for 24 h. The effects of phototherapy are expressed as ratios of serum total (TB) and unbound (UB) bilirubin before and after exposure to each LED. Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) was measured by HPLC before and after exposure to each LED to determine photo-oxidative stress.
Results: Values < 1.00 indicate effective phototherapy. The ratios of TB and UB were decreased to 0.85, 0.89, 1.07, 0.90, and 1.04, and 0.85, 0.94, 0.93, 0.89, and 1.09 after exposure to blue, filtered blue, FB50, and filtered blue mixed with green LED, respectively. In contrast, urinary 8-OHdG increased to 2.03, 1.25, 0.96, 1.36, 1.31, and 1.23 after exposure to blue, filtered blue, FB50, mixed, green LED, and control, indicating side-effects (>1.00), respectively.
Conclusions: Blue plus green phototherapy is as effective as blue phototherapy and it attenuates irradiation-induced oxidative stress.
Practice implications: Combined blue and green spectra might be effective against neonatal hyperbilirubinemia.

1. Introduction

The fact that natural sunlight reduces neonatal jaundice was discovered in 1975 [1]. Cremer and colleagues then investigated the effective range of visible light and determined that bilirubin absorbs sunlight in the blue spectrum *in vitro* [2]. They developed a phototherapy device that emitted blue light superimposed upon white light for clinical applications [1,2]. Thereafter, blue light was considered more effective than white light [3]. Blue fluorescence light without the < 400-nm wavelength at the near ultraviolet end of the spectrum has been applied in Japan. However, a shorter wavelength that might damage cellular DNA [4] was still included in the blue fluorescence device. Thus, a device emitting green fluorescence was developed for phototherapy against jaundice [5,6] and this device has also been applied in Japan to treat neonatal jaundice. Green fluorescent light was initially considered to reduce serum total bilirubin to a level equivalent to that of blue light [5,6].

Durable, blue light-emitting diodes (LED) have recently been used predominantly to reduce electrical power consumption, and phototherapy with blue LED also has proven as effective as the use of blue fluorescent light [7–9].

Our retrospective study at Nara Medical University NICU found that entirely blue light with a narrow spectrum emitted by an LED together with a device emitting broad-spectrum green fluorescence with some blue component similarly mitigated neonatal unconjugated jaundice [10]. However, neither of these devices alone could treat serious jaundice caused by blood type incompatibility or accompanied by sepsis, whereas a combination of both was effective against unconjugated jaundice (unpublished data). In addition, we hypothesized that combined blue and green light might reduce total bilirubin in addition to oxidative stress resulted in on neonates by exposure to powerful blue light. The present study therefore aimed to confirm whether or not

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http://dx.doi.org/10.1016/j.earlhumdev.2015.04.010
0378-3782/

blue and/or green light emitted by customized LED devices can mitigate unconjugated jaundice in Gunn rats and whether or not various wavelengths impose changes in oxidative stress.

2. Materials and methods

2.1. Animals and environment

The models of unconjugated jaundice comprised 12 four-week-old homozygous male Gunn rats (Slc-j/j) (Nihon SLC Inc., Hamamatsu, Japan) that were acclimated for one week before the next three weeks trials. All experiments including control trials proceeded in the same room. This room was controlled facility at 23 °C and had no windows, and light cycle was controlled at the Laboratory Animal Research Center, Nara Medical University as follows: lights on, 08:00–20:00 h; lights off, 20:00–08:00 h (mimicking an artificial circadian rhythm of night-and-day). And even during phototherapy, we could not change the lighting because of the central control. The Ethics Committee at Nara Medical University approved the study protocol and the animals were handled and maintained according to institutional guidelines (Approval numbers: 10160 and 10489). They always could access to the CE-2CLEA rodent diet (CLEA Japan Inc., Tokyo, Japan) and water *ad libitum*. One of the 12 rats (No.9 rat; Table 1.) died while under anesthesia for shaving and venipuncture at six weeks of age. His data of the 5-weeks-old were involved in this analysis (Table 1.).

2.2. Wavelength and intensity of LED units

The following (P4630) LED units (Ushio Inc., Tokyo, Japan) were custom-built for this study. The wavelength ranges (with peak emissions; color) were 420–520 (450; blue), 440–520 (455; filtered blue: FB), 440–590 (bimodal peak, 455, 515; mixed), and 490–590 (515; green) nm (Fig. 1). The mean energy of light intensity was 669, 671, 336, 671, and 738 μW/cm^2 for the blue, filtered blue, filtered blue at 50% intensity (FB50), mixed, and green LED, respectively. Spectral intensity was obtained by integrating each spectrum as a function of wavelength measured using a calibrated HR4000 spectrometer (Ocean Optics, Dunedin, FL, USA) at a distance of 12.5 cm. During phototherapy, the LED unit was suspended in a canopy 12.5 cm above 3700M071 metabolic cages (Tecniplast, Buguggiate, Italy).

Table 1
Light-emitting diodes and laboratory data at start of phototherapy.
Schedule of phototherapy. Serum TB and UB levels at start of phototherapy and urinary 8-OHdG at 6 h thereafter did not significantly differ among rats (Student's *t*-test). Rat No. 9 died while under anesthesia for shaving and venipuncture at six weeks of age. TB, serum total bilirubin; UB, serum unbound bilirubin.

LED TB, UB and 8-OHdG at start of phototherapy			
Rat no.	5-weeks old	6-weeks old	7-weeks old
1	Green 4.8, 0.75, 628.52	Mixed 4.7, 0.76, 335.99	Filtered blue 4.1, 0.63, 351.32
2	Mixed 5.1, 0.7, 486.26	Filtered blue 5.9, 0.76, 1817.46	Green 4.5,0.74, 827.67
3	Filtered blue 5.4, 0.88, 469.93	Green 5.5,0.78, 643.99	Mixed 4.4, 0.68, 197.65
4	Green 5.2, 0.7, 775.48	Mixed 6.0, 0.93, 633.40	Filtered blue 5.5, 0.80, 388.83
5	Mixed 4.9, 0.7, 453.99	Filtered blue 5.0,0.77, 555.98	Green 5.1, 0.74, 254.95
6	Filtered blue 5.0/0.79: 172.20	Green 5.4/0.85: 163.35	Mixed 5.1/0.79: 161.80
7	Control NT NT 557.17	FB50 4.3, 0.66, 593.56	FB50 4.5,0.82, 868.95
8	FB50 6.2, 0.96, 557.17	Control NT NT 681.42	FB50 5.3, 0.81, insufficient sample volume
9	FB50 4.7, 0.76, insufficient sample volume	Dead	Dead
10	Blue 5.8, 0.89, 472.81	Blue 6.2, 0.90, 492.53	Blue 4.9, 0.82, 364.59
11	Blue 7.2, 1.10, 216.88	Blue 5.4, 0.83, 213.94	Blue 6.1, 0.98, 146.51
12	Control NT NT 598.86	Control NT NT 634.80	Control NT NT 485.96

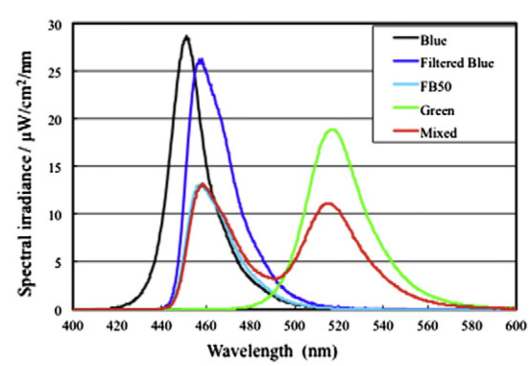
LED, light-emitting diode; NT, not tested; TB (mg/dL), UB (μg/dL); serum obtained by venipuncture at 0 h phototherapy. 8-OHdG (μg/g Creatinine); urine collected between 0–6 h of phototherapy (pre-phototherapy).

2.3. Phototherapy procedures

The flanks and backs of five to seven-week-old Gunn rats weighing 60–170 g were shaved, then blood and urine samples were collected before and after exposure to continuous phototherapy for 24 h. The rats were allowed to breed normally for six days and were then exposed to different wavelengths for 24 h. Phototherapy with blue LED, filtered blue LED, FB50 LED, mixed LED, and green LED was applied to rats bred from the first until the last exposure to phototherapy for three weeks. Control rats were not exposed to illumination. All experiments including control trials proceeded consecutively from 06:00 h to 06:00 h on the following day in the same room and under the light cycle environment that mentioned "Animals and environment". Table 1 shows the phototherapy schedule and the measured parameters (serum total bilirubin (TB), unbound bilirubin (UB), and 8-OHdG; 8-hydroxy-2′-deoxyguanosine) at the start of phototherapy. All rats underwent three 24-h phototherapy sessions. None of the measured parameters were influenced by prior studies (TB: 5 weeks old vs. 6 weeks old, $p = 0.79$; 6 weeks old vs. 7 weeks old, $p = 0.13$; UB: 5 weeks old vs. 6 weeks old, $p = 0.48$; 6 weeks old vs. 7 weeks old, $p = 0.57$; 8-OHdG: 5 weeks old vs. 6 weeks old, $p = 0.38$; 6 weeks old vs. 7 weeks old, $p = 0.20$; Student's *t*-test), confirming that the six-day interval between studies was sufficient.

2.4. Serum total and unbound bilirubin analysis

Concentrations of TB and UB were determined from blood (0.05 mL) collected from tail veins under inhaled isoflurane anesthesia into sodium heparinized micro-hematocrit capillary tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 0 and 24 after starting phototherapy. Serum was immediately separated by centrifugation for 5 min at 11,800 ×g (Kubota Corp., Tokyo, Japan) and then TB and UB were



		Blue	Filtered Blue	FB 50	Mixed	Green
Total irradiance	(/μW/cm^2)	669	671	336	738	671
Wavelength	(nm)	420-520	440-520	440-520	440-590	490-590
Peak wavelength	(nm)	450	455	455	455, 515	515

Fig. 1. Emission spectra of light-emitting diode (LED) devices used for phototherapy. Irradiance of each device was adjusted to minimize differences except for FB50 which was filtered blue light with 50% intensity achieved by omitting wavelengths < 440 nm. Mixed refers to LED device comprising half filtered blue and half green lamps and it has bimodal peak.