Blue LED phototherapy in preterm infants

van der Schoor, Lori W E; van Faassen, Martijn H J R; Kema, Ido; Baptist, Dyvonne H; Olthuis, Annelies J; Jonker, Johan W; Verkade, Henkjan J; Groen, Henk; Hulzebos, Christian V

Published in:
ARCHIVES OF DISEASE IN CHILDHOOD-FETAL AND NEONATAL EDITION

DOI:
10.1136/archdischild-2019-317024

Publication date:
2020

Blue LED phototherapy in preterm infants: effects on an oxidative marker of DNA damage

Lori W E van der Schoor,[1] Martijn H J R van Faassen,[2] Ido Kema,[2] Dyvonne H Baptist,[3] Annelies J Olthuis,[3] Johan W Jonker,[1] Henkjan J Verkade,[4] Henk Groen,[5] Christian V Hulzebos[3]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/archdischild-2019-317024).

[1]Department of Pediatrics, Beatrix Children's Hospital, University Medical Center Groningen, Groningen, The Netherlands
[2]Department of Laboratory Medicine, University Medical Center Groningen, Groningen, The Netherlands
[3]Department of Neonatology, Beatrix Children's Hospital, University Medical Centre Groningen, Groningen, The Netherlands
[4]Department of Pediatric Gastroenterology and Hepatology, Beatrix Children's Hospital, University Medical Center Groningen, Groningen, The Netherlands
[5]Department of Epidemiology, University Medical Center Groningen, Groningen, The Netherlands

Correspondence to
Lori W E van der Schoor, Department of Pediatrics, University Medical Center Groningen, Groningen, Netherlands; lwevanderschoor@gmail.com

Received 11 February 2019
Revised 8 March 2020
Accepted 11 March 2020

To cite: van der Schoor LWE, van Faassen MHJR, Kema I, *et al*. *Arch Dis Child Fetal Neonatal Ed* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/archdischild-2019-317024

ABSTRACT

Background Phototherapy is used on the majority of preterm infants with unconjugated hyperbilirubinaemia. The use of fluorescent tube phototherapy is known to induce oxidative DNA damage in infants and has largely been replaced by blue light-emitting diode phototherapy (BLP). To date, it is unknown whether BLP also induces oxidative DNA damage in preterm infants.

Objective To determine whether BLP in preterm infants induces oxidative DNA damage as indicated by 8-hydroxy-2′deoxyguanosine (8-OHdG).

Design Observational cohort study.

Methods Urine samples (n=481) were collected in a cohort of 40 preterm infants (24–32 weeks' gestational age) during the first week after birth. Urine was analysed for the oxidative marker of DNA damage 8-OHdG and for creatinine, and the 8-OHdG/creatinine ratio was calculated. Durations of phototherapy and levels of irradiance were monitored as well as total serum bilirubin concentrations.

Results BLP did not alter urinary 8-OHdG/creatinine ratios (B=0.2, 95% CI −6.2 to 6.6) at either low (10–30 μW/cm^2/nm) or high (>30 μW/cm^2/nm) irradiance: (B=2.3, 95% CI −5.7 to 10.2 and B=−3.0, 95% CI −11.7 to 5.6, respectively). Also, the 8-OHdG/creatinine ratios were independent on phototherapy duration (B=−0.1, 95% CI −0.3 to 0.1).

Conclusions BLP at irradiances up to 35 μW/cm^2/nm given to preterm infants ≤32 weeks' gestation does not affect 8-OHdG, an oxidative marker of DNA damage.

What is already known on this topic?

- Phototherapy is the gold standard for treating unconjugated hyperbilirubinaemia, and it is used in more than 80% of preterm infants admitted to a neonatal intensive care unit.
- Fluorescent tube phototherapy is associated with oxidative stress and DNA damage in full-term neonates.
- Fluorescent tube phototherapy is being replaced by blue light-emitting diode (LED) phototherapy, which allows delivery of higher irradiances without significant heat production.

What this study adds?

- Blue LED phototherapy up to 35 μW/cm^2/nm is not associated with an increase in the oxidative marker of DNA damage 8-hydroxy-2′deoxyguanosine (8-OHdG) in preterm infants ≤32 weeks' gestation.
- 8-OHdG/creatinine ratios increase during the first week after birth and are highest in infants weighing <1000 g.

INTRODUCTION

Neonatal jaundice as a result of unconjugated hyperbilirubinaemia occurs in up to 80% of preterm infants in the first week after birth.[1] If severe and left untreated, unconjugated hyperbilirubinaemia may lead to permanent neurological damage or even death. From 1968 onward, the standard treatment for neonatal hyperbilirubinaemia has been phototherapy[2] and presently given to >80% of preterm infants admitted to a neonatal intensive care unit.[3] Since the introduction of phototherapy, the use of exchange transfusions has diminished drastically.[4] Nevertheless, conventional phototherapy using fluorescent tubes (FTs) has several known side effects, including oxidative stress (OS) and DNA damage in full-term infants,[5–8] and a tendency towards increased mortality in preterm infants.[9 10] In the long-term, FT phototherapy has been associated with diabetes, asthma and epilepsy, and a slight increased incidence of infant cancer,[11–15] but the mechanisms involved have never been established.

Over the past 60 years, phototherapy devices incorporating FTs have been largely replaced by devices with blue light-emitting diodes (LEDs), which makes it easier and safer to administer high irradiances with less heat production.[16] It remains largely unclear whether using blue LED phototherapy (BLP) in preterm infants causes OS and thereby DNA damage. Recently, we analysed effects of BLP at irradiances up to 100 μW/cm^2/nm in hyperbilirubinaemic rats and found no oxidative DNA damage as indicated by the markers 8-hydroxy-2′-deoxyguanosine (8-OHdG) or gamma-H2AX.[17] It is unclear, however, whether we can reliably extrapolate these results to preterm infants, who are more susceptible to oxidative damage in comparison with full-term infants.[18] In the present study, we determined the effects of BLP on the oxidative marker of DNA damage 8-OHdG in preterm infants of ≤32 weeks' gestational age.

METHODS

Research population

In this observational cohort study, we enrolled 40 infants. Inclusion criteria were a gestational age ≤32 weeks, admission to the neonatal intensive care unit of the Beatrix Children's Hospital, University Medical Center Groningen, the Netherlands within 24 hours after birth and phototherapy treatment during the first postnatal week. Infants were recruited between November 2016 and April 2017. Exclusion criteria included congenital malformations or syndromes and infants with non-Dutch or non-English speaking parents. Parental informed consent was obtained for all participants.

Urine collection

On admission, urine samples were collected as soon as possible and serially collected during the first week, resulting in 0–5 urine samples per day for seven consecutive days. We collected the urine using PeeSpots (Hessels+Grob, Apeldoorn, the Netherlands).[19] The PeeSpots were placed and collected by the nursing staff at each routine diaper change. The time of collection was recorded for each urine sample. At each collection, the PeeSpots were placed in a tube and stored at −20°C for maximally 4 weeks. Prior to analysis, the tubes were thawed and centrifuged for 1 min at 200XG to retrieve the urine. We chose to collect urine using PeeSpots instead of gauze pads. This decision was based on a pilot study in which retrieval of both 8-OHdG and creatinine was 100% from the PeeSpots, whereas retrieval of 8-OHdG from a gauze was only 78% (data not shown).

Urinary analyses of 8-OHdG

We measured the oxidative marker of DNA damage 8-OHdG, the breakdown product of guanine, in urine. Guanine is the DNA base that is most susceptible to OS, and 8-OHdG is completely excreted in the urine.[20] Thus, 8-OHdG was obtained in a non-invasive manner and it specifically served as a marker of OS-induced DNA damage. It is the most commonly used OS marker in oncology and environmental toxicology and is very stable as repeated measurements of urinary 8-OHdG in samples stored at −20°C even for 15 years and did not show any decline.[20 21] 8-OHdG and creatinine were measured as previously described.[17] In order to obtain the 8-OHdG/creatinine ratio, the concentration of 8-OHdG was divided by the concentration of creatinine to correct for urine osmolality.

Phototherapy

Phototherapy was administered using either a neoBLUE mini (Natus Medical Incorporated, San Carlos, California, USA) or a Mavi LED Phototherapy System (Inspiration Healthcare, Leicester, UK). Both systems emit blue LED light between 450 nm and 470 nm and were used at irradiances ranging from 10 to 35 μW/cm^2/nm. Phototherapy devices were positioned at ~35 cm distance from the infants, which were naked except for diapers and eye protection. Starting and ending times of phototherapy sessions and the irradiance used at each session were recorded. Irradiances were measured daily at the infant's head, trunk and knees using a BiliBlanket Light Meter II (GE Healthcare, Madison, Wisconsin, USA), and the mean irradiance was calculated based on these three measurements.

For every urinary sample collected under phototherapy, we recorded the corresponding irradiance level (μW/cm^2/nm) at that time as well as the duration of phototherapy on the days before collection of that particular urine sample. Generally, a distinction was made between high (≥30 μw/cm^2/nm) and low (<30 μW/cm^2/nm) irradiance.

Clinical parameters

For all infants, we recorded gender, gestational age, birth weight, Apgar score and umbilical pH, CO_2 and base excess. In addition, we recorded whether the infants were part of twins and whether they suffered from sepsis. Furthermore, we assessed daily whether the infant received respiratory support and their daily amount of enteral and breastfeeding.

Statistics

Sample size was calculated using Power Analysis and Sample Size 2019 software.[22 23] Using an expected detected difference (d) of 0.5 SD, an α of 0.05 and β of 0.8 resulted in a required sample size of 34 infants. Instead, we recruited 40 infants to ensure a sufficiently large sample.

Changes in the 8-OHdG/creatinine ratios (8-OHdG/creatinine) over time were analysed using generalised estimating equations (GEEs). This technique allows the longitudinal analysis of datasets with different numbers of measurements among the participants. GEE takes into consideration that measurements from the same individual are correlated with each other. Thereby, GEE corrects for this correlation without discarding individual measurements and maximises the use of available data without artificially increasing the sample size.[24]

Phototherapy use, irradiance and duration were entered as predictors in a GEE model to assess their influence on the variation in 8-OHdG/creatinine during the first postnatal week. Potential confounding clinical parameters, for example, breast feeding or respiratory support, were entered as predictors. Since all possible effects needed to be corrected for postnatal age, univariable analyses were performed by entering each parameter individually in a GEE model, together with postnatal day (table 1/online supplementary table S3). In addition, to assess a possible interaction between birth weight and phototherapy parameters, we assessed the predictive power of the respective interaction terms.

For all GEE analyses, an exchangeable working correlation matrix was used, assuming a fixed correlation between measurements within one participant. On entering specific categorical parameters (eg, phototherapy vs no phototherapy), the model could predict 8-OHdG/creatinine for each predictor category: the estimated marginal mean (EMM). These EMMs reflect 8-OHdG/creatinine more accurately than sample means because infants with many urine samples will contribute more to the sample mean than infants with few samples. Therefore, all figures display EMMs on the y-axes. GEE analyses were performed on raw 8-OHdG/creatinine ratios because logarithmical transformation reduced the skewness but still did not result in a normal distribution (Kolmogorov-Smirnov $p<0.005$), and it did not significantly affect the linearity of the association (homoscedasticity of residuals). In the text, data are displayed as (regression coefficient (B) (CI).

RESULTS

Table 2 shows the clinical characteristics of the enrolled infants. On average, infants received 74 (median) hours of phototherapy at median irradiance of 15 (14 to 21) μW/cm^2/nm. The phototherapy duration per case is shown in online supplementary table S1. One infant received phototherapy within 24 hours after birth, and 17 received phototherapy on day 1. The number of

Table 1 Generalised estimating equations (GEE) model to predict the 8-OHdG/creatinine ratios (µg/g creatinine)

Parameter*	Categories	Regression coefficient	95% CI lower	95% CI upper
Postnatal day	Day 0 (n=22 samples)	0		
	Day 1–6 (n=459 samples)	9.5	5.8	13.2
Birth weight (g)	≥1000 (n=311 samples)	0		
	<1000 (n=170 samples)	8.8	1.6	16.0
No phototherapy versus phototherapy samples	No phototherapy (n=168 samples)	0		
	Phototherapy (n=287 samples)	0.2	−6.2	6.6
Before versus during vs after phototherapy	Before phototherapy (n=100 samples)	0		
	During phototherapy or <2 hours after phototherapy (n=286 samples)	−2.6	−16.1	10.9
	2–12 hours after phototherapy (n=25 samples)	−7.9	−22.8	7.0
Low irradiance versus high irradiance (µW/cm^2/nm)) phototherapy	No lamp (n=164 samples)	0		
	Low irradiance (<30) (n=171 samples)	2.3	−5.7	10.2
	High irradiance (≥30) (n=39 samples)	−3.0	−11.7	5.6
Total phototherapy duration (hours)		−0.12	−0.33	0.09
Total serum bilirubin (µmol/L)		−0.06	−0.14	0.01

*All results originated from individual testing of each parameter in a GEE model together with postnatal day in order to correct for the effect of postnatal age.
8-OHdG, 8-hydroxy-2′deoxyguanosine.

urine samples collected per case is shown in online supplementary table S2.

The 8-OHdG/creatinine ratios depend on postnatal age

Both 8-OHdG (median 2.3 (interquartile range 1.6 to 3.5) µg/L) and creatinine (median 0.11 (interquartile range 0.08 to 0.16) g/L) were measured in all urine samples (n=481 samples). This allowed us to calculate 8-OHdG/creatinine (median 20.6 (interquartile range 14.4 to 28.6) µg/g creatinine) for all infants. We found that postnatal day was a significant predictor of 8-OHdG/creatinine and that 8-OHdG/creatinine was lower on the day of birth than on all subsequent days (figure 1A/table 1). This indicated that 8-OHdG/creatinine depends on postnatal age.

The 8-OHdG/creatinine ratios did not increase by phototherapy

For each urine sample, we recorded whether it had been collected under phototherapy, defined as collection after at least 2 hours of continuous phototherapy or within 2 hours after phototherapy had stopped. Before choosing this collection period, we evaluated different collection periods, which all resulted in similar results. The collection period after phototherapy discontinuation was not a significant predictor of 8-OHdG/creatinine and 8-OHdG/creatinine was not different before, during or after phototherapy (figure 1B/1C/table 1). Phototherapy irradiance and total phototherapy duration were not significant predictors of 8-OHdG/creatinine in the GEE prediction model during the first postnatal week (figure 1D/table 1). We also assessed the effect of phototherapy duration by analysing the correlation between the mean 8-OHdG/creatinine on each postnatal day and the total phototherapy duration, which did not result in significant correlations (data not shown).

8-OHdG/creatinine ratios were higher in extremely low birthweight infants, irrespective of phototherapy use or bilirubin levels

Plotting 8-OHdG/creatinine of infants in different birthweight categories (figure 2A) showed that infants weighing <1000 g (n=13) had significantly higher 8-OHdG/creatinine (table 1/figure 2B), indicating that these smallest infants might experience more oxidative DNA damage. Theoretically, this could be caused by either their longer duration of phototherapy exposure (B=27.4 (95% CI 8.2 to 46.6) hours) or by potentially increased susceptibility to phototherapy-induced OS. To assess whether birth weight affects the relationship between phototherapy and 8-OHdG/creatinine, we built interaction terms with birth weight and phototherapy use, irradiance and duration and entered these in a 8-OHdG/creatinine GEE prediction model. Whereas birth weight alone was our significant predictor, the interaction terms with phototherapy use (B=−0.5 (95% CI −16.0 to −15.0), phototherapy irradiance (low irradiance B=−5.2 (95% CI −22.5 to 12.1), high irradiance B=7.9 (95% CI −10.2 to 25.9)) and phototherapy duration (B=−0.09 (95% CI=−0.39 to 0.22)) were not significant predictors. This shows that the relationship between the 8-OHdG/creatinine and phototherapy is not different in low birthweight infants and indicates that the higher 8-OHdG/creatinine in infants <1000 g are merely birthweight mediated and not phototherapy mediated. In addition, since these infants have lower total serum bilirubin levels, we tested whether bilirubin levels affected with the relationship between birth weight and 8-OHdG/creatinine, which was not the case (interaction term birth weight and bilirubin: B=−0.04 (95% CI 0.25 to 0.17)). This shows that higher 8-OHdG/creatinine in infants<1000 g are not bilirubin mediated.

In addition to phototherapy parameters and birth weight, we recorded several clinical parameters as well as medications that could possibly affect OS or 8-OHdG production. With the exception of birth weight, none of these parameters were significant predictors of 8-OHdG/creatinine when tested in our GEE model as shown in online supplementary table S3.

DISCUSSION

We showed that for early preterm infants, BLP at irradiances of up to 35 µW/cm^2/nm was not associated with an increase in the oxidative marker of DNA damage 8-OHdG. We measured 8-OHdG/creatinine before and during phototherapy, and during different time periods after discontinuation of phototherapy, but found no significant difference (figure 1B/1C/table 1). Even in urine samples collected between 12 and 24 hours after

Table 2 Clinical characteristics

Clinical characteristics	Descriptives
Phototherapy	
Phototherapy duration (hours)	74 (45–92)
Phototherapy irradiance (μW/cm²/nm)*	15 (14–21)
Head	16 (13– 22)
Trunk	20 (15–28)
Knees	15 (14–21)
Phototherapy irradiance	
Low irradiance <30 μW/cm²/nm	30 infants (75)
High irradiance ≥30 μW/cm²/nm	10 infants (25)
Total serum bilirubin (μmol/L)	119 (90–154)
Clinical parameters	
Birth weight (g)	1150 (830–1550)
Intrauterine growth restriction (birth weight <10th percentile for gestational age)	12 infants (30)
Gestational age (weeks)	29 (27–30)
Boys/girls	23 boys (58)/17 girls (42)
Part of twins (yes/no)	15 infants (38)/25 infants (62)
Apgar score at 5 min	8 (7–9)
Umbilical pH	7.3 (7.2–7.3) (n=35 infants)
Umbilical CO_2	7.1 (5.7–7.9) (n=34 infants)
Umbilical base excess	−4 (−6 to −2) (n=35 infants)
Respiratory support	
Mechanical	28 infants (70)
Continuous positive airway pressure (CPAP)	38 infants (95)
High flow	12 infants (30)
Low flow	3 infants (8)
Minimal oxygen %	21 (21–21)
Maximal oxygen %	22 (21–30)
Nutrition	
Parenteral feeding	40 infants (100)
Mother's milk	40 infants (100)
Formula feeding	34 infants (85)
Enteral feeding (mL/kg)	38 (14–67)
Mother's milk (mL/kg)	23 (5–54)
Mother's milk % of enteral feeding	94 (47–100)
Sepsis, number †	
Early onset sepsis	2 infants (5)
Late onset sepsis	10 infants (25)

Data are presented as numbers of infants (% of total) or medians (IQR).
*Two infants were briefly treated with 50 μW/cm²/nm, but too few samples were collected during this time for reliable interpretations. These samples were excluded from analyses.
†Early onset was defined as a positive blood culture during the first 48 hours after birth. Late onset was defined as a positive blood culture after the first 48 hours.

discontinuation of phototherapy, we found similar 8-OHdG/creatinine (data not shown). We found no correlation between phototherapy duration or irradiance and 8-OHdG/creatinine. We confirmed that birth weight was a significant predictor of the 8-OHdG/creatinine ratio with infants weighing <1000 g having the highest levels predominantly during the first three postnatal days. This suggested that these infants had higher levels of oxidative DNA damage. Although infants <1000 g received longer phototherapy, the differences could only be explained by birth weight and not by phototherapy (table 1). This is in line with previous measures that reported a significant correlation between birth weight and 8-OHdG. We also showed that, in addition to clinical characteristics, total bilirubin levels were not a significant predictor of 8-OHdG/creatinine. The fact that infants <1000 g had higher 8-OHdG/creatinine could theoretically be caused by photons penetrating deeper into their tissues as their skin thickness of is less when compared with term counterparts. However, we also showed that within this low birth weight group, phototherapy use does not correlate with higher 8-OHdG/creatinine. Furthermore, the 8-OHdG/creatinine ratio increases over time, and does their skin thickness, making an association between skin thickness and 8-OHdG/creatinine unlikely.

Several studies in full-term infants reported an increase in DNA damage after FT phototherapy. FTs could be expected to cause more OS and DNA damage than BLP. BLP allows higher treatment irradiances without producing significant increases in heat. The heat produced by FTs can induce hyperthermia, which is known to enhance OS.[25 26] Second, FTs are known to emit small amounts of ultraviolet light, especially close to the lamp.[27] This is true particularly when the ultraviolet protective screens become damaged over time or are removed.

There are few studies that report on blue FT or LED phototherapy and OS in full-term neonates. Demirel *et al* observed an increased OS index after exposure to FT phototherapy but not after BLP,[28] whereas Kale *et al* and El-Farrash *et al* observed increased OS after both phototherapy types.[29 30] In these studies, however, the compared FT and LED irradiances were neither in the same range nor measured accurately. All studies assessed OS by measuring total oxidant status (TOS) and/or total antioxidant capacity (TAC) in plasma. The validity of these assays in this context has been questioned, because bilirubin acts as an antioxidant and can therefore affect the outcome of TOS and TAC. Since bilirubin is reduced by phototherapy, phototherapy can therefore potentially interfere with both TOS and TAC.[31 32]

In our study, we circumvented these interactions by determining the physiologically relevant effect of OS by using a marker that is only produced as a result of DNA damage. This marker, 8-OHdG, is known to be produced in many OS-induced diseases in preterm infants, such as bronchopulmonary dysplasia, necrotising enterocolitis and retinopathy of prematurity.[33–35] It also has been described to correlate with other frequently used markers of oxidative marker of DNA damage in neonates, such as the Comet assay and malondialdehyde levels.[36 37] In addition, 8-OHdG is the direct oxidation product of the DNA base guanine and is therefore directly correlated with DNA oxidation and damage. Furthermore, using a urinary marker allows for frequent noninvasive sampling.

Limitations

Although we studied 8-OHdG/creatinine longitudinally per child, our study design did not involve continuous measurements. Moreover, no infants received irradiances higher than 35 μW/cm²/nm in this study, and the relatively small sample size did not allow us to assess the influence of all potential confounders. Our study did not include a negative control group that received no phototherapy at all. This limitation is intrinsic to the prematurity of our cohort; the majority of preterm infants developed hyperbilirubinaemia and received phototherapy.

Strengths

First, our prospective longitudinal design allowed accurate monitoring of phototherapy duration and irradiance, whereas GEE analysis allowed us to correct for the effect of age. Most clinical studies only determine markers of damage once before and after phototherapy. Such a design harbours an inherent methodological confounder on account of the fact that certain OS markers

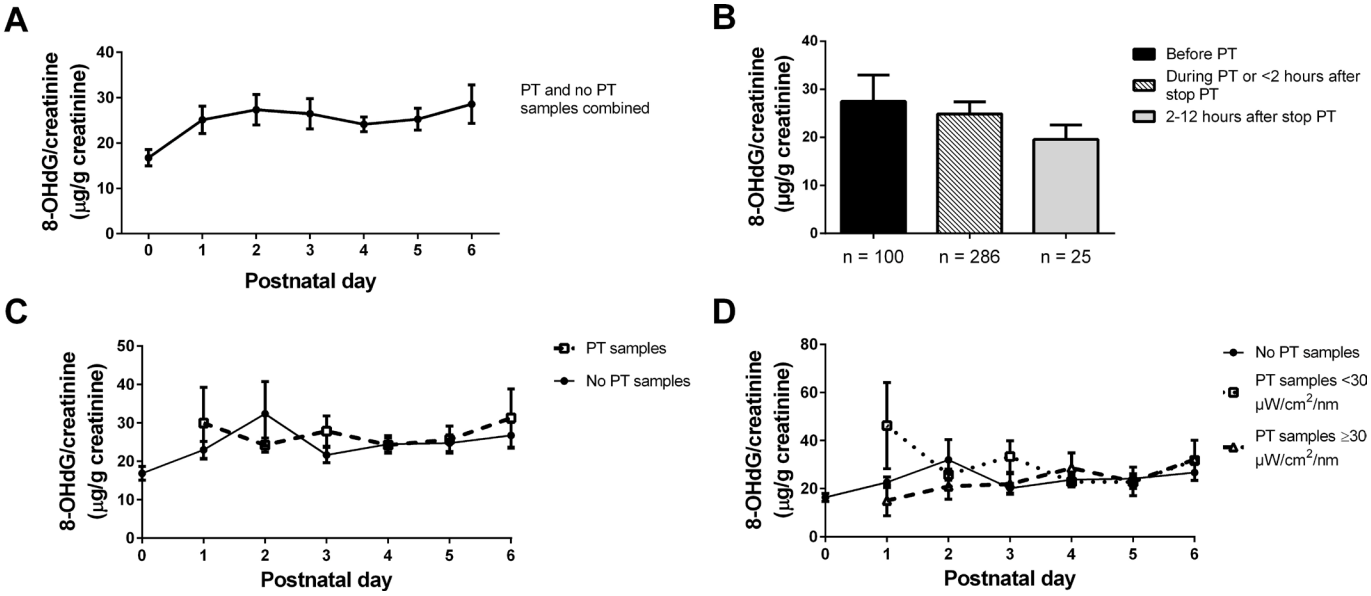

Figure 1 The 8-OHdG/creatinine ratio during the first week after birth and the effect of phototherapy. (A) The postnatal course of the 8-OHdG/creatinine ratio during the first week after birth. (B) The 8-OHdG/creatinine ratio in urine samples collected before phototherapy (PT), during or within 2 hours after phototherapy discontinuation, or 2–12 hours after phototherapy discontinuation. (C) The 8-OHdG/creatinine ratio in urine samples collected under phototherapy or less than 2 hours before or after phototherapy. (D) The 8-OHdG/creatinine ratio in samples collected more than 2 hours before or after phototherapy or under low irradiance (< 30 µW/cm²/nm) or high irradiance (≥ 30 µW/cm²/nm) phototherapy. All values on the y-axes represent EMMs. 8-OHdG, 8-hydroxy-2′deoxyguanosine; EMMs, estimated marginal means.

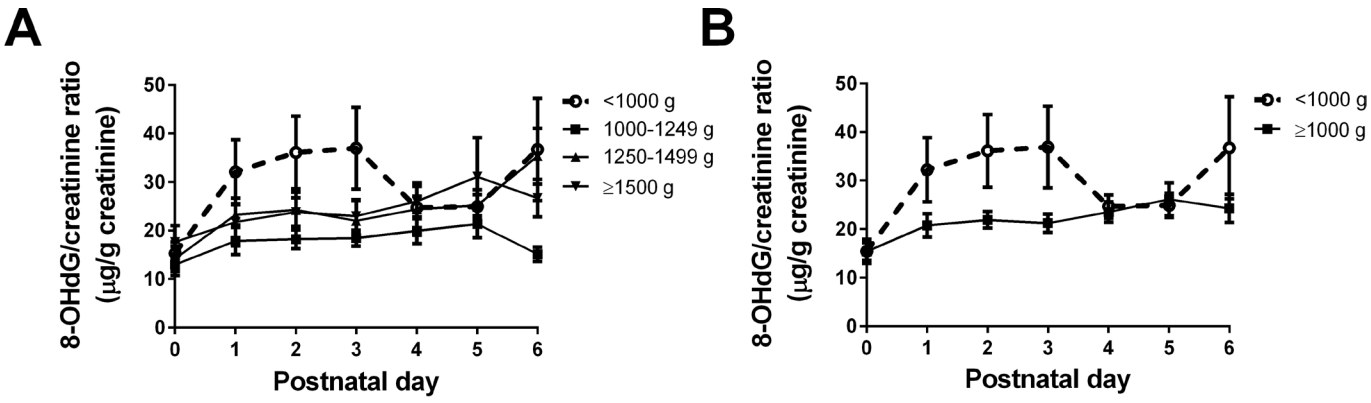

Figure 2 The effect of birth weight on the 8-OHdG/creatinine ratio and total bilirubin. (A) The 8-OHdG/creatinine ratio was divided into four birthweight (BW) categories, corresponding to the categories used for the hyperbilirubinaemia treatment guidelines. (B) The 8-OHdG/creatinine ratio in samples of infants with a BW of <1000 g versus a BW of ≥1000 g. All values on the y-axes represent EMMs. 8-OHdG, 8-hydroxy-2′deoxyguanosin; EMMs, estimated marginal mean.

increase with postnatal age.[38 39] We are therefore cautious about concluding that increased levels of OS after phototherapy in these studies are caused by phototherapy instead of merely by age. For 8-OHdG, we and others have shown that postnatal day is a significant predictor of 8-OHdG/creatinine.[38] We therefore accounted for the influence of postnatal age when assessing phototherapy effects. Second, in contrast to most existing studies, we chose to test our hypothesis in infants of ≤32 weeks, which is the most relevant patient population, because the vast majority of them receive phototherapy and their antioxidative capacities are the weakest.

CONCLUSION

BLP is gradually replacing FT phototherapy and thereby the door is opened to high-irradiance phototherapy with no heat-induced side effects. Thus far, however, the clinical implementation of LED phototherapy has been impeded by concerns about long-term side effects. We did not find that BLP resulted in increased levels of the OS marker 8-OHdG in preterm infants ≤32 weeks' gestation. Our data do not exclude other potential harmful effects that could cause previously reported long-term adverse effects. It also remains to be determined whether higher phototherapy irradiances of ≥50 µW/cm²/nm can induce oxidative DNA damage in early preterm infants.

Acknowledgements We greatly appreciate the help of T van Wulfften Palthe in correcting the English manuscript.

Contributors LWEvdS: designed the set-up of the study, coordinated the study and performed 8-hydroxy-2′deoxyguanosine (8-OHdG) sample analyses, data management and statistical analyses. She wrote the majority of the manuscript. MHJRvF: assisted in 8-OHdG sample analyses and designed, validated and optimised the respective method. IK: created the idea for the 8-OHdG analyses and supervised the development of this technique. DHB and AJO: supervised the sample collection

by the NICU nurses and performed phototherapy intensity measurements. JWJ: was responsible for daily supervision of all laboratory work, analyses and project planning. HJV: responsible for supervision of the project and regular critical evaluations of study design, study course and manuscript. HG: provided statistical advice during set-up of the study, statistical analyses and drafting of the manuscript and reviewed the manuscript to assure statistical correctness. CVH: created the idea for the study, performed daily supervision of clinical activities, data management, statistical analyses and writing of the manuscript. All authors contributed to the writing of the manuscript.

Funding This study was funded by the Dr. C.J. Vaillant foundation and 'Stichting Vrienden Beatrix Kinderziekenhuis'.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Medical Ethics Committee of the UMCG (METC 2016/437).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

REFERENCES

1 Chou S-C, Palmer RH, Ezhuthachan S, *et al*. Management of hyperbilirubinemia in newborns: measuring performance by using a benchmarking model. *Pediatrics* 2003;112:1264–73.
2 Lucey J, Ferriero M, Hewitt J. Prevention of hyperbilirubinemia of prematurity by phototherapy. *Pediatrics* 1968;41:1047–54.
3 Mukherjee D, Coffey M, Maisels MJ. Frequency and duration of phototherapy in preterm infants <35 weeks gestation. *J Perinatol* 2018;38:1246–51.
4 Brown AK, Kim MH, Wu PY, *et al*. Efficacy of phototherapy in prevention and management of neonatal hyperbilirubinemia. *Pediatrics* 1985;75:393–400.
5 Gathwala G, Sharma S. Phototherapy induces oxidative stress in premature neonates. *Indian J Gastroenterol* 2002;21:153–4.
6 Tatli MM, Minnet C, Kocyigit A, *et al*. Phototherapy increases DNA damage in lymphocytes of hyperbilirubinemic neonates. *Mutat Res* 2008;654:93–5.
7 Kahveci H, Dogan H, Karaman A, *et al*. Phototherapy causes a transient DNA damage in jaundiced newborns. *Drug Chem Toxicol* 2013;36:88–92.
8 Yahia S, Shabaan AE, Gouida M, *et al*. Influence of hyperbilirubinemia and phototherapy on markers of genotoxicity and apoptosis in full-term infants. *Eur J Pediatr* 2015;174:459–64.
9 Tyson JE, Pedroza C, Langer J, *et al*. Does aggressive phototherapy increase mortality while decreasing profound impairment among the smallest and sickest newborns? *J Perinatol* 2012;32:677–84.
10 Arnold C, Pedroza C, Tyson JE. Phototherapy in ELBW newborns: does it work? is it safe? the evidence from randomized clinical trials. *Semin Perinatol* 2014;38:452–64.
11 Dahlquist G, Kallen B. Indications that phototherapy is a risk factor for insulin-dependent diabetes. *Diabetes Care* 2003;26:247–8.
12 Newman TB, Wu YW, Kuzniewicz MW, *et al*. Childhood seizures after phototherapy. *Pediatrics* 2018;142:e20180648.
13 Maimburg RD, Olsen J, Sun Y. Neonatal hyperbilirubinemia and the risk of febrile seizures and childhood epilepsy. *Epilepsy Res* 2016;124:67–72.
14 Wickremasinghe AC, Kuzniewicz MW, Grimes BA, *et al*. Neonatal phototherapy and infantile cancer. *Pediatrics* 2016;137:e20151353.
15 Aspberg S, Dahlquist G, Kahan T, *et al*. Is neonatal phototherapy associated with an increased risk for hospitalized childhood bronchial asthma? *Pediatr Allergy Immunol* 2007;18:313–9.
16 Ebbesen F, Hansen TWR, Maisels MJ. Update on phototherapy in jaundiced neonates. *Curr Pediatr Rev* 2017;13:176–80.
17 van der Schoor LWE, Hulzebos CV, van Faassen MH, *et al*. LED-phototherapy does not induce oxidative DNA damage in hyperbilirubinemic Gunn rats. *Pediatr Res* 2019;85:1041–7.
18 Davis JM, Auten RL. Maturation of the antioxidant system and the effects on preterm birth. *Semin Fetal Neonatal Med* 2010;15:191–5.
19 van den Belt SM, Gracchi V, de Zeeuw D, *et al*. Comparison of urine collection methods for albuminuria assessment in young children. *Clin Chim Acta* 2016;458:120–3.
20 Wu LL, Chiou CC, Chang PY, *et al*. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta* 2004;339:1–9.
21 Loft S, Svoboda P, Kasai H, *et al*. Prospective study of 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion and the risk of lung cancer. *Carcinogenesis* 2006;27:1245–50.
22 Zar JH. *Biostatistical analysis*. Englewood Cliffs, New Jersey: Prentice-Hall, 1984.
23 Machin D, Campbell M, Favers P, *et al*. *Sample size tables for clinical studies (second edition*. Massachusetts: Blackwell Science. Malden, 1997.
24 Ziegler A. Generalized estimating equations: Springer Science & Business Media 2011.
25 Flanagan SW, Moseley PL, Buettner GR. Increased flux of free radicals in cells subjected to hyperthermia: detection by electron paramagnetic resonance spin trapping. *FEBS Lett* 1998;431:285–6.
26 Kletkiewicz H, Rogalska J, Nowakowska A, *et al*. Effects of body temperature on post-anoxic oxidative stress from the perspective of postnatal physiological adaptive processes in rats. *J Physiol Pharmacol* 2016;67:287–99.
27 Moseley H, Ferguson J. The risk to normal and photosensitive individuals from exposure to light from compact fluorescent lamps. *Photodermatol Photoimmunol Photomed* 2011;27:131–7.
28 Demirel G, Uras N, Celik IH, *et al*. Comparison of total oxidant/antioxidant status in unconjugated hyperbilirubinemia of newborn before and after conventional and led phototherapy: a prospective randomized controlled trial. *Clin Invest Med* 2010;33:335–41.
29 Kale Y, Aydemir O, Celik Ülker, *et al*. Effects of phototherapy using different light sources on oxidant and antioxidant status of neonates with jaundice. *Early Hum Dev* 2013;89:957–60.
30 El-Farrash RA, El-Shimy MS, Amer ST, *et al*. Effect of phototherapy on oxidant/antioxidant status: a randomized controlled trial. *Free Radic Res* 2018:1–291.
31 Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004;37:277–85.
32 Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;38:1103–11.
33 Ates O, Alp HH, Caner I, *et al*. Oxidative DNA damage in retinopathy of prematurity. *Eur J Ophthalmol* 2009;19:80–5.
34 Joung KE, Kim H-S, Lee J, *et al*. Correlation of urinary inflammatory and oxidative stress markers in very low birth weight infants with subsequent development of bronchopulmonary dysplasia. *Free Radic Res* 2011;45:1024–32.
35 Chen C-M, Chou H-C. Hyperoxia disrupts the intestinal barrier in newborn rats. *Exp Mol Pathol* 2016;101:44–9.
36 Isabel R-RM, Sandra G-A, Rafael V-P, *et al*. Evaluation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) adduct levels and DNA strand breaks in human peripheral blood lymphocytes exposed in vitro to polycyclic aromatic hydrocarbons with or without animal metabolic activation. *Toxicol Mech Methods* 2012;22:170–83.
37 Negi R, Pande D, Kumar A, *et al*. In vivo oxidative DNA damage and lipid peroxidation as a biomarker of oxidative stress in preterm low-birthweight infants. *J Trop Pediatr* 2012;58:326–8.
38 Drury JA, Jeffers G, Cooke RW. Urinary 8-hydroxydeoxyguanosine in infants and children. *Free Radic Res* 1998;28:423–8.
39 Buonocore G, Perrone S, Longini M, *et al*. Oxidative stress in preterm neonates at birth and on the seventh day of life. *Pediatr Res* 2002;52:46–9.