The impact of hemoglobin on the efficacy of phototherapy in hyperbilirubinemic infants

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BACKGROUND: Phototherapy is the routine treatment for neonatal hyperbilirubinemia. Absorption of light in the skin transforms the native Z,Z-bilirubin to photobilirubins. This study investigates whether the hemoglobin concentration has an impact on efficacy of phototherapy, expressed by the decline of total serum bilirubin concentration (TsB).

METHODS: A trial was conducted on 93 infants, gestational age ≥ 33 weeks, with uncomplicated hyperbilirubinemia. The infants were treated with conventional phototherapy using LED light for 24 h. The median light irradiance was $66.8 \mu\text{W}/\text{cm}^2/\text{nm}$.

RESULTS: The median decrease in TsB after 24 h was 121 (57–199) $\mu\text{mol}/\text{l}$; the median hemoglobin was 12.0 (7.0–14.7) mmol/l . There was a significant effect of hemoglobin concentration on the decrease in TsB of $-3.61 \mu\text{mol}/\text{mmol}$ hemoglobin ($P=0.022$), after adjusting for initial TsB and postnatal age. That is, assuming the same initial TsB and postnatal age, for each mmol/l increase in hemoglobin, the decrease in TsB was $3.61 \mu\text{mol}/\text{l}$ smaller. In our hemoglobin range, the decrease in TsB is reduced by $28 \mu\text{mol}/\text{l}$ (23%).

CONCLUSION: Increasing hemoglobin levels led to a decrease in the efficacy of phototherapy. Our data provide additional support for the conclusion that the transformation of bilirubin to photobilirubins takes place mainly in the superficial capillaries of the skin.

Jaundice in neonates is common, with 2–6% of infants requiring treatment for this condition. Over the last 50 years, phototherapy has been used to treat neonatal hyperbilirubinemia to prevent extremely high levels of bilirubin that may cause bilirubin-induced encephalopathy. The treatment has improved as the light sources and strategy for using these have been optimized. Exchange transfusions have been increasingly replaced by phototherapy because of both the efficacy and the superior safety profile of the latter (1).

It is well known that phototherapy transforms unconjugated bilirubin from the native, non-polar, and neurotoxic Z,Z-bilirubin to more polar isomers called photobilirubins

that consists of configurational isomers Z,E- and E,Z-bilirubin and structural isomers E,Z- and E,E-lumirubin (2). These isomers can then be excreted without conjugation into the bile and the structural isomers into the urine (3). Whether the biochemical processes during phototherapy occur predominantly intravascularly or extravascularly has long been debated, although recent evidence strongly supports the idea that the biochemical processes during phototherapy predominantly take place intravascularly. In these *in vitro* studies, it has also been found that hemoglobin is a major competitor with bilirubin for the absorption of light during light exposure (4,5). The authors predicted that a high hematocrit would reduce the therapeutic efficacy of phototherapy; however, this has never been confirmed *in vivo* (4,5). Confirming this hypothesis in a clinical setting is an important step in understanding the basic mechanisms of phototherapy, and could have implications for the treatment strategy for neonates with hyperbilirubinemia.

In the present clinical study, we investigated whether the efficiency of phototherapy, expressed as the decline in the total serum bilirubin concentration (TsB), is dependent on the concentration of hemoglobin. We hypothesized that the efficacy of phototherapy will decrease with increasing hemoglobin concentrations.

METHODS

A prospective trial was conducted between 15 June 2014 and 15 June 2015 in the neonatal intensive care unit at Aalborg University Hospital, Denmark. Inclusion criteria were otherwise healthy term or late preterm (gestational age ≥ 33 weeks) newborn infants with hyperbilirubinemia, without signs of hemolytic disease, birth weight $\geq 1,800$ g, postnatal age > 24 h and ≤ 7 days, who had not previously received phototherapy, and who could be treated in a crib. Infants fulfilling the indications for exchange transfusion or double phototherapy due to a very high initial or rapidly increasing TsB were not enrolled. The indications for phototherapy followed the existing guidelines of the North Denmark Region.

The attending physician enrolled the infants consecutively in the study. One hundred infants were eligible for the study. The parents of six of the infants did not wish to participate, and one infant was excluded from the study because of Rhesus isoimmunization. Thus, the study cohort consisted of 93 infants who received phototherapy with blue LED light from above at a distance of 30 cm between the light source and the mattress. The distances between the lamps and

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the mattress were adjusted with a wood template for each infant. The average distance from the light source to the surface of the infant was 21.5 cm.

All infants received phototherapy for 24 h. They were naked except for diapers and eye pads. Phototherapy was administered continuously except during 30 min of feeding and nursing care every third hour. Previous studies have shown that the infants received phototherapy 85% of the time on average with this regime (6). The weight of the infants was registered before and after phototherapy.

Measurements

The phototherapy device was the neoBLUE LED phototherapy system (Natus Medical, San Carlos, CA) that emits blue light in the 420–520 nm spectrum, centered at 458 nm with a bandwidth of 450–470 nm, which is defined as the spectral range at half peak irradiance. Irradiance was measured with a neoBLUE radiometer (Natus Medical), which measures spectral irradiance from 420 to 500 nm with maximum sensitivity in the spectrum of 440–480 nm. The device was calibrated just before initiation of the study.

During phototherapy, irradiance was measured three times, approximately every eighth hour, at the infants' head, abdomen, and knees, and the mean value was calculated. Because of the height of the radiometer, these measurements were obtained 3.5 cm above the infant, which results in higher levels of irradiance than at the skin level. The ratio between the irradiance at the skin level and the measured irradiance was 0.93. This correction factor was employed.

The irradiance of the phototherapy device was measured in a footprint containing the illuminated area measuring 21 × 28 cm. It was measured in a grid consisting of 12 squares of 7 × 7 cm, 8.5 cm above the mattress level, corresponding to the average height over the mattress of the exposed skin of the infants. The mean irradiance of the footprint was 64.8 μW/cm²/nm. We also measured the irradiance in a bigger illuminated area measuring 28 × 49 cm, in a grid consisting of 28 squares of 7 × 7 cm (ref. 7). The mean irradiance was 53.1 μW/cm²/

nm. The levels of irradiance did not change during the study.

TsB was measured at the start (TsB₀) and after 24 h (TsB₂₄) of treatment in capillary blood drawn by a heel stick. It was analyzed at the Department of Clinical Biochemistry, Aalborg University Hospital, with the diazo method using Cobas 8000 (Roche Diagnostics International, Mannheim, Germany) (8). The imprecision expressed as coefficient of variation was 3.0 and 1.4% at TsB levels of 176 and 333 μmol/l, respectively. External quality assessment showed a bias below 5% in the interval 10–1,000 μmol/l (7).

Hemoglobin was measured in the capillary blood at the start of phototherapy, drawn concurrently with TsB₀. It was analyzed at the Department of Clinical Biochemistry, Aalborg University Hospital with the photometric method using ABL (Radiometer, Copenhagen, Denmark). The imprecision expressed as coefficient of variation was <0.5% in the hemoglobin range 5–12 mmol/l, and generally the variation was smaller at higher concentrations (validated based on control material from Radiometer, at the Department of Clinical Biochemistry, Aalborg University Hospital).

Information was registered about gender, gestational age, ethnicity, birth weight, postnatal age, and feeding type for the infants.

Ethics

The Regional Committee on Health Research Ethics in North Denmark Region approved the study. Verbal and written informed consent was obtained from the parents of the infants. The study was registered at ClinicalTrials.gov.

Statistical Analysis

On the basis of a pilot study ($n=22$), at least 90 infants were required to detect an effect size of 8 μmol/l decrease in TsB per 1 mmol/l increase in hemoglobin. The SDs used were 1.2 mmol/l for hemoglobin and 30 μmol/l for decrease in TsB. This sample size was sufficient to achieve 80% power with a significance level of $\alpha=0.05$ in an unadjusted linear regression.

TsB₀, TsB₂₄, and change in TsB in μmol/l after 24 h of treatment (Δ TsB_{0–24}) as well as hemoglobin concentration are presented as the medians with their respective ranges.

Simple linear regression analyses were performed with Δ TsB_{0–24} as the response variable and hemoglobin, TsB₀, gestational age, birth weight, postnatal age, infants receiving formula (exclusively or as supplementation), and light irradiance as explanatory variables. The explanatory variables that were statistically significant in each of the simple linear regressions were included in a multivariable linear regression. Besides, a sensitivity analysis was carried out to investigate the effect of outliers.

Statistical analyses were performed in Stata 13 (StataCorp, College Station, TX, USA). Results with P -values below 0.05 were considered statistically significant.

RESULTS

Baseline demographic and clinical data of the 93 infants are presented in Table 1. TsB₀, TsB₂₄, Δ TsB_{0–24}, and hemoglobin concentrations are presented in Table 2. As shown, the median TsB decrease was 121 μmol/l after 24 h of phototherapy. Hemoglobin ranged from 7.0 to 14.7 mmol/l, median 12.0 mmol/l.

The regression analyses are presented in Table 3. The simple linear regression analyses showed that the following variables had a significant impact on Δ TsB_{0–24}: hemoglobin, TsB₀, and postnatal age. The multivariable regression model therefore included these three statistically significant parameters. Δ TsB_{0–24} was associated negatively with the hemoglobin concentration and positively with TsB₀ and postnatal age. The estimated regression coefficient for hemoglobin was -3.61 ($P=0.022$).

Table 1. Baseline demographic, clinical, and irradiance data

Infants (n)	93
Gender: female/male (n)	45/48
Non-Caucasian ethnicity (n)	9 ^a
Age	
Gestational age (days) ^b	269 (239–293)
Postnatal age (h) ^b	78 (41–164)
Weight	
Birth weight (g) ^b	3,270 (1,835–5,090)
Weight change from birth to phototherapy (%) ^b	–4.7 (–10.8–2.6)
Weight change during phototherapy (%) ^b	0.6 (–3.8–7.6)
Feeding during phototherapy (n)	
Exclusively breast-fed	39
Exclusively formula-fed	6
Mixed formula/breast-fed	48
Light irradiance, measured (μW/cm ² /nm) ^{b,c}	71.8 (48.9–84.0)
Adjusted light irradiance (μW/cm ² /nm) ^{b,d}	66.8 (45.5–78.1)

^aSeven Asian, one African, and one Middle Eastern.

^bMedian (range).

^cLight irradiance: mean of all three measurements for each infant.

^dThe irradiance was measured 3.5 cm above the infant because of the dimensions of the light sensor. A correction factor of 0.93 was employed.

Assuming a postnatal age of 78 h and an initial TsB of 270 $\mu\text{mol/l}$, the infant with a hemoglobin concentration of 7.0 mmol/l would experience a 140 $\mu\text{mol/l}$ TsB decrease compared with a 112 $\mu\text{mol/l}$ decrease for the infant with a hemoglobin concentration of 14.7 mmol/l. That is, a decrease of 28 $\mu\text{mol/l}$ in TsB within the observed hemoglobin range. These observed maximum and minimum hemoglobin values might not be considered normal in a clinical setting. Therefore, we chose to look at the difference in effect between 9.0 and 14.0 mmol/l, which are the 5% and the 95% percentiles of hemoglobin. The decrease in TsB was 18 $\mu\text{mol/l}$ smaller at the upper end of this hemoglobin range than at the lower end.

Scatter plots of change in TsB vs. hemoglobin concentration are presented in Figure 1. The figure shows both the unadjusted regression line, and the regression line adjusted for TsB₀ and postnatal age in hours. At any hemoglobin level, there was a large variation in the decrease of TsB during the treatment as seen in the scatter plot.

A sensitivity analysis was performed excluding the infant with a hemoglobin concentration of 7.0 mmol/l. It only had a minor influence on the results; the hemoglobin coefficient was -5.82 ($P=0.007$) in the simple regression and -3.47 ($P=0.036$) in the multivariable regression.

No side effects of phototherapy other than loose stools were observed. No infants had hypo- or hyperthermia, and no rashes were seen.

DISCUSSION

Table 2. Changes in total serum bilirubin concentration after 24 h of phototherapy, and hemoglobin concentration at initiation of phototherapy

TsB ₀ , $\mu\text{mol/l}$ ^a	270 (181–338)
TsB ₂₄ , $\mu\text{mol/l}$ ^a	146 (52–209)
ΔTsB_{0-24} , $\mu\text{mol/l}$ ^a	121 (57–199)
Hemoglobin, mmol/l ^a	12.0 (7.0–14.7)

TsB₀, total serum bilirubin concentration at the start of phototherapy; TsB₂₄, total serum bilirubin concentration after 24 h of phototherapy; ΔTsB_{0-24} , difference between TsB₀ and TsB₂₄.

^aMedian (range).

Table 3. Regression analyses

Explanatory variables	Simple linear regressions		Multivariable linear regression	
	Coefficient (95% CI)	P value	Coefficient (95% CI)	P value
Hemoglobin, mmol/l	-5.29 ($-9.20, -1.38$)	0.009	-3.61 ($-6.69, -0.53$)	0.022
TsB ₀ , $\mu\text{mol/l}$	0.43 (0.30, 0.56)	<0.001	0.36 (0.25, 0.48)	<0.001
Gestational age, days	0.25 ($-0.16, 0.66$)	0.23		
Birth weight, kg	-1.76 ($-10.65, 7.13$)	0.70		
Postnatal age, h	0.51 (0.34, 0.69)	<0.001	0.32 (0.16, 0.49)	<0.001
Formula feeding	-9.52 ($-21.06, 2.03$)	0.11		
Irradiance, $\mu\text{W}/\text{cm}^2/\text{nm}$	-0.10 ($-0.94, 0.74$)	0.81		

CI, confidence interval; TsB₀, total serum bilirubin concentration at the start of phototherapy; TsB₂₄, total serum bilirubin concentration after 24 h of phototherapy; ΔTsB_{0-24} , difference between TsB₀ and TsB₂₄.

Our overall results showed that the efficacy of phototherapy was significantly dependent on the concentration of hemoglobin. For every 1.0 mmol/l increase in hemoglobin concentration, the decrease in TsB during 24 h of phototherapy was 3.61 $\mu\text{mol/l}$ smaller. To our knowledge, this is the first prospective clinical study corroborating that hemoglobin is a competitor with bilirubin in phototherapy, an observation made in *in vitro* studies.

In 1983, Granati *et al.* (9) showed *in vitro* that, as hematocrit declined, bilirubin photodegradation increased; however, the correlation was not confirmed *in vivo*. In another *in vitro* study, by Lamola *et al.* (4), using a semi-empirical skin model, it was confirmed that hemoglobin in the skin competes with bilirubin for absorption of light during phototherapy. This was strongest at the wavelength 400–460 nm, also found *in vitro* by Linfield *et al.* (5) in 2016. On the basis of these results, it was predicted that a high hematocrit would reduce the therapeutic efficacy of phototherapy *in vivo*.

From a theoretical perspective, there is increasing, although indirect, evidence that isomerization of bilirubin *in vivo* primarily occurs in the skin capillary circulation (10,11). Our

Figure 1. Scatter plots of change in total serum bilirubin concentration during 24 h of phototherapy vs. hemoglobin concentration.

findings support this understanding. Previously, the clinical observation that the yellow color of the skin in the jaundiced infant diminishes during the first hours of phototherapy had led to the understanding that transformation of Z,Z-bilirubin to photobilirubins mainly takes place extravascularly in the skin. Pursuant to this understanding, infants in phototherapy were routinely alternated from prone to supine position to increase the efficacy of phototherapy (12). However, this routine was largely discontinued when it was found that the efficacy of phototherapy is independent of alternating the position of the infant (10,13–15). Other observations also support the hypothesis that the effect of phototherapy is predominantly intravascular. Thus, 15 min after phototherapy is initiated, a substantial amount of the photobilirubin Z,E-bilirubin can be measured in plasma, corresponding to ~10% of total bilirubin (11). This finding seems inconsistent with diffusion from the extravascular to the intravascular compartment, as diffusion is a much slower process.

Our finding of an inverse relationship between hemoglobin and the efficacy of phototherapy could be explained by an intravascular effect of phototherapy on bilirubin isomerization (4). The hemoglobin in the erythrocytes will absorb light, which implies that a fraction of the light will not reach the bilirubin molecules, i.e., hemoglobin in the erythrocytes will compete with the bilirubin molecules and thereby influence the efficacy of phototherapy. This has also been investigated by measuring the photobilirubins. The formation of Z,E-bilirubin is reversible, and Z,E-bilirubin accumulates rapidly in plasma at commonly used levels of irradiance because of a fast formation and a slow excreting rate. Within a few hours of phototherapy, the ratio of Z,E-bilirubin to Z,Z-bilirubin reaches a plateau, which means that a photo-equilibrium occurs between these two substances (16). Mreihil *et al.* (11) in 2015 compared the rate and level of 4Z, 15E photoisomerization during intensive phototherapy and did a *post hoc* analysis of hemoglobin data from 36 of their patients. They found a significant, negative correlation between hemoglobin concentration and percentage of Z,E-bilirubin after 15, 30, and 60 min; however, it disappeared at the later time points. This was explained by the fact that when an equilibrium between Z,Z-bilirubin and Z,E-bilirubin is obtained in plasma, the percentage of Z,E-bilirubin only depends on the emission spectrum of the light, and is independent of the irradiance. The authors concluded that the hemoglobin concentration would influence the efficacy of phototherapy until an equilibrium between Z,Z- and Z,E-bilirubin occurs.

However, we found an effect of hemoglobin on the bilirubin decline in plasma after 24 h of phototherapy. We suggest that this is explained by the fact that the production of E,Z-lumirubin is the most important bilirubin-lowering process during intensive phototherapy, and an equilibrium between E,Z-lumirubin and Z,Z-bilirubin does not occur (3,16,17). Therefore, the decline of bilirubin is dependent on hemoglobin concentration throughout the whole treatment period and not just during the first 60 min, as shown by Mreihil *et al.* (11).

From a clinical perspective, our results may be beneficial for clinicians treating hyperbilirubinemic infants with phototherapy. We have included infants with gestational ages ranging from 33 to 41 weeks; the mean hemoglobin concentration increases in this period of gestation from 10.4 to 11.5 mmol/l (ref. 18). Over a period of 24 h, the average decrease in TsB will be ~18 µmol/l less in infants with a hemoglobin concentration of 14.0 mmol/l than in infants with a hemoglobin concentration of 9.0 mmol/l, which is equivalent to a decrease of 15% less. Thus, infants with high hemoglobin concentration are likely to need a longer duration of treatment, higher irradiance, or a larger body-surface area exposed to phototherapy, compared with infants with a lower hemoglobin concentration, in order to achieve the same decrease in TsB. This could be important for the decision of when to draw the next blood sample. An algorithm predicting the effect of phototherapy should probably include hemoglobin concentration. In a “crash cart” case where the level of TsB should be reduced as fast as possible, the influence of the variation in the hemoglobin level on the decrease of TsB during phototherapy might be considered as minimal.

Our study has some limitations. The bilirubin production rate was not measured directly as the end expiratory excretion rate of CO (CoSense[®], CAPNIA, Redwood City, CA) because this method was not available at the start of the study.

Another limitation is that a higher hemoglobin level may be associated with a higher bilirubin production rate appearing as a higher rate of bilirubin increase in plasma. The shown negative relationship between ΔTsB_{0-24} and hemoglobin could be explained by a higher rate of bilirubin production at higher hemoglobin concentrations. However, ΔTsB_{0-24} was adjusted for TsB_0 and postnatal age. TsB_0 in relation to postnatal age is the increase in TsB from birth to the start of phototherapy, and is therefore an estimate of the bilirubin production. During the first days of life, the relationship between the increase in TsB and postnatal age is linear.

The strengths of the study were that the exposed population was homogenous and healthy except for that they were hyperbilirubinemic. In addition, from a methodological standpoint, the neoBLUE radiometer with maximum sensitivity from 440 to 480 nm matches well with the neoBLUE phototherapy system centered at 458 nm, and the imprecision of the methods for determination of both hemoglobin and TsB was small.

CONCLUSION

We found that hemoglobin had a significant effect on the efficacy of phototherapy: the higher the hemoglobin concentration, the smaller the decrease in TsB. This knowledge is important for clinicians who treat hyperbilirubinemic infants with phototherapy. These results are compatible with the understanding that the transformation of bilirubin to photobilirubins during phototherapy takes place mainly in the superficial capillaries in the skin.

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