Review Article

Phototherapy for neonatal hyperbilirubinemia

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Abstract

Approximately 60 years ago in England, phototherapy for neonatal hyperbilirubinemia was introduced in clinical practice. It was introduced in Japan approximately 50 years ago. At that time, the mechanism underlying the serum bilirubin concentration decrease by phototherapy was still unknown. The mechanism was identified by chemists, biochemists, and pediatricians. Clarification started with the report that unconjugated bilirubin was excreted into bile after phototirradiation in Gunn rats. After confirmation of the molecular structure of bilirubin on X-ray analysis, the mechanism for bile excretion of unconjugated bilirubin was verified based on geometric configurational photoisomers in the Gunn rat. Finally, the reaction and excretion of structural bilirubin photoisomers was proved to be the main mechanism for the decrease in serum bilirubin during phototherapy for neonatal hyperbilirubinemia, which differs from the mechanism in the Gunn rat. The most effective and safest light source and the optimal method to evaluate phototherapy, however, remain unknown. Moreover, as for bronze baby syndrome, which is a well-known adverse reaction to phototherapy, the etiology is unclear. Hence, we review phototherapy for hyperbilirubinemia including a fundamental understanding of the bilirubin photochemical reactions, and discuss the subclinical carcinogenic risk of phototherapy and the increased mortality rate of extremely low-birthweight infants due to aggressive phototherapy, which is becoming an increasing problem.

Key words bilirubin photoisomer, bronze baby syndrome, green light, human serum albumin, subclinical carcinogenic risk.

The effectiveness of phototherapy for neonatal hyperbilirubinemia was reported for the first time by Cremer et al.1 in 1958, and was introduced in Japan by Onishi in 1968.2 The mechanism of bilirubin excretion in a neonate during phototherapy, however, was unknown. The confirmation of the molecular structure of bilirubin on X-ray analysis by Bonnett et al.3 in 1976 led to clarification of the mechanism. Moreover, Lightner et al.4 reported that bilirubin oxidation was a minor pathway in vivo in 1984, and research on the oxidative reactions of bilirubin, believed to be the main mechanism at that time, was ceased. After the molecular conformation of bilirubin, research turned to the photochemical reaction of bilirubin in a tetrapyrrolic state. Based on PubMed, the last basic bilirubin photochemical study on phototherapy for neonatal hyperbilirubinemia, including the pathway of the photochemical reaction, was reported by Ennever and Dressing in 1991.5

In contrast, the subclinical carcinogenic risk in vitro of phototherapy has been investigated since 1970. In the clinical field, however, the adverse reaction to phototherapy is widely known as bronze baby syndrome, and other critical adverse reactions have not been reported until the present. Recently, an epidemiologic survey reported that the risk of childhood cancer was increased by previous phototherapy.6,7 and the mortality rate among infants weighing 501–750 g at birth was increased by aggressive phototherapy.8

From these reports, phototherapy may be positioned similarly to drug therapy.9 In order to verify its effectiveness and safety, it is important to continuously develop the light sources and irradiation methods based on photochemical reaction (like drug dosage and treatment) and discuss the methods to evaluate these phototherapy instruments (like therapeutic drug monitoring).

Geometric configurational bilirubin photoisomers

The chemical name of bilirubin is 1,10,19,21,23,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxo-3,18-devinylbilin-8, 12-dipropanoic acid, or 8,12-bis(2-carboxyethyl)-10,21,23,24-tetrahydro-2,7,13,17-tetramethyl-3,18-devinyl-bilin-1,19-dion, or 8,12-bis(carboxyethyl)-2,7,13,17-tetramethyl-3,18-divinyl (10H, 21H,24H)bilin-1,19-dione or 2,17-deiethenyl-1,10,19,22,23,24-hexahydro-3,7,13,18-tetramethyl-1,19-dioxo-21H-biline-8, 12-dipropanoic acid.10 Although the plane structure and molecular weight are clear, we could not explain the hydrophobic character of bilirubin despite the existence of many polar groups (2 carbonyl, 4 imino, 2 carboxyl). This was resolved by the X-ray analysis of Bonnett et al.3 in 1976. The polar groups are confined inside the bilirubin molecule by intramolecular hydrogen bonds and the surface structure has only hydrophobic groups (4 methyl, 2 vinyl; Fig. 1). Generally, the structural formula is shown omitting intramolecular
hydrogen bonds (Fig. 2). The possibility of cis and trans (Z and E) geometric isomerization about the meso carbon–carbon double bonds at C4=C5 and C15=C16 of bilirubin was recognized by Lemberg in 1939.13 In order to develop the structure reported by Bonnett et al., which forms intramolecular hydrogen bonds, it must have a Z configuration. Therefore, the trivial name of bilirubin in consideration of the stereoisomeric form is (ZZ)-bilirubin. The geometric photoisomers of (ZZ)-bilirubin are: (ZE)-bilirubin, (EZ)-bilirubin, and (EE)-bilirubin (Fig. 3).14

The dynamics of the geometric isomers under light irradiation to each bilirubin solution are as follows: (ZE)/(EZ)-bilirubin in dimethyl sulfoxide reaches a photoequilibrium with (ZZ)-bilirubin within 1 min of light irradiation at 11.8 μW/cm²/nm.15 The (ZE)-bilirubin/(ZZ)-bilirubin ratio in bilirubin–human serum albumin solution reaches a state of photoequilibrium as irradiation time is prolonged, irrespective of the amount of irradiation energy.16

**Structural (constitutional) bilirubin photoisomers**

Regarding structural isomerization (intermolecular dipyrrole exchange), bilirubin-IXα can rearrange in various acid catalytic solutions to a mixture of bilirubin-IIIα, -IXα, and -XIIIα (Fig. 4). This isomerization reaction caused many erroneous results in vitro,17 but this reaction plays no important role in bilirubin photoisomerization in vivo.18

The main structural bilirubin photoisomers in phototherapy for neonatal hyperbilirubinemia were detected on separation analysis methods such as thin layer chromatography (TLC)19 and high-performance liquid chromatography (HPLC), 20–23 and the reaction materials were identified. The structural photoisomer was first identified by McDonagh and Palma using 1H nuclear magnetic resonance (NMR) spectra (Fig. 5),24 but two structural isomers were possible with their method, and Bonnett et al.19 confirmed the most likely structure using infrared-absorption spectra. Onishi et al.25 identified the same structure based on 1H- and 13C-NMR spectra in the same period as Bonnett et al.19 Despite using the same structural formula, the nomenclature was different. McDonagh and Palma, Bonnett et al., and Onishi et al. named it (Z)-lumirubin, photobilirubin IIA,19 and (EZ)-cyclobilirubin,25 respectively. Moreover, the geometric photoisomer with an E-conformation in the double bond of C15-C16 was named (E)-lumirubin, photobilirubin IIB,19 and (EE)-cyclobilirubin,25 respectively.

Regarding the reaction pathway from (ZZ)-bilirubin to (EZ)-cyclobilirubin in the bilirubin–human serum albumin complex, McDonagh et al.24,26 reported that (EZ)-cyclobilirubin was generated directly from (ZZ)-bilirubin. Itoh and Onishi, Bonnett and Ioannou, and Ennever and Dresing, however, verified
that (EZ)-bilirubin was an intermediate in photochemical changes from (ZZ)-bilirubin to (EZ)-cyclobilirubin using a kinetic method,\textsuperscript{27} (Z)-vinylneoxanthobilirubic acid,\textsuperscript{28} and purified (EZ)-bilirubin,\textsuperscript{5} respectively.

The dynamic states of (EZ)-cyclobilirubin in the dimethyl sulfoxide solution of bilirubin\textsuperscript{15} and in the bilirubin–human serum complex solution\textsuperscript{16} show a dose–response relationship between irradiation time and production of (EZ)-cyclobilirubin, and between the light dose and (EZ)-cyclobilirubin production, respectively. The relationship between (EZ)-cyclobilirubin and (EE)-cyclobilirubin in the bilirubin–human serum albumin complex solution is a state of photoequilibrium, the same as that between (ZZ)-bilirubin and (ZE)-bilirubin.\textsuperscript{23}

**Mechanism of phototherapy for human neonatal hyperbilirubinemia**

Early in vivo research elucidated the production and excretion of bilirubin photoisomers on light irradiation of Gunn rats. Ostrow found that unconjugated bilirubin was excreted into bile on photoirradiation of Gunn rats.\textsuperscript{29} The biological importance of the geometric configurational photoisomerization of
(ZZ)-bilirubin to photobilirubin (mixture of (ZE)-, (EZ)-, and (EE)-bilirubin) in this phenomenon was first reported by McDonagh and Ramonas. This phenomenon was proved on HPLC of the exact geometric bilirubin photoisomers. The results using Gunn rats, however, differed from those in human newborn infants. The main form of bilirubin excreted into bile during phototherapy was the geometric isomer in Gunn rats and the structural isomer in human newborn infants. This was proved by analyzing the bilirubin photoisomers in bile during phototherapy. In contrast, (EZ)-(EE)-cyclobilirubin is increased by only a small amount in human neonatal serum during phototherapy in vivo, although (EZ)-(EE)-cyclobilirubin in bilirubin–human serum complex solution increased with the photoradiation time in vitro. This is explained by the fact that the overall elimination of (EZ)-(EE)-cyclobilirubin into bile was far more rapid than that of (ZE)-bilirubin. (ZZ)-Bilirubin returns to (ZZ)-bilirubin in bile and is reabsorbed from the intestinal tract into the enterohepatic circulation. Moreover, blue light-emitting diode (LED) is widely used as a light source for phototherapy in Japan. There is sufficient cyclobilirubin production on blue LED phototherapy in vitro, but high-level production of (ZE)-bilirubin occurs at the same time. This may reduce the effect of phototherapy in vivo because (ZE)-bilirubin may compete with cyclobilirubin for liver uptake. Therefore, (ZE)-bilirubin will be inefficiently excreted out of the body during phototherapy.

The development of phototherapy instruments for human hyperbilirubinemic neonates using the concept described in the previous section is important in clinical practice. For that purpose, it is necessary to identify the wavelength that generates (EZ)-cyclobilirubin the most efficiently. Onishi et al. and Itoh et al. found that the most efficient wavelength was 500–520 nm in the green region. Ennever and Dresing reached the same conclusion by calculating the quantum yield for the formation of (EZ)-cyclobilirubin from (EZ)-bilirubin. The basis of the clinical effect of green light phototherapy has been confirmed and a clinical effect has been confirmed using LED light closer to monochromatic light. The development of a radiometer to evaluate light sources, however, is not progressing, and evaluation has to be carried out in consideration of light sources with several wavelengths, reported by

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Kuboi et al. (ZE)-Bilirubin, which is less markedly generated using the long wavelength region of visible light, has to be re-evaluated regarding its in vivo effect in phototherapy for human neonatal hyperbilirubinemia.

**Adverse reactions of phototherapy for neonatal hyperbilirubinemia**

Important adverse reactions of phototherapy for neonatal hyperbilirubinemia include the subclinical carcinogenic risk, the effect of aggressive phototherapy on extremely low-birthweight (ELBW) infants, and bronze baby syndrome.

**Subclinical carcinogenic risk and effect of aggressive phototherapy in ELBW infants**

Many in vitro studies have reported on the subclinical carcinogenic risk using a conventional light source. It was thought that a wavelength 400–450 nm, which induces the photosensitization of riboflavin, an endogenous substance, would pose a problem in phototherapy. Serious adverse reactions, however, were not reported in phototherapy using blue light including the white light generally used in clinical field, possibly because of the shielding effect of the Soret band of the hemoglobin at wavelengths of 400–500 nm.

The same authors from California recently published two papers on an epidemiologic survey of phototherapy. The first report focused on the onset of cancer in infants under 1 year of age. Those who received phototherapy were 1.6-fold more likely to develop cancer (95%CI: 1.2–2.0; P = 0.002). The second focused on children diagnosed with cancer at any age. The crude data suggested an increased risk following phototherapy (relative risk, 1.4). Furthermore, Morris et al. randomly assigned 1,974 ELBW infants (≤1,000 g) at 12–36 h of age to either aggressive or conservative phototherapy. The mortality rates were 39% with aggressive phototherapy and 34% with conservative phototherapy for infants with birthweight 501–750 g (relative risk. 1.13; 95% CI: 0.96–1.34). Morris et al. hypothesized about the difference in the mortality rate due to antioxidant levels or oxidative injury. In a clinical setting, however, in such small infants often need higher inotropes or fluid to maintain appropriate circulation during phototherapy.

Given that a new phototherapy light source that does not produce wavelengths 400–450 nm and is clinically effective has been developed and the radiometer can evaluate the energy of the light source based on the theoretical evidence of phototherapy for neonatal hyperbilirubinemia, it is necessary to examine whether such adverse reactions will decrease using them. The adverse reactions of green light phototherapy include effects on the observer’s vision and the phototoxicity of bilirubin to human cells in vitro. We reported that the optical stimulus could be reduced by adding a complementary color light (pink light) to the green light, and the mutagenicity caused by green light was less than that by blue light in vitro. The bilirubin phototoxicity on human cells caused by green light may arise from singlet oxygen via the photochemical reaction of (EZ)-cyclobilirubin. (EZ)-Cyclobilirubin is generated by not only green light but also by blue light, and it is not accumulated in the physiological state. Especially, in phototherapy for ELBW infants, the phototherapy light source must be selected to ensure the correct light wavelength and light energy level, because the capacity of ELBW infants for (EZ)-(EE)-cyclobilirubin elimination, and the antioxidant level, are low.

Furthermore, ELBW infants also required high-level hemodynamic management accompanying phototherapy.

**Bronze baby syndrome**

In 1972, Kopelman et al. first reported bronze baby syndrome as an adverse reaction to phototherapy in hyperbilirubinemic neonates. In 1971 at the 74th Annual Meeting of the Japan Pediatric Society, however, Onishi had already reported a patient with the greenish-brown tone of serum as an adverse reaction to phototherapy in hyperbilirubinemic neonates, and considered that it was related to methohemalbumin or biliverdin. Kopelman et al. considered the accumulation of photo-oxidation substances of bilirubin as the origin, because neonates with the symptoms of bronze baby syndrome were complicated by obstructive jaundice. In 1978, Onishi conducted an analysis of the predisposing factors and the bronze pigment of bronze baby syndrome. Using a questionnaire to analyze the predisposing factors, the complication rate of obstructive jaundice was the highest (Table). On analysis of the bronze pigments, the molecular weight was >600 Da on gel-permeation chromatography. Thus, the bronze pigments were considered to be polymerization products caused by the free radical reactions of the degradation products of bilirubin (dipyromethene) due to phototherapy.

Thereafter, we succeeded in the separation of bilirubin photoisomers on reversed-phase HPLC. Based on that analysis, the peak retention time was shorter than the peak of (ZE)-(EZ)-bilirubin, and we named it the unknown pigment. We also reported that neonates with symptoms of bronze baby syndrome had higher levels of the unknown pigment in the serum than other hyperbilirubinemic neonates without bronze baby syndrome during phototherapy. The structure of the unknown pigment was determined on 1H- and 13C-NMR and

| Table | Predisposing factors of bronze baby syndrome (%) |
|-------|-----------------------------------------------|
| Factors |                                     |
| Obstructive jaundice | 68 |
| Exchange transfusion and blood transfusion | 53 |
| Hemorrhage | 47 |
| Infection | 28 |
| Hepatic damage (sAST, sALT: elevated) | 26 |
| Laparotomy | 14 |
| Down syndrome | 11 |
| Very low-birthweight infants | 11 |
| Hemolysis and/or erythroblastosis | 8 |

sALT, serum alanine aminotransferase; sAST, serum aspartate aminotransferase.
mass spectrometry, and named (EZ)-cyclobilirubin. This substance has the structure of the azacyclopentadiene ring, and von Dobeneck reported that the structure could promote free radical reaction polymerization.

The copper porphyrin theory was reported by Rubaltelli et al. in 1983, and many studies on the theory were reported after that. The evidence to support the copper porphyrin theory is based on a peculiar peak at ≥500 nm (λmax, 585, 619, 670 nm) identified on spectral analysis of the samples from bronze baby syndrome. Porphyrin in the samples was analyzed, two kinds of Cu2+-proto and Cu2+-coproporphyrin were found, and Cu2+-uroporphyrin was detected in the aqueous layer on TLC. Copper and porphyrin in the blood increased with the obstruction of the biliary tract, combined, and a copper–porphyrin complex was formed. It was hypothesized that the copper–porphyrin complex changed to a brown color with the photosensitization of bilirubin by light irradiation. They proved those processes based on the absorption and fluorescence spectra. This hypothesis, and the analytical methods, however, were associated with some problems: bilirubin becomes a photosensitizer and Cu2+-porphyrin is degraded by the effects of photosensitizers. We examined whether the copper–protoporphyrin complex would accelerate the generation of (EZ)-/(EE)-cyclobilirubin and polymerization of cyclobilirubin, and the results were negative (data not shown). Moreover, McDonagh examined whether bilirubin would change the copper–porphyrin complex with the absorption spectrum, and proved that the phenomenon does not exist. Therefore, the copper–porphyrin complex does not influence the development of bronze baby syndrome in neonates.

Given, however, that the etiology of bronze baby syndrome is unclear, we must identify the photoproducts when treating bronze baby syndrome. We believe that the cause of the bronze color is the polymerized substances of bilirubin photoisomers and that those substances accumulate in the body of the neonatal infant. We consider that the double bond between N of the B ring of (EZ)-/ (EE)-cyclobilirubin and C6 breaks, and that C6 of (EZ)-/(EE)-cyclobilirubin covalently binds to N of both sides of the end ring of the E-type photoisomers and they polymerize (Fig. 7).

**Conclusion**

In conclusion, the most effective wavelength of phototherapy for human neonatal hyperbilirubinemia lies near the green region rather than the blue region. Based on this idea, we are able to perform safer phototherapy by avoiding the wavelength 400–500 nm, which belongs to the blue region and which can be harmful. The current method to assess light energy in phototherapy is limited to that for blue light, therefore it is necessary to develop a new method for evaluation and comparison of light sources other than blue light. The control of light energy in consideration of the excretion dynamic state of (EZ)-/(EE)-cyclobilirubin and the antioxidant action of bilirubin is required for phototherapy, especially in that for ELBW infants.

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Disclosure

The authors declare no conflict of interest.

Author contributions

S.I. contributed to the conception and design of this review and drafted the manuscript; H.O. prepared the figures and table; T.K. reviewed the section of light sources of phototherapy; T.K. critically reviewed the manuscript. All authors read and approved the final manuscript.

References

1 Cremer R, Perryman PW, Richards DH. Influence of light on the hyperbilirubinemia of infants. *Lancet* 1958; 1: 1094–7.
2 Ogawa J, Ogawa Y, Onishi S et al. Five years' experience in phototherapy. In: Brown AK, Showare J (eds). *Phototherapy for Neonatal Hyperbilirubinemia*. DHEW Publication No. (NIH). US Government Printing, Washington, 1976; 49–66.
3 Bonnett R, Dabies JE, Hursthouse HB. Structure of bilirubin. *Nature* 1976; 262: 326–8.
4 Lightner DA, Linnane WP III, Ahlfors CE. Bilirubin photoxidation products in the urine of jaundiced neonates receiving phototherapy. *Pediatr. Res.* 1984; 18: 696–700.
5 Ennever JF, Perryman PW, Richards DH. Influence of light on albumin-bound bilirubin. Selective binding of intramolecularly hydrogen-bonded conformational enantiomers. *J. Biol. Chem.* 1986; 261: 6034–8.
6 Wickremasinghe AC, Kuznieczw MW, Grimes BA, McCulloch CE, Newman TB. Neonatal phototherapy and infantile cancer. *Pediatrics* 2016; 137: pii: e20151354.
7 Newman TB, Wickremasinghe AC, Walsh EM, Grimes BA, McCulloch CE, Kuzniecwz MW. Retrospective cohort study of phototherapy and childhood cancer in northern California. *Pediatrics* 2016; 137: pii: e20151354.
8 Morris BH, Oh W, Tyson JE et al. Aggressive vs. conservative phototherapy for infants with extremely low birth weight. *N. Engl. J. Med.* 2008; 359: 1885–96.
9 Itoh S, Kusaka T, Sugihara S et al. Is phototherapy for neonatal jaundice the same as drug therapy?. *Jpn. J. Dev. Pharmacol. Therapeutics* 1994; 7: 34–6. (in Japanese).
10 Lightner DA. Structure, photochemistry, and organic chemistry of bilirubin. In: Heirwegh KPM, Brown SB (eds). *Bilirubin Volume I Chemistry*. CRC Press, Boca Raton, FL, 1982; 1–58.
11 Onishi S, Itoh S, Isobe K. Basic and clinical studies for metabolism of bilirubin and its photoisomers in neonatal jaundice – the way to clarification of the mechanism of phototherapy for neonatal jaundice. *Acta Neonatol. Jpn.* 1999; 35: 589–642. (in Japanese).
12 Lightner DA, Reisinger M, Landen GL. On the structure of albumin-bound bilirubin. Selective binding of intramolecularly hydrogen-bonded conformational enantiomers. *J. Biol. Chem.* 1986; 261: 6034–8.
13 Lemberg R. The physiological disintegration of haemoglobin. *Aust. Chem. Inst. J. Proc.* 1939; 6: 170–9.
14 Lightner DA, Woolridge TA, McDonagh AF. Photobilirubin: an early bilirubin photoprotein detected by absorbance difference spectroscopy. *Proc. Natl Acad. Sci.* USA 1979; 76: 29–32.
15 Onishi S, Kawade N, Itoh S, Isobe K, Sugiyama S. High-pressure liquid chromatographic analysis of anaerobic photoproduits of bilirubin IXα in vitro and its comparison with photoproduits in vivo. *Biochem. J.* 1980; 190: 527–32.
16 Yasuda S, Itoh S, Imai T, Isobe K, Onishi S. Cyclobilirubin formation by in vitro photolrradiation with neonatal phototherapy light. *Pediatr. Int.* 2001; 42: 270–5.
17 Blanckaert N, Gollan J, Schmid R. Mechanism of bilirubin diglucuronides formation in intact rat: bilirubin diglucuronide formation in vivo. *J. Clin. Invest.* 1980; 65: 1332–42.
18 McDonagh AF. Thermal and photochemical reactions of bilirubin IXα. *Ann. NY Acad. Sci.* 1975; 244: 533–69.
19 Bonnett R, Buckley DG, Hamzetash D, Hawkes GE, Ioannou S, Stoll MS. Photobilirubin II. *Biochem. J.* 1984; 219: 1035–6.
20 Onishi S, Itoh S, Kawade N, Isobe K, Sugiyama S. The separation of configurational isomers of bilirubin by high pressure liquid chromatography and the mechanism of Jaundice phototherapy. *Biochem. Biophys. Res. Commun.* 1979; 12: 890–6.
21 Onishi S, Itoh S, Kawade N, Isobe K, Sugiyama S. Accurate and sensitive analysis of ethyl anthranilate azopigments from bile by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 1980; 182: 105–9.
22 McDonagh AF, Palma L. Phototherapy for neonatal jaundice. Configurational isomers of bilirubin. *J. Am. Chem. Soc.* 1982; 104: 6865–7.
23 Itoh S, Isobe K, Onishi S. Accurate and sensitive high-performance liquid chromatographic method for geometric and structural photoisomers of bilirubin IXα using the relative molar absorptivity values. *J. Chromatogr.* A 1999; 848: 169–77.
24 McDonagh AF, Palma LA. Phototherapy for neonatal jaundice. Stereospecific and regioselective photoisomerization of bilirubin bound to human serum albumin and NMR characterization of intramolecularly cyclized photoproduits. *J. Am. Chem. Soc.* 1982; 104: 6867–9.
25 Onishi S, Miura I, Isobe K et al. Structure and thermal interconversion of cyclobilirubin IXα. *Biochem. J.* 1984; 218: 667–76.
26 McDonagh AF, Agati G, Fusi F, Pratesi R. Quantum yields for laser photocyclization of bilirubin in the presence of human serum albumin. Dependence of quantum yield on excitation wavelength. *Photochem. Photobiol.* 1989; 50: 305–19.
27 Itoh S, Onishi S. Kinetic study of the photochemical changes of (ZZ)-bilirubin IXα bound to human serum albumin. *Biochem. J.* 1985; 226: 251–8.
28 Bonnett R, Ioannou S. Phototherapy and the chemistry of bilirubin. *Molec. Aspects Med.* 1887; 9: 457–71.
29 Ostrow JD. Photocatabolism of labeled bilirubin in the congenitally jaundiced (Gunn) rat. *J. Clin. Invest.* 1971; 50: 707–18.
30 McDonagh AF, Ramonas LM. Jaundice phototherapy: micro flow-cell photometry reveals rapid biliary response of Gunn rats to light. *Science* 1978; 201: 829–31.
31 Onishi S, Ogiino T, Yokoyama T et al. Biliary and urinary excretion rates and serum concentration changes of four bilirubin photoproduits in Gunn rats during total darkness and low or high illumination. *Biochem. J.* 1984; 221: 717–21.
32 Onishi S, Isobe K, Itoh S et al. Metabolism of bilirubin and its photoisomers in newborn infants during phototherapy. *J. Biochem.* 1986; 100: 789–95.
33 Onishi S, Itoh S, Yamakawa T et al. Comparison of kinetic study of the photochemical changes of (ZZ)-bilirubin IXα bound to human serum albumin with that bound to rat serum albumin. *Biochem. J.* 1985; 230: 561–7.
34 Iwase T, Itoh S. (EZ)-cyclobilirubin formation from bilirubin in complex with serum albumin derived from various species. *J. Photochem. Photobiol.* 2010; 12: 138–43.

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35 Onishi S, Kawade N, Itoh S et al. Kinetics of biliary excretion of the main two bilirubin photoproducts after injection into Gunn rats. Biochem. J. 1981; 198: 107–12.

36 Ennever JF, Knox I, Denne SC, Speck WT. Phototherapy for neonatal jaundice: in vivo clearance of bilirubin photoproducts. Pediatr. Res. 1985; 19: 205–8.

37 Okada K, Abe T, Itoh Y, et al. In vitro production of bilirubin photoisomers by light irradiation using neoBLUE. Pediatr. Int. 2007; 49: 318–21.

38 Onishi S, Itoh S, Isobe K. Wavelength-dependence of the relative rate constants for the main geometric and structural photoisomerization of bilirubin IXα bound to human serum albumin: demonstration of green light at 510 nm as the most effective wavelength in photochemical changes from (ZZ)-bilirubin IXα to (EZ)-cyclobilirubin IXα via (EZ)-bilirubin. Biochem. J. 1986; 236: 23–9.

39 Itoh S, Onishi S, Isobe K, Manabe M, Yamakawa T. Wavelength dependence of the geometric and structural photoisomerization of bilirubin bound to human serum albumin. Biol. Neonate 1987; 51: 10–7.

40 Vecchi C, Donzelli GP, Miogliorini MG, Shrama G. Green light in phototherapy. Pediatr. Res. 1983; 17: 461–3.

41 Okada H, Masuya K, Kurono Y et al. Change of bilirubin photoisomers in the urine and serum before and after phototherapy compared with light source. Pediatr. Int. 2004; 46: 640–4.

42 Kuboi T, Kusaka T, Okazaki K et al. Efficacy of green LED phototherapy for neonatal hyperbilirubinemia: an in vivo study. J. Jpn Soc. Neonatal Health Dev. 2015; 27: 280–6. (in Japanese).

43 Ebbesen F, Madsen PH, Vandborg PK, Jakobsen LH, Trydal T, Vreman HJ. Bilirubin isomer distribution in jaundiced neonates during phototherapy LED light centered at 497 nm (turquoise) vs. 495 nm (blue). Pediatr. Res. 2016; 80: 511–5.

44 Kuboi T, Kusaka T, Yasuda S, Okubo K, Isobe K, Itoh S. Management of phototherapy for neonatal hyperbilirubinemia: is a new radiometer applicable for all wavelengths and light source types? Pediatr. Int. 2011; 53: 689–93.

45 Kopelman AE, Brown RS, Odell GB. The “bronze” baby syndrome: a complication of phototherapy. J. Pediatr. 1972; 81: 466–72.

46 Speck WT, Chen CC, Rozenkranz HS. In vitro studies of effects of light and riboflavin on DNA and HeLa cells. Pediatr. Res. 1975; 9: 150–3.

47 Balowowitz L, Bunjamin A, Handefeld F, Lietz L, StÜttgen G, Wirjadi D. Effect of riboflavin on Gunn rats under phototherapy. Pediatr. Res. 1979; 13: 1307–15.

48 Ennever JF, Speck WT. Photodynamic reaction of riboflavin and deoxyguanosine. Pediatr. Res. 1981; 15: 956–8.

49 Ennever JF, Speck WT. Photochemical reaction of riboflavin; covalent binding to DNA and to Poly (dA) Poly (dT). Pediatr. Res. 1983; 17: 234–6.

50 Kusaka T, Sugihara S, Murao C et al. The significance of Soret band in phototherapy of neonatal jaundice by blue-white light. Photomed. Photobiol. 1993; 15: 119–21.

51 Yaguchi Y, Itoh S, Isobe K, Onishi S. Phototherapy for neonatal hyperbilirubinemia: comparison of various forms of phototherapy. J. Jpn Soc. Neonatal Health Dev. 1997; 9: 147–56. (in Japanese).

52 Bohm F, Drygalla F, Charlesworth P, Bohm K, Truscott TG, Jokiel K. Bilirubin phototoxicity to human cells by green light phototherapy in vitro. Photochem. Photobiol. 1995; 62: 980–3.

53 Ando M, Itoh S, Kusaka T, Onishi S. Pathophysiologic significance of neonatal jaundice in defense system against active oxygen in the neonatal period: comparison of bilirubin photochemical reactions of blue-white light and green light in the presence of Flavin mononucleotide. J. Jpn Soc. Neonatal Health Dev. 1990; 2: 96–103. (in Japanese).

54 Okada H, Masuya K, Yasuda S et al. Developmental changes in serum half-life of (EZ)-cyclobilirubin. Early Hum. Dev. 2005; 81: 619–22.

55 Kunikata T, Itoh S, Ozaki T, Kondo M, Isobe K, Onishi S. Formation of propentypophenols and biliverdin, oxidized metabolites of bilirubin, in infants receiving oxygen therapy. Pediatr. Int. 2000; 42: 331–6.

56 Onishi S. Phototherapy for hyperbilirubinemia. J. Jpn. Pediatr. Soc. 1971; 75: 778. (in Japanese).

57 Onishi S. Bronze baby syndrome and its allied disease. Jpn. Med. Assoc. J. 1978; 21: 53–6.

58 Itoh M. Study on bronze baby syndrome: predisposing factor and pathogenesis. J. Nagoya City Med. Assoc. 1977; 28: 1125–38.

59 Onishi S, Itoh S, Isobe K, Togari H, Kitoh H, Nishimura Y. Mechanism of development of bronze baby syndrome in neonates treated with phototherapy. Pediatrics 1982; 69: 273–6.

60 von Dobeneck H. The Stokovis reaction. In: Dolfont B (ed.). The Purporthes, Vol. 4. Academic Press, New York, 1979; 651–62.

61 Rubaltelli FF, Jori G, Reddi E. Bronze baby syndrome: a new porphyrin-related disorder. Pediatr. Res. 1983; 17: 327–30.

62 Jori G, Reddi E, Rubaltelli FF. Bronze baby syndrome: an animal model. Pediatr. Res. 1990; 27: 22–5.

63 Rubaltelli FF, Da Riol R, D’Amore ESG, Jori G. The bronze baby syndrome: consequence of impaired excretion of (EZ)-bilirubin. J. Jpn Soc. Neonatal Health Dev. 1984; 35: 87–103. (in Japanese).