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Metalloporphyrins in the management of neonatal hyperbilirubinemia

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Summary

Neonatal jaundice in the first week of life is a common problem in newborns. It is due to an imbalance of bilirubin production and its elimination, which can lead to significantly elevated levels of circulating bilirubin or hyperbilirubinemia. Use of phototherapy and/or exchange transfusion are the current modes for treating neonatal hyperbilirubinemia and preventing any neurologic damage. These strategies, however, only remove bilirubin that has already been formed. Preventing the production of excess bilirubin may be a more logical approach. Synthetic heme analogs, metalloporphyrins, are competitive inhibitors of heme oxygenase (HO), the rate-limiting enzyme in bilirubin production, and their use has been proposed as an attractive alternative strategy for preventing or treating severe hyperbilirubinemia.

Keywords

Bilirubin; Heme oxygenase; Hyperbilirubinemia metalloporphyrin; Neonatal jaundice

[A] Introduction

The proposed use of metalloporphyrins (Mps) in the management of neonatal hyperbilirubinemia represents a targeted therapeutic intervention for the prevention of a transitional condition, which is sometimes exacerbated by exogenous factors.^{1–3} Therefore, a thorough understanding of the causes of neonatal jaundice is required and serves as a foundation for the rationale to reduce or inhibit the production of bilirubin as a way of controlling neonatal hyperbilirubinemia after birth.^{1,2,4,5} It is important to understand that neonatal jaundice is a syndrome with a variety of contributing causes. Historically, it has been the ‘jaundice syndrome’ that has been addressed categorically by non-specific maneuvers to eliminate excessive bilirubin from the body, after it has been produced, irrespective of the complex causation of its accumulation in an individual infant.^{1–3} The most popular first-line approach to treatment continues to be phototherapy, using light (actually blue light, a discrete part of the spectrum – from the mid-400 to low-500 nm range) to photoconvert the bilirubin molecule and form photoisomers that are excreted in bile without the need for hepatic conjugation to water-soluble glucuronides,^{6,7} the latter process

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Conflict of interest statement

None declared.

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being poorly developed in most infants in the first week after birth^{1–3} and genetically limited in some beyond that timeframe.⁸ Exchange transfusion is an even more invasive and risky treatment for severe hyperbilirubinemia^{1–3} or for hyperbilirubinemia unresponsive to phototherapy and is the last resort to prevent acute bilirubin-induced neurologic dysfunction (BIND) or rescue a patient in the context of BIND.⁹ An important point to be made is that there are limitations of such non-specific therapeutic interventions – they do not reflect personalized medicine, nor are they preventive. In fact, traditional classifications of pathologic conditions based on ‘appearance’, such as the condition of being jaundiced, are often not informing with respect to directing specific therapies to eliminate or mitigate any contributing causes of the pathologic condition. Moreover, any potential for prevention is lost because the therapies are non-specific and designed only to decrease jaundice after its appearance. In fact, much of medicine is reactive in this way and conditions are defined by deviations from the norm, with treatments mostly retrenching from pathology back towards normalcy.

[A] Neonatal hyperbilirubinemia

The first step then is to understand the phenotype of neonatal jaundice. It can be best defined as the result of an imbalance between bilirubin production and its elimination such that, when the rate at which bilirubin is produced exceeds the rate at which bilirubin is eliminated, the bilirubin load in the body increases.^{1,3,10} A certain amount of bilirubin can be retained in circulation, mainly bound to albumin. Even when this binding is sufficient, some bilirubin still can move outside the circulation and into tissues like the skin, with the infant becoming ‘visibly’ jaundiced. Visible jaundice is a sign that the bilirubin load is increasing, but it is a poor predictor of the concentration of bilirubin in circulation or other body compartments like the brain.^{11,12} Because bilirubin elimination is compromised in all babies in the first weeks after birth, bilirubin production becomes the major contributing cause to many kinds of pathologic jaundice in the newborn. Even the normal term newborn has increased bilirubin production (about two to threefold higher) compared to the adult, mainly due to an increased red cell mass and a shorter red cell lifespan.¹³ There are many other factors that can further enhance the production of the pigment, but hemolysis arising from a variety of causes is one of the most common and potentially most dangerous.^{1–3} The danger of hemolysis is its association with a greater risk for neurologic injury in the presence of severe hyperbilirubinemia. It is likely that an increased production of bilirubin in general confers a similar increased risk in any jaundice situation in which it is encountered, because it increases the load of bilirubin in the body and the amount of bilirubin that is likely to be in tissue for a given binding capacity. The rationale then for controlling production of the pigment in order to mitigate hyperbilirubinemia and avoid the increased risk for injury associated with hyperbilirubinemia in the context of increased bilirubin production becomes clearer and more persuasive.

[A] Inhibition of bilirubin production

The logical target for modulating bilirubin production is heme oxygenase (HO), the first and rate-limiting step in the production of bilirubin. Like most biologic targets, it is not singular in nature, but really a target in a context, which is complex. Moreover, there is more than one kind of HO,^{14,15} the inducible HO-1 and the constitutive HO-2, and possibly a third, about which less is known.¹⁶ In fact, the heme catabolic pathway can be described as a signaling network with many different connected pathways. The heme catabolic pathway itself represents a complex series of chemical reactions including the first and rate-limiting step which is catalyzed by HO and in the presence of NADPH (derived from the cytochrome P450 system) and molecular oxygen (O₂) in a series of oxidations and reductions ultimately leading to the breaking of the IX-alpha methene bridge, creating biliverdin and releasing

carbon monoxide (CO) and iron (Fe^{++}) in equimolar proportions.¹⁷ The second step in the process also requires NADPH and is catalyzed by biliverdin reductase yielding bilirubin, also in equimolar relationship to CO and Fe^{++} .

Because the pathway catabolizes heme, a pro-oxidant, and produces a potent antioxidant, bilirubin, it can be characterized as an antioxidant system, with the biliverdin–bilirubin interconversion ‘shuttle’ contributing to the redox state of the cell.^{18,19} Although iron, CO and bilirubin are all toxic at high levels, they are also all important molecules in connecting systems. The biliverdin–bilirubin shuttle may have antioxidant,²⁰ anti-inflammatory,¹⁸ and anti-apoptotic effects.¹⁸ CO can cause vessel relaxation mediated directly through calcium-dependent potassium channels or through activation of soluble guanylyl cyclase (sGC), similar to nitric oxide (NO), increasing cyclic GMP and signaling vessel^{21,22} and smooth muscle relaxation²³ as well as other effects on platelet aggregation,²⁴ apoptosis,²⁵ cell proliferation,^{26,27} and neurotransmission.^{26,27} CO may also inhibit proinflammatory cytokines through p38 MAPK²⁸ and cause angiogenesis through increases in vascular endothelial growth factor (VEGF).²⁹ Iron, through its participation in the ferritin-iron ATPase pump, may have antioxidant, anti-inflammatory, and anti-apoptotic effects.³⁰ There are also many regulatory interactions between the HO and CO system and the nitric oxide synthase (NOS) and NO system.^{22,31} Some are positive and others are negative, and they are often countering. Thus, HO inhibitors not only affect heme catabolism and the production of bilirubin, a potential toxin in the newborn under some conditions; but they can also affect many other systems indirectly.^{31,32}

Because CO and bilirubin are produced in equimolar amounts during the catabolism of heme, CO production, as estimated by carboxyhemoglobin (COHb) in circulation, end-tidal CO concentration corrected for ambient CO (ETCO_c) and pulmonary excretion rates of CO (VeCO) can be used to estimate total body bilirubin formation,³³ and thus can be used clinically to identify high risk situations in which bilirubin production is increased and the bilirubin load is likely to be high. The identification of high producers of bilirubin represents the direct targeting of individual infants who would benefit from modulation of bilirubin production as a contributing cause to their pathologic jaundice. Such biologic targeting avoids the inhibition of HO to below physiologic activity levels in babies who do not have abnormally elevated heme catabolism, reflecting not only normal transitional changes in heme catabolism, some of which are inducible, but also constitutive heme catabolism mediated by HO-2, and further avoids any unnecessary downstream direct or indirect effects on other important signaling systems.

Although CO production can serve as a good index of bilirubin production, it is important to recognize that there are other endogenous sources of CO, some of which may be important under some conditions, for example, photo-oxidation and lipid peroxidation.³⁴ However, under most circumstances encountered in the newborn who does not have pulmonary disease with exposure to high inspired O_2 , heme degradation accounts for greater than 80% of the CO produced and excreted, with 70% of that derived from senescing red blood cells, 10% from ineffective erythropoiesis, and 20% from the degradation of other hemoproteins.^{1,3} Under some of the pathologic conditions encountered in the newborn, such as hematoma formation, polycythemia, and hemolytic disease, the proportion of CO coming from heme degradation may be even greater. Thus, CO in breath is probably the most sensitive index of clinically important hemolysis and the best way to target babies with increased production of bilirubin.³³ Although clinical and epidemiologic risk factors can be used to target potential candidates for drug therapy, such targeting is unavoidably less precise. For example, only half of the babies with ABO heterospecificity and a positive direct Coombs’ test have hemolysis.³⁵ Conversely, some babies with ABO heterospecificity and a negative direct

Coombs' test have increased hemolytic rates.³⁵ These circumstances are readily identifiable by estimating CO production.³⁶

Early work by Maines, Drummond and Kappas,^{4,37,38} began a systematic study of Mps, synthetic structural analogues or metalloporphyrins (Mps) of heme, as a new class of drugs for the modulation of bilirubin production. Over several decades, they were joined by a host of other investigators.^{5,39–44} Many different Mps have been investigated with respect to their efficacy and safety. Only tin protoporphyrin (SnPP)⁴⁵ and later tin mesoporphyrin (SnMP)^{46–48} have been studied in human neonates. SnPP was abandoned early because of its photosensitizing properties,^{49,50} despite being highly efficacious. Although SnMP is also photosensitizing,⁵¹ its greater potency has allowed for its use at lower doses with minimal apparent photoreactivity in the clinical setting, especially if light exposure is avoided or restricted to a narrow part of the spectrum.⁷ In mouse studies, we have found that at high oral doses [30 $\mu\text{mol/kg}$ /body weight (BW)], SnMP significantly inhibits brain HO activity as well as inhibits liver HO activity. Furthermore, significant induction of HO-1 expression was found in the liver and brain. When lower doses were tested, significant inhibition of brain HO activity was still observed at a dose of 7.5 $\mu\text{mol/kg}$ BW, but induction of HO-1 in brain and liver were minimal.⁵² At still lower doses (3.75 $\mu\text{mol/kg}$ BW), long-term potent inhibition of HO is still observed with negligible effects on HO-1 transcription,⁵³ as well as avoiding potential direct effects on other enzyme systems, such as sGC and NOS.³² The use of SnMP for the prevention of severe neonatal hyperbilirubinemia has also been reviewed in considerable detail.⁵⁴

Nonetheless, the ideal antihyperbilirubinemic drug would contain a biocompatible central metal, cause potent HO inhibition, have negligible degradation, inhibition of other enzymes, photoreactivity, HO-1 upregulation, and have an optimal duration of action (which would be no more than days) (Table I).^{54,55} Alternative Mps to SnMP do exist and are being studied because they have many of these desirable features, including being photo-inert, which have been reviewed in detail previously.⁵⁵ Some, such as chromium mesoporphyrin (CrMP)^{56,57} and zinc bis glycol (ZnBG),^{58,59} are orally absorbable; and zinc protoporphyrin (ZnPP) is naturally occurring and has no apparent photoreactivity in vivo. In fact, orally absorbed compounds may be more likely to exhibit effects in the liver and spleen with less distribution to other tissues particularly if they can be used at lower doses. Some of these compounds also appear not to upregulate substantially the HO-1 gene (e.g. ZnPP and ZnBG), while maintaining their inhibitory potency.^{60,61}

CrMP at a dose of 15 $\mu\text{mol/kg}$ BW is orally absorbed by the newborn mouse, inhibits liver HO activity in an age-dependent manner 24 h after administration without affecting spleen and brain HO activity.⁶² When the in vivo effects of prophylactic oral CrMP on HO activity following a single oral heme load (30 $\mu\text{mol/kg}$ BW) were investigated, CrMP abolished the heme-mediated increases in liver and spleen significantly.⁵⁶ After a second heme load, a low dose of CrMP (3.75 $\mu\text{mol/kg}$ BW) was effective in inhibiting heme-induced increases in liver HO activity in the spleen, but not in the brain.⁵⁶

ZnBG is also effective towards inhibiting liver HO activity at a lower dose (3.75 $\mu\text{mol/kg}$ BW) and more quickly (with 3 h of administration) than CrMP, and does not affect spleen or brain HO activity.⁵⁹ Neither compound appears to induce any changes in HO-1 protein or gene transcription levels. These properties also make CrMP and ZnBG attractive alternative compounds to SnMP since they have great efficacy of short duration with minimal or no long-term effects on HO-1 gene regulation.

There is now additional motivation for using an Mp HO-1 inhibitor or some other drugs to inhibit HO-1 or lower bilirubin levels transiently after birth, because of recent findings in a

randomized controlled trial of aggressive versus conservative phototherapy for infants with extremely low birth weight.⁶³ Although the primary outcome of the study revealed no clear difference between both groups with respect to death or neurodevelopmental impairment, a subgroup analysis suggested that infants weighing between 501 and 750 g in the aggressive phototherapy group, the smallest and most translucent babies, might have an increased risk of death. This was further supported by Bayesian analyses. More study is required to demonstrate that aggressive phototherapy does not increase the mortality of infants ≤ 750 g, but there is biologic plausibility for this possibility. In order to resolve this question, a randomized controlled trial to compare aggressive phototherapy with Mp therapy in order to avoid severe hyperbilirubinemia is needed.

Still, a pharmacologic approach to managing newborn jaundice in the smallest babies could be an attractive alternative. Other compounds structurally different from Mps have been found to possess HO-inhibiting properties. For example, peptide inhibitors, originally developed for use in transplantation survival studies, have been found to inhibit HO activity in vitro in a dose-dependent manner.⁶⁴ However, administration of peptides in mice resulted in an upregulation of HO-1 mRNA and protein, as well as HO activity in liver, spleen, and kidney. Consequently, human studies using these peptides for the treatment of hyperbilirubinemia have not been performed. In addition, imidazole dioxolanes, compounds designed to inhibit cholesterol production, have also been found to inhibit in vitro and in vivo HO activity.^{65–68} These compounds have a high selectivity for inhibiting the inducible HO-1.^{66,68} Some of these compounds have been found to affect other important enzymes, such as NOS and sGC, in rat tissues.⁶⁵ In in-vivo studies using one of these compounds, Azalanstat, we demonstrated that HO activity in the spleen, brain, and liver could be inhibited. However, 24 h after treatment, spleen HO activity, HO-1 protein, and HO-1 mRNA levels are significantly increased.⁶⁷

[A] Conclusion

The use of Mps in the management of neonatal hyperbilirubinemia represents an opportunity to introduce targeted therapeutic intervention for prevention of neonatal jaundice into clinical practice. Furthermore, an anticipatory approach to neonatal jaundice is the ideal tactic, perhaps even with antenatal diagnosis of genetic vulnerabilities, such as polymorphisms known to be associated with jaundice, for example, having fewer than normal GT repeats in the HO-1 promoter, or mutations in UGT1A1 or OATP1B1 contributing to impaired conjugation or hepatic uptake and slower elimination of the pigment.^{8,11,69,70} The interaction of genes could also be anticipated – for example, glucose-6-phosphate dehydrogenase (G6PD) deficiency and Gilbert's syndrome, as has been reported by Kaplan et al.^{71,72} With such anticipation, infants could then be monitored non-invasively after birth for increased production of bilirubin, with early prophylactic treatment of high producers and poor eliminators with antihyperbilirubinemic drugs. Such genetic and phenotypic targeting would finally bring rationality to the management of neonatal jaundice and largely avoid the risk of toxicity from bilirubin.

Practice points

- To understand the causation of neonatal hyperbilirubinemia.
- To understand the use of metalloporphyrins (Mps) or other potential alternative compounds to prevent or treat severe neonatal hyperbilirubinemia.
- To understand the potential side-effects and safety concerns of Mps.
- To understand the current state of Mp research.

Research directions

- Comparison of the efficacy of aggressive phototherapy with Mp therapy to prevent the development of severe hyperbilirubinemia.
- Evaluation of the potential phototoxic effects, if any, of chromium mesoporphyrin and zinc bis glycol porphyrin.

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Table 1

Most desirable properties of an anti-hyperbilirubinemia drug

Property	SnMP	CrMP	ZnBG	ZnPP
Biocompatible central metal			X	X
Potent HO inhibitor	X	X	X	
Absorbed orally		X ^a	X ^a	X ^a
Not photoreactive		X		X
Optimal duration of action	X	X	X	X
Negligible effects on other enzymes		X		
Negligible HO-1 upregulation			X	

SnMP, tin mesoporphyrin; CrMP chromium mesoporphyrin; ZnBG, zinc bis glycol; ZnPP, zinc protoporphyrin; HO, heme oxygenase.

^aBased on studies performed on mice.