Phenobarbitone in Rh Hemolytic Disease of the Newborn: A Randomized Double-Blinded Placebo-Controlled Trial

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Summary

Objective: To evaluate the efficacy of prophylactic oral phenobarbitone (PB) in neonates with Rh hemolytic disease of the newborn.

Study Design: In this double-blind randomized trial conducted in a tertiary care unit, we randomly allocated neonates with Rh hemolytic disease of the newborn born at or after 32 weeks’ gestation to PB (10 mg/kg/day on day 1 followed by 5 mg/kg/day on days 2–5) (n = 23) or oral glucose (n = 21). The primary outcome was the duration of phototherapy.

Results: Baseline variables were comparable. There was no difference in the median duration of phototherapy [54 (range: 0–180) vs. 35 h (0–127); p = 0.39] and in the incidences of failure of phototherapy or significant rebounds of serum bilirubin. However, the proportion of infants with cholestasis was significantly lower in the PB group (0 vs. 19%; p = 0.04).

Conclusions: PB does not reduce duration of phototherapy or its episodes. Its potential to reduce cholestasis needs validation in larger studies.

Key words: Rh hemolytic disease of the newborn (Rh-HDN), phenobarbitone, phototherapy.

Introduction

Rh hemolytic disease of the newborn (Rh-HDN) is associated with rapid rise of serum bilirubin levels soon after delivery, necessitating active interventions to prevent the potential bilirubin-induced neurotoxicity [1]. The available interventions to prevent bilirubin-induced neurotoxicity arising from Rh-HDN include double-volume exchange transfusion (DVET), intensive phototherapy and intravenous immunoglobulin (IVIG).

Although the disease has been more or less eradicated from most of the developed countries with the use of anti-D γ-globulin, there remains a substantial, yet unrecognized, burden of this disease in the low-income countries. Based on the annual Rh-negative pregnancies and the prevalence of usage of anti-Rh prophylaxis, a recent article estimated the annual burden of Rh-HDN to range from 14,000 to 56,000 among various communities globally [2].

Phenobarbitone (PB) is a potent inducer of the hepatic UDP-glucuronoyltransferase (UDPGT) [3], and has, in addition, multiple actions at various levels of bilirubin metabolism in the liver [4]. PB has been used in the prevention of neonatal jaundice for very-low-birth-weight babies [5] and in the treatment of hyperbilirubinemia of Crigler–Najjar syndrome [3], Dubin–Johnson syndrome [4] and neonatal cholestasis.

Its role in the background of hemolysis has been evaluated in the settings of ABO incompatibility [6] and glucose-6-phosphate dehydrogenase (G6PD) deficiency [7] and has resulted in mixed results, with interventions spilling into the antenatal period [8] in some of these studies.

In view of paucity of evidence of potential role of PB in the setting of Rh-HDN and considering its different mechanisms of actions to reduce bilirubin load compared with the available therapies, we hypothesized that addition of prophylactic PB to a standard regimen for treating Rh-HDN would result
in better clinical outcomes such as reduced duration of phototherapy and failure of phototherapy.

Methods

Subjects and setting
We conducted this randomized double-blind placebo-controlled trial from July 2008 to December 2009 at the All India Institute of Medical Sciences, New Delhi. We enrolled neonates born at or after 32 weeks’ gestation and diagnosed with Rh-HDN (positive direct Coombs test in the neonates’ sera and/or history of intrauterine blood transfusions in the setting of Rh blood group incompatibility). All the mothers were antenatally screened at the Department of Obstetrics of the Institute for anemia in the fetus by middle cerebral artery peak systolic velocity measurements and received intrauterine transfusions of packed red blood cells when necessary. The gestational assessment of the enrolled patients was calculated from the first day of last menstrual period if the mother was sure of dates; if not, we calculated it based on the first trimester ultrasound assessment or by expanded New Ballard Score. We excluded infants exposed to antenatal PB for any indication and those with neonatal convulsions before enrollment or major congenital malformations. We obtained informed written consent from the parents before enrolling their infants. The institutional ethics committee had cleared the study protocol. The study was registered in the Clinical Trials Registry of India (CTRI-2010 000218).

Randomization and blinding
Enrolled neonates were stratified based on period of gestation (Group I: 32–34 6/7 weeks and Group II: 35 weeks or more) and were randomly allocated to either oral PB or placebo using computer-generated random sequence numbers. The hospital pharmacist prepared identical sachets of both PB and placebo in two different strengths (10 and 5 mg). The sachets contained similar-looking white powders. The strength of the drug—10 mg or 5 mg—was clearly mentioned on each sachet. Seven sachets each of both 10 and 5 mg strengths were then kept in serially numbered transparent plastic pouches. As PB is bitter, we administered both the drugs by orogastric tube so as to ensure blinding.

The investigators as well as the treating team were masked to the process of randomization and treatment group allocation. Only the pharmacist was aware of the codes. The codes were revealed to the investigators only after the final analysis.

Intervention
Eligible infants were given the intended drug by orogastric tube by pre-mixing the contents of the sachets in 5 ml of feeds (preferably expressed breast milk). The drug was administered at a dose of 10 mg/kg on the first day, followed by 5 mg/kg on the subsequent 4 days. The doses were administered by the on-duty nursing staff at the scheduled intervals. The dose was repeated in case of vomiting within 2 h of administration. In case of anticipated requirement of DVET soon after delivery, the first dose was administered after the procedure. The remaining doses were administered by either the parents or the nurses in the postnatal ward. We ensured compliance by checking the remaining pouches on a daily basis until completion of the study.

As a part of the unit protocol, the cord serum bilirubin levels and packed cell volume (PCV) measurements are estimated immediately after delivery for all infants with Rh setting. PCV was measured by a hematocrit scale after centrifugation of microcapillaries at 10,000 rpm for 3 min, and the separated serum in the sample was used to measure the total serum bilirubin (TSB) by means of twin-beam microbilirubinometer (Gineveri Technologie Biomediche, Italy). If the infant did not require DVET immediately after birth (see later in the text), IVIG was administered at a dose of 1 g/kg over 4 h. In the remaining infants, IVIG was administered after the procedure.

Outcomes and their measurement
The primary outcome was the total duration of phototherapy. Secondary outcomes were the incidence of failure of phototherapy (defined as a rise of TSB while on phototherapy to a level necessitating DVET) and significant rebounds of TSB (defined as a rise of TSB within 24–48 hours after stopping phototherapy to a level that required restart of phototherapy), and the proportion of infants with cholestasis (defined as a level of direct bilirubin >2 mg/dl or the fraction of direct bilirubin ≥20% of the TSB) during the hospital stay. In addition, we also looked at the proportion of infants with lethargy (excessive sleepiness as observed by the caregivers) and apnea (cessation of breathing for >20 s and/or associated with bradycardia and cyanosis) during the period of intervention.

Phototherapy was provided by 2 U of compact fluorescent light (Special blue light, Bird, Meditech, Mumbai) applied at 35–40 cm to provide an irradiance of 25–30 μW/cm²/nm. The phototherapy units were checked periodically to confirm the irradiance, and the lights were replaced after 1000 phototherapy hours. For infants born at or after 35 weeks, we initiated phototherapy based on the American Academy of Pediatrics (AAP) guidelines [9]. For the remaining infants, phototherapy was initiated based on the birth weight—<1000 g: 5 mg/dl; 1001–1500 g: 7 mg/dl; 1501–2000 g: 10 mg/dl and 2001–2500 g: 12 mg/dl. Phototherapy was stopped when
two values of TSB taken 8 h apart were below the
defined cut-off levels. The criteria for restart of
phototherapy were the same as those for initiation.
We decided a priori to estimate TSB and PCV at an
interval of 8 ± 2 h during the course of study until
24 h of cessation of phototherapy. The duration of
phototherapy was recorded on a printed Performa
specifying the time and the total duration was
added up.

DVET was performed immediately after birth if the
cord TSB was >5 mg% and PCV <30%. Subse-
quently, we used the cut-offs provided in the guide-
lines for infants born at or after 35 weeks, and TSB
levels of 5 mg% above the levels defined for photo-
therapy for those born before 35 weeks.

As only TSB was measured by the twin-beam tech-
nique, the onset of cholestasis was assessed clinically
in all babies by determining the development of
greenish-brown discoloration of the skin and passage
of high colored urine. Following this, blood samples
were sent to the laboratory for estimation of the frac-
tions of serum bilirubin. The infants were continu-
ously monitored for apnea and excessive sleepiness
while in the nursery by the on-duty staff, and while
the infant was shifted out, the caregivers were appro-
priately educated to look for these signs and report.
The same were monitored by the principal worker at
regular intervals.

Statistical analysis
Patient information was collected in a proforma.
Data entry was done using MS Access 2007
(Microsoft, Redmond, CA). Data analysis was done
using Stata 11.0 (Stata Corp, College Station, TX).
Continuous data with normal distribution were ana-
lyzed by Student’s t-test, whereas non-normally dis-
tributed data were analyzed by Mann–Whitney U
test. Categorical data were analyzed by Fisher
exact/chi-square test. P value of <0.05 was taken as
significant.

The sample size was calculated based on the data
from a previous randomized trial comparing two
doses of IVIG conducted in our unit [10]. The mean
(SD) duration of phototherapy in the 1 g/kg
IVIG group in that trial was 96 (24) h. Assuming
that PB would reduce the duration of phototherapy
by 24 h with a standard deviation of 24 h in each
group, power of 90% and a two-tailed \( z \) of .05, we
needed to enroll 22 babies in each group.

Results
Forty-four infants were enrolled between March
2010 and Jan 2011 (Fig. 1). Baseline variables were
comparable for the severity of the Rh-HDN disease,
as assessed antenatally by the indirect Coombs test
titers, significant fetal anemia (middle cerebral artery
peak systolic velocity >1.5 MoM) and requirement
of intratraborine transfusions, and postnatally by cord
bilirubin, cord PCV, presence of hydrops and re-
quirement of DVET in first 6 h of life. The postnatal
morbidities of infants were also comparable among
the two groups (Table 1).

Primary outcome
Figure 2 depicts the primary outcome—total dur-
ation of phototherapy. There was no significant dif-
ference in the duration between the two groups
[median (range): 54 (0–180) vs. 35 (0–127) h;
\( p = 0.39 \)].

In total, two babies in the PB group and three
babies in the placebo group did not receive any
phototherapy. The difference in duration of photo-
therapy was not significant between the two groups
even after exclusion of these infants [median (range):
58.5 (12–180) vs. 46 (16–127) h; \( p = 0.98 \)].

Secondary outcomes
There was no significant difference in the incidence of
failure of phototherapy necessitating DVET after
first 6 h of life or the rebounds of TSB requiring
restart of phototherapy. The proportion of infants
with cholestasis was significantly lower in the PB
group \([0 \text{ vs. } 4 (19%); \ p = 0.04]\). The median day of
onset of clinically evident cholestasis in the babies
was at 4.5 days of life. All the babies who developed
cholestasis required at least one DVET. In the ab-
sence of alternative diagnosis in these babies, a clin-
cal diagnosis of inspissated bile syndrome was made.
All these babies with cholestasis were started on
open-label PB as per our unit protocol, to which
they responded.

Two babies (one in each group) had TSB levels of
20 mg% during the course of disease and none of the
babies in either group had any features of bilirubin-
induced neurological dysfunction.

Discussion
Rh-HDN continues to be a major burden in the de-
veloping countries. With an annual birth of approxi-
mately 26 million babies in India and an existing
prevalence rate of 5% of Rh negativity, the rate of
Rh-negative pregnancies is 1.3 million per year. The
estimated prevalence of the Rh D gene in the popu-
lation is 0.78, thus accounting for approximately
1 million Rh-negative pregnancies at risk of deliver-
ing an Rh-positive baby. Based on the actual number of
Rh immunoglobulin distributed in the country, only
25% of these pregnancies are protected, estimating an
annual prevalence of Rh-HDN of 56 672 [2]. Our cen-
ter being a referral hospital for in utero management of
Rh-HDN has an annual delivery rate of approxi-
mately 50 such babies. In fact, in our study, almost
57% of the subjects required in utero transfusion in
both the arms (Table 1).

In view of the potential role of PB in reducing the
surges of serum bilirubin in various settings [3–8],
we believed its addition into the management protocol of Rh-HDN would have a beneficial effect in the reduction of serum bilirubin, translating into useful clinical outcomes, besides being a low-cost intervention. As there were no published studies on the use of PB in Rh-HDN, we chose the dosage and duration based on the studies that had demonstrated the efficacy of PB in other conditions like G6PD deficiency [3, 11–15]. A primary outcome of duration of phototherapy was considered, as we believed that this parameter would result in clinically useful outcome of lesser duration of hospital stay and early transfer of the infant to the mother.

Our study found that addition of PB to the available standard modalities of treatment consisting of IVIG, DVETs and intensive phototherapy in Rh-HDN did not result in the reduction of clinically relevant outcomes of duration of phototherapy, failure of phototherapy requiring DVETs or rebounds of bilirubin necessitating restart of phototherapy.

The classic studies by Peronar et al. [16] have shown clinically significant effects of PB emanating from 72 to 120 h after start of therapy. Similar effects were shown in rat models by Crigler, wherein endoplasmic reticular proliferation of hepatocytes, suggestive of increased enzyme induction, was noticed after PB administration [3]. The maximum effects were seen between 4 and 6 days of starting treatment in various studies, although PB was stopped before the effect of the maximal decline [3, 16, 17, 18, 19]. In view of this, we planned to administer the dose of PB for 5 days so as to strike an optimal duration of therapy with least side effects. It is possible that the cumulative dose of PB used in our study was lesser than required, as some studies that showed benefit had used higher doses (8 mg/kg/d [15] and 15 mg/kg/d [17, 18]) or longer duration of therapy (6 [18] and 7 days [19]). It is possible that a loading dose of PB of 20 mg/kg might have been useful. But in absence of studies using such high doses in settings of jaundice and possible side effects of high dosage, it was not used.

The published studies on the efficacy of PB in various settings had administration of PB as sole intervention, and its effects were measured in terms of requirement of DVET and duration/need of

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Fig. 1. Trial flow of the study. POG (period of gestation), PB (phenobarbitone).
However, in our study of babies with Rh-HDN, wherein the onset of hemolysis is *in utero*, DVET and phototherapy were part of the management protocol. In fact, almost 70% of the infants required DVET in the first 6 h (Table 1). These measures would have led to a rapid reduction of bilirubin load and could have eclipsed the potential effects of PB, which are normally apparent from the third day of treatment initiation. These measures of early DVET and phototherapy based on cut-off values of TSB at various time frames were essential for reduction of the existing bilirubin load to prevent neurotoxicity. In a study on the efficacy of PB in a setting of G6PD deficiency, Murki et al. found no effect of the drug on the need for phototherapy. The lack of effect in their study was cited probably due to late onset of action of PB, reaching a steady state at approximately 51 h, vs. an early onset of rise of TSB in a setting of G6PD deficiency. A similar mechanism may be a contributing factor in our study, as the etiopathogenesis of early hemolysis is common to both, except that Rh-HDN is a more severe disease than G6PD deficiency. As the onset of hemolysis is *in utero*, it is plausible that antenatal administration of the drug would be useful. In fact, antenatal administration of PB was found to reduce the need for exchange transfusions significantly in one study [20]. The effects of antenatal administration of PB could be secondary to its prolonged effects on the fetus in maturation of the hepatic UDPGT enzyme before delivery. However, these studies were retrospective and need further validation. The effect of PB is genetically mediated through its action on the PBREM region of the promoter region of the UDPGTIAI gene [21]. There is a possibility of genetic variation among study subjects resulting in varied response to a standard drug regimen.

We found a significant reduction in the development of cholestasis in the intervention group (Table 2). Given the biological plausibility of the mechanisms of action of PB in increasing the bile salt-independent bile flow, hepatic blood flow, secretion of bile acids, ligandin formation, formation of bile-acid glucuronides and stimulation of hydroxylatation of bile acids by microsomal cytochrome P450-dependent enzyme [4], this outcome stands to reason...

### Table 1
**Baseline characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phenobarbitone group (n = 23)</th>
<th>Placebo group (n = 21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICT titers</td>
<td>128 (16–512)</td>
<td>120 (4–512)</td>
<td>0.85</td>
</tr>
<tr>
<td>Antenatal IVIG</td>
<td>2 (8.7%)</td>
<td>2 (9.5%)</td>
<td>1.0</td>
</tr>
<tr>
<td>MCA PSV &gt;1.5 MoM</td>
<td>12 (52.2%)</td>
<td>13 (61.9%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Received IUTs</td>
<td>13 (56.8%)</td>
<td>12 (57.1%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Number of IUTs received</td>
<td>1 (0–8)</td>
<td>2 (0–4)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Neonatal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>16 (69.6%)</td>
<td>17 (80.9%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Gestation (wk)</td>
<td>34.8 ± 2.3</td>
<td>34.7 ± 1.9</td>
<td>0.85</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2393 ± 407</td>
<td>2293 ± 515</td>
<td>0.48</td>
</tr>
<tr>
<td>Birth weight groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1500 g</td>
<td>0</td>
<td>1 (4.7%)</td>
<td>0.87</td>
</tr>
<tr>
<td>1500–2499 g</td>
<td>13 (56.5%)</td>
<td>12 (57.1%)</td>
<td>0.87</td>
</tr>
<tr>
<td>&gt;2500 g</td>
<td>10 (43.4%)</td>
<td>8 (38%)</td>
<td>0.87</td>
</tr>
<tr>
<td>5-min Apgar score</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>0.66</td>
</tr>
<tr>
<td>Cord PCV (%)</td>
<td>33 ± 14</td>
<td>32 ± 14</td>
<td>0.83</td>
</tr>
<tr>
<td>Cord TSB</td>
<td>3.5 (0.3–7.6)</td>
<td>3.7 (1–10)</td>
<td>0.36</td>
</tr>
<tr>
<td>Requirement of DVET in first 6 h</td>
<td>15 (66.2%)</td>
<td>14 (66.7%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Presence of hydrops</td>
<td>4 (17.0%)</td>
<td>4 (19.0%)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Co-morbid conditions and interventions received</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory distressa</td>
<td>5 (21.7%)</td>
<td>5 (23.8%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Sepsisb</td>
<td>6 (26.1%)</td>
<td>4 (19%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Antibiotic administration</td>
<td>8 (34.8%)</td>
<td>6 (28.6%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>5 (21.7%)</td>
<td>3 (14.3%)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD, median (range) or number (percentage).

ICT, indirect Coombs test; IVIG, intravenous immunoglobulin; MCA PSV, middle cerebral artery peak systolic velocity; MoM, multiples of median; PCV, packed cell volume; PET, partial exchange transfusion; TSB, total serum bilirubin; DVET, double-volume exchange transfusion; IUT, intrauterine transfusion.

*a*Includes respiratory distress syndrome, pneumonia or transient tachypnea of new born requiring respiratory support.

*b*Clinical suspicion with positive screen for sepsis and/or positive blood culture.
but needs further studies, measuring this outcome primarily. This is further heightened by the fact that cholestasis in a setting of Rh-HDN is a relatively late phenomenon, which matches with the delayed onset of action of PB [1, 3, 16].

The strengths of the study are its robust study design with no loss to follow-up. In addition, our study was conducted in a tertiary teaching hospital, ensuring highest standard of care, and used multiple interventions based on current best available evidence. Our study has a few limitations—first, it was not powered enough to detect a small but significant difference in the incidence of failure of phototherapy or rebounds of TSB. Second, we did not estimate conjugated bilirubin in all the enrolled infants; it was estimated only in those who were suspected to have direct hyperbilirubinemia by the clinical team. However, the chance of measurement bias is almost negligible because the clinical team as well as the investigators were blinded to the nature of the intervention.

**Conclusions**

Addition of oral PB to a standardized protocol of management of Rh-HDN does not confer advantage...
in terms of reductions of total duration of phototherapy, episodes of failure of phototherapy or significant rebounds of serum bilirubin. Addition of PB, however, reduces cholestasis during the hospital stay. This clinical benefit needs to be validated in larger studies.

References