ORIGINAL ARTICLE

Efectiveness of ursodeoxycholic acid *vs* phenobarbital for the treatment of neonatal cholestasis: a cross-randomized clinical trial

Silvia Romero Maldonado[1], Noemí Caritina Godínez Téllez[2], Gabino Yescas-Buendía[3], Luis Alberto Fernán-Carrocera[3], Olga Leticia Echaniz-Avilés[4], and Edna Rocely Reyna Ríos[2]

Abstract

Background. The prevalence of neonatal cholestasis varies from 7–57%. Part of the treatment includes ursodeoxycholic acid (UDCA) and phenobarbital, both with little supporting evidence in the literature.

We undertook this study to compare the effectiveness of phenobarbital *vs* UDCA in reducing the direct serum bilirubin levels in patients with cholestasis and weighing from 1000 to 2000 g.

Methods. Using a cross-randomized clinical trial, 18 patients were included with 36 treatments. Each subject randomly received one of the two interventions: UDCA (10 mg/kg/day) every 12 h or phenobarbital (3 mg/kg/day, every 24 h for 7 days) continuing with 7 days of wash-out to return to their initial state and to subsequently receive the other treatment. At the beginning and at the end of the administration of each medication, bilirubin concentrations and hepatic test functions were measured. Central tendency and dispersion measurements were applied according to the type of variable. For hypothesis confirmation, paired t-test was carried out.

Results. The obtained results indicate that with UDCA at a dose of 10 mg/kg/day every 12 h for 7 days, serum bilirubin levels decreased to 2.7 mg/dL (p <0.01*)*. Phenobarbital had no effect in reducing bilirubin concentration.

Conclusion. Use of UDCA is recommended at a dose of 10 mg/kg/dose every 12 h (PO) as a coadjuvant in the treatment of neonatal cholestasis.

Key words: cholestasis, direct hyperbilirubinemia, ursodeoxycholic acid, phenobarbital.

Introduction

Cholestasis is clinically defined as the presence of direct bilirubin (DB) >2 mg/dL during the first 3 months of life.[1,2] Physiologically, it is a process in which there is a decrease of biliary flow with bile pigment deposits in hepatocytes and biliary ducts, favoring serum increase of DB.[3] The liver of the newborn has the disadvantage that the volume of biliary acids and the expression of the hepatobiliary carriers are decreased in comparison to older children and adults.[4] This hepatocellular dysfunction predisposes to hemorrhages, encephalopathy and hepatorenal syndrome in some cases, whereas the deficit of biliary salts may give rise to liposoluble vitamin deficits, osteopenia, hypocalcemia (vitamin D deficiency), malnutrition, hemorrhage (vitamin K deficiency), resistance to the action of growth hormone and peripheral neuropathy (vitamin E deficiency). It is

[1]Terapia Intermedia al Recién Nacido; [2]Ex-Residente; [3]Terapia Intermedia; [4]Neonatología, Instituto Nacional de Perinatología, México, D.F., México

Received for publication: 12-8-09
Accepted for publication: 4-14-10

also important to mention that biliary salt retention causes pruritus.[2,5-7]

The incidence of cholestasis varies between 7 and 57%, although the frequency of each individual disorder varies according to race and gender. The main causes are toxicity due to total parenteral nutrition (TPN),[8,9] idiopathic neonatal hepatitis (1/4,800-9,000), extrahepatic biliary duct atresia (1/8,000-18,000), lack of intrahepatic biliary ducts (1/70,000), 1-alpha-antitrypsin deficit in neonatal cholestasis (1/10,000-20,000), Byler disease (1/50,000-100,000), and choledochal cyst (1/13,000-2,000,000).[10]

At the Instituto Nacional de Perinatología (INPer) a 21% incidence of cholestasis was estimated in neonates who received TPN during the first 7 days and 31% for those who received it for >15 days.[11]

Management of cholestasis can be divided into three categories:

1) Specific treatment
2) Surgical
3) Unspecific and/or medical treatment (includes treatments with choleretics designed to improve bile flow and prevent or treat sequelae such as malnutrition)

The majority of patients with cholestasis benefit from this treatment regardless of the underlying disease.[9]

Phenobarbital is an inductor of microsomal liver enzymes and has a choleretic action by increasing biliary flow independent of the type of biliary salts.[5] It is not known if its capacity in reducing pruritus is due to its action on biliary flow or to its sedating effects. Oral dose is 3-5 mg/kg/day and the main secondary effects, which limit its use on cholestatic patients, are sedation and alteration of vitamin D metabolism. Gleghorn, in a retrospective study, concluded that phenobarbital is not useful for the treatment of cholestasis; however, the study design lacked methodological precision.[12]

In a prospective study, Blommer included 15 patients with cholestasis (without including controls) and used phenobarbital at a dose of 3-5 mg/kg/day. He concluded that phenobarbital decreases bilirubin number by 2 mg/dL per week and controls pruritus.[13] In 2004, Venigalla and Gourley suggested that phenobarbital be included for the treatment of cholestasis;[14] however, another review by the gastroenterology group of the Hospital Infantil de Mexico concluded that phenobarbital has not been consistently used for decreasing pruritus, suggesting that its use should be limited due to its sedating effects.

UDCA has a cytoprotective and stabilizing effect of the hepatocyte membrane due to the increase of hydrophilic biliary acids that replace the hydrophobics, which are hepatotoxic. It is also an inductor of biliary flow and reduces intestinal reabsorption of biliary acids, besides having an antipruritic effect.[15] The usual dose in pediatric patients is 10-15 mg/kg/day. It has few secondary effects, among which are nausea, vomiting, abdominal pain, constipation, gas, and diarrhea, with the latter being the most frequent.

The existing literature on UCDA is based on reports focused on pediatric and adult populations.[13-15] Neonatal literature lacks scientific rigor because most reports have no controls or the controls are historical.

Treatment with UDCA may be tolerated in infants and there may be a significant effect in reducing DB levels.[16] The first reports of UDCA use were conducted in Japan in 1957 as therapy for different types of liver diseases. Chen-Yi et al. carried out a retrospective study that included 30 patients: 12 treated with UDCA and 18 controls; patients who received UDCA at doses of 10-30 mg/kg/day had cholestasis for fewer days (62.8 vs. 92.4, 0.006).[17]

Spagnuolo et al. conducted a pilot study that included a total of seven patients: four boys and three girls who had UDCA administered. There were no controls and the author found that serum bilirubin disappeared after 4 weeks in all patients.[18] Al-Hathlol et al. performed a retrospective study that included 13 patients with intractable cholestasis secondary to TPN. The authors used UDCA at doses of 15-20 mg/kg/day and found a reduction of serum bilirubin with a value of $p \leq 0.0001$, as well as the aminotransferase enzyme. No side effects were reported.[19]

Cholestasis associated with TPN continues being one of the most common complications. In fact, it is a limiting factor in the use of this resource. Only catheter-associated complications surpass it in frequency.[20,21] For this reason, we posed the question, can phenobarbital and UCCA be equally effective in the treatment of neonatal cholestasis associated with TPN? To answer the question, we aim to determine the effectiveness of phenobarbital and UDCA to reduce the numbers of DB in preterm infants of 1000-2000 g birth weight who had TPN and who developed cholestasis.

Patients and Methods

In a tertiary level hospital of the Department of Health, during the period from July 1, 2004 to June 31, 2005 a randomized, cross-over clinical trial was carried out with sequential controls. Included in the study were all premature patients born in the institution who received TPN for at least 2 weeks and who developed cholestasis (direct bilirubin ≥2 mg/dL, weight 1000-2000g during the first 15 days of life, without congenital malformations of the digestive tract, who received ≥100 ml/kg/day of milk via enteral route, and with written consent of the responsible family member. Excluded were those patients with intraventricular hemorrhage (IVH) grade III and IV and patients who developed seizures. All patients had liver ultrasound, neonatal screening, TORCH complex titration performed to rule out other causes of cholestasis. Patients with innate errors of metabolism were eliminated. All patients were managed with TPN 24 h after their birth as per the institutional norms: proteins 3 g/kg/day (aminoacids at 10%), glucose 6 mg/kg/min, lipids 1.5 g (lipids at 20%), calcium gluconate at 200 mg/kg/day, trace elements 0.3 mL, multivitamins 2 mL/kg/day, magnesium 0.5 mg/kg/day.

Phenobarbital (in tablet form) was prepared by the hospital service, at a concentration of 3 mg per 0.5 mL, as well as UDCA prepared in envelopes of 5 mg. For monitoring of phenobarbital serum concentrations were taken at 5 half lives. All had weekly determinations of total and direct serum bilirubin, alkaline phosphatase, alanine-aminotransferase, aspartate-aminotransferase, γ-glutamyl transpeptidase. Also recorded were gestational age, weight, height, days of fasting, days of start, and days of TPN, diagnoses such as sepsis, neurological, digestive, respiratory, metabolic disorders, anatomical problems, toxic medications. The definition of cholestasis was considered to be a serum direct bilirubin of ≥2 mg/dL.

Statistical analysis was performed in the following manner: for continuous quantitative variables, average and standard deviation; for nominal variables, percentages and for ordinals, mean. Paired t-test was used for testing hypothesis.

The size of the sample was calculated on the basis of the formula of bioequivalents and taking into account the study by Blommer and Boyer[13] who found a decrease of bilirubin of 2 mg/dL/week.

$$N = (\sigma)^2(Z\infty + Z\beta)^2/d^2$$

where N = is the size of the sample for each group of treatment, σ = standard deviation of the effectiveness of treatment (0.45), $Z\infty$ = 1.64, $Z\beta$ = 1.28, d = is the difference between

the effects of normal and new treatment that are considered clinically significant =15% (2 x 0.15 = 0.3). Therefore,

$$N = (0.45)^2(1.64 + 1.28)^2/(0.3)^2 = 19.1 \text{ patients}$$

The analysis was performed using the SPSS program v. 12 for Windows. Of 22 patients who initially met the criteria of cholestasis, four were eliminated in the first phase of the study: two when they were included with the phenobarbital group (one for presenting seizures and the other was discharged) and the other two when being treated with UDCA (one had seizures and another secondary to catheter sepsis).

The group was composed of 18 patients (36 treatments) who received both drugs at different times. In the first intervention, ten patients received phenobarbital and eight patients received UDCA. In the second intervention, the 10 patients who started with phenobarbital later received UDCA and the other eight patients received phenobarbital. A description and comparison of patient characteristics was performed at both times to assess their condition at the time of receiving treatment.

Coadjuvant treatment for all patients was similar, taking into consideration the INPer institutional guidelines. Once cholestasis was diagnosed, patients were treated with human milk and/or hydrolyzed protein and soluble vitamins A, E, D and K, and vitamin C were administered. There was no need to modify the TPN because at the time of admission to the study the patients were receiving a total of 100 ml/kg/day of enteral nutrition.

Results

The group was comprised of 18 patients (36 treatments). There was no difference regarding demographic characteristics of the patients during the study period (Table 1). Of the group of

Table 1. Description of the study population

Initial group	Phenobarbital	UDCA	*p*
Phase I			
Gender			0.5
• Male	6	4	
• Female	3	5	
Weight (g)			0.5
• Average	1630.5	1670.1	
• SD	±475	±420	
Gestational age (weeks)			0.5
• Average	32.6	33.1	
• SD	±2.53	±2.6	
Days of fasting			0.5
• Average	10.57	11.11	
Days of TPN	21.43 ± 3.2	20.3 ± 2.3	0.5
Bilirubin (mg/dL)	5.4 ±2.63	6.65 ±2.5	0.05
Phase II			
Gender			0.5
• Male	4	6	
• Female	5	3	
Weight (g)	1890 ±900	1 905 ±400	0.5

UDCA, ursodeoxycholic acid; SD, standard deviation; TPN, total parenteral nutrition.

patients, 95% were born via cesarean section, 80% of the patients had varying degrees of respiratory distress, average days of ventilation was 12.9 ± 6.3, the average time of initiation of TPN was 40 ± 8 h and the average duration was 20.3 ± 8.7 days. Average time of initiation of enteral feeding was 12.23 ± 1.2 days. One patient from each group had sepsis, with no statistical difference.

The results obtained from the treatment maneuvers indicate that phenobarbital at a dose of 3 mg/kg/day administered every 24 h enterally for 1 week had no effect in reducing the numbers of DR. On the contrary, during the first phase of treatment results showed a slight increase in the numbers of DB (Table 2). When the analysis of the 18 treatments was done and compared with the UDCA, the lack of decrease in the numbers for DR was again shown.

Serum concentration of phenobarbital was 19 ± 7 µg/dL. Patients did not receive sedation or have other secondary effects. With the UDCA, a dose of 10 mg/kg/day divided in two doses, administered enterally, a reduction in the serum bilirubin figures was observed, with a statistically significant difference ($p \leq 0.01$) (Table 3). There were no secondary effects with this medication. Cumulative overall and reported incidence of all the processes that cause cholestasis vary between 7 and 57%, although the frequency of each individual disorder varies by race and gender.

Technological advances in neonatal therapies lead to routine use of TPN. However, prolonged use produces complications, among which is cholestasis and is considered the second cause in frequency of complications of TPN.[22]

The results obtained in the present study indicate that there is a significant reduction in the numbers of bilirubinemia utilizing UDCA in comparison with phenobarbital, with a statistically significant difference. These results are in agreement with other reports such as that of Chen-Yi et al.[17] and more recently that of Al-Hathlol et al. [19] in which despite the controls being historic, which report satisfactory results without secondary effects.[19] Other reports, despite the small sample size, also conclude that they are cost-beneficial treatments for their use.[23,24]

Regarding the UCDA dose utilized, it is not known if there is a dose response effect considering that there are reports in which a dose of 15-20 mg/kg/day is used, or as that of Spagnuolo et al. who used doses as high as 30 mg/kg/day without reporting secondary effects in the seven patients treated. The controversy is due to the size of the sample and to the fact that there were no controls.[20]

Regarding liver enzymes, the present study was not designed to analyze such effect. However, other authors report improvement in liver enzymes after a treatment period of 4-8 weeks.[19,20] We did not find these results in the present investigation

Table 2. Concentrations of direct bilirubin (mg/dL) in the study groups during the first phase of treatment

Intervention	Average ± SD	Average ± SD	*p*
Phase I	**Initial**	**Final**	
• Phenobarbital	5.43 ± 2.63	6.07 ± 3.7	0.5
• UDCA	6.65 ± 3.28	4.36 ±1.13	0.01
Wash-out phase			
Phase II			
• UDCA	4.4 ± 2.1	2.7 ± 0.91	0.05
• Phenobarbital	6.05 ± 2.50	5.7 ± 0.91	0.3

UDCA, ursodeoxycholic acid; SD, standard deviation.

Table 3. Final concentrations of bilirubin (mg/dL) after cross-over treatments

Treatment (*n* =18)	Concentrations Average ± SD	*p*
Phenobarbital	-1.67 ± 3.70	0.38
UDCA	2.29 ± 2.29	0.010

UDCA, ursodeoxycholic acid; SD, standard deviation. Paired t test was used for differences of concentrations before and after treatment. Statistical significance was considered when $p \leq 0.05$.

because the intent was to demonstrate the early effect in reduction of DB, which was achieved with UDCA and not with PBB. It is important to mention that once the treatment protocol was concluded, the patients continued with conventional treatment and both medications until conclusive results of the project were obtained.

Regarding phenobarbital, although it is mentioned as treatment for cholestasis,[14] a dose of 3 mg/kg/day as a solitary dose for 7 days did not show any effect on the reduction of DB in the results of the present study, which is in agreement with the data by Gleghorn et al.[12] Therefore, we cannot suggest its use for lowering numbers of DB. However, a doubt exists whether at higher doses it may have some beneficial effect. In a study by Blommer and Boyer[13] who report using the dose of 3-5 mg/kg/dose and to found a reduction in the levels of DB. For this medication there are no figures reported of toxicity or sedation in the patients.

It is important to mention that there is a lack of studies of choleretic medications with adequate methodology in the neonatal stage, and those that exist are focused on the pediatric or adult population.[19] Therefore, we consider that the present study represents a great advance in the support of the pharmacology of the medication, which has been studied by Williams who described the action mechanisms of UCDA, which highlights its cytoprotective effect due to incorporation of the cellular membrane.[19]

The therapy stabilizes and protects due to the choleretic effect and provides biliary flow. It induces excretion of biliary acids, favoring the reabsorption of biliary acids in the intestinal lumen as well, decreasing the intracellular concentration of the toxic biliary acids.

In conclusion, UDCA at a dose of 10 mg/kg/dose every 12 h administered enterally for 7 days may decrease figures of DB in patients with neonatal cholestasis secondary to TPN. In the present work, the size of the effect was 2.29 mg/dL. No secondary effects were reported such as vomiting or constipation, making it a safe alternative for patients with neonatal cholestasis. Even when surgical patients are not included, UDCA may be useful for treatment of cholestasis in these patients once oral feeding has begun. Phenobarbital at a dose of 3 mg/kg/day administered orally had no effects on the reduction of DB. However, a doubt remains if at elevated doses it would have some effect on the reduction of bilirubin, taking into consideration that a higher dose may have a secondary sedating effect, which is its principal limiting factor.

Correspondence to: Dra. Silvia Romero Maldonado
Terapia Intermedia al Recién Nacido
Instituto Nacional de Perinatología
México, D.F., México
E-mail: silviarmzeta@yahoo.com.mx

References

1. Peden VH, Witzleben CL, Skelton MA. Total parenteral nutrition. J Pediatr 1971;78:180-181.
2. Balistreri WF. Liver disease in infancy and childhood. In: Schiff ER, Sorrell MF, Madrey WC, eds. Schiff¨s Disease of the Liver. Philadelphia: Lippincott-Raven, 1999; pp. 1357-1512.
3. Worona-Dibner LB, García-Aranda JA. Colestasis neonatal. Bol Med Hosp Inf Mex 2003:60;334-348.
4. Karpen SJ. Update on the etiologies and management of neonatal cholestasis. Clin Perinatol 2002;29:159-180.
5. Suchy FJ. Approach to the infant with cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. Philadelphia: Lippincott, Williams and Wilkins, 2001; pp.187-194.
6. Young TE. Manual de drogas neonatológicas. In: Neofax, Editorial Panamericana, 2002.
7. Bernstein J, Chang CH, Brough AJ, Heidelberger KP. Conjugated hyperbilirubinemia in infancy associated with parenteral alimentation. J Pediatr 1997;90:361-367.
8. Coger RH. Hepatic complications of parenteral nutrition. Semin Liver Dis 1983;3:216-224.

9. Moss RL, Das JB, Raffensperger JG. Total parenteral nutrition-associated cholestasis: clinical and histopathologic correlation. J Pediatr Surg 1993;28:1270-1274.
10. Manzanares L, Medina E. Colestasis en el recién nacido y lactante: orientación diagnóstica. Ann Pediatr 2003;58:162-167.
11. Romero-Maldonado S. Incidencia de colestasis neonatal asociada a nutrición parenteral total (Tesis). Instituto Nacional de Perinatología, México D.F., 2002.
12. Gleghorn EE, Merrit RJ, Subramanian N, Ramos A. Phenobarbital does not prevent total parenteral nutrition-associated cholestasis in noninfected neonates. J Parenter Enteral Nutr 1986;10:282-283.
13. Blommer JR, Boyer JL. Phenobarbital effects in cholestatic liver disease. Ann Int Med 1975;82:310-317.
14. Venigalla S, Gourley GR. Neonatal cholestasis. Semin Perinatol 2004;28:348-355.
15. Kapen S. Update on the etiologies and management of neonatal support in patients with chronic cholestasis disease. Ann Pediatr 2003;58:174-180.
16. Pegev RH. Tratamiento de colestasis neonatal en el síndrome de Dubin Johnson. Rev Esp 2004;96:60-73.
17. Chen-Yi C, Po-nen T, Huey-Ling C, Hung-Chen C, Wu-Shun H, Mei-Hwei C. Ursodeoxycholic acid (UDCA) therapy in very low birth weight infants with parenteral nutrition associated cholestasis. J Pediatr 2004;145:317-321.
18. Spagnuolo MI, Iorio R, Vegnete A, Guarino A. Ursodeoxycholic acid for treatment of cholestasis in children on long-term total parenteral nutrition: a pilot study. Gastroenterology 1996;111:716-719.
19. Al-Hathlol K, Al-Madani A, Al-Saif S, Abulaimoun B, Al-Tawil K, El-Demerdash A. Ursodeoxycholic acid therapy for intractable total parenteral nutrition-associated cholestasis in surgical very low birth weight infants. Singapore Med J 2006;47:147-151.
20. Rodgers BM, Hollenbeck JI, Bonnelly WH, Talbert JL. Intrahepatic cholestasis with parenteral alimentation. Am J Surg 1976;13:14-55.
21. Pereira GR, Sherman MS, DiGiacomo J, Ziegler M, Roth K, Jacobowski D. Hyperalimentation-induced cholestasis and severity in premature infants. Am J Dis Child 1981;135:842-845.
22. Benjamin DR. Hepatobiliary disfunction in infants and children associated with long-term total parenteral nutrition. A clinicopathologic study. Am J Clin Pathol 1981;76:276-283.
23. Scher H, Bishop WP, McCray PB. Ursodeoxycholic acid improves cholestasis in infants with cystic fibrosis. Ann Pharmacother 1997;31:1003-1005.
24. Pasha T, Heathcote J, Gabriel S, Cauch Dudek K, Jorgensen R, Therneau T, et al. Cost effectiveness of ursodeoxycholic acid therapy in primary biliary cirrhosis. Hepatology 1999;29:21-26.