

Early treatment with ursodeoxycholic acid for cholestasis in children on parenteral nutrition because of primary intestinal failure

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Publication data

Submitted 13 September 2005

First decision 3 October 2005

Resubmitted 29 March 2006

Resubmitted 24 April 2006

Accepted 25 April 2006

SUMMARY

Background

There is conflicting evidence as to whether ursodeoxycholic acid (UDCA) reduces the incidence of parenteral nutrition-associated cholestasis.

Aim

To investigate the efficacy of UDCA on parenteral nutrition-associated cholestasis in children with intestinal failure due to short bowel syndrome or to other causes.

Methods

Children with cholestasis received 30 mg/kg/day UDCA. Improvement or normalization of parenteral nutrition-associated cholestasis was evaluated at 6 months of therapy and at the last follow-up. In a subgroup of children, serum UDCA levels were measured while receiving UDCA and after 4 weeks withdrawal.

Results

Twelve children were treated with UDCA. Full remission or partial improvement of parenteral nutrition-associated cholestasis occurred in 11 of 12 children. In three of four children, withdrawal of UDCA was associated with a rebound rise of cholestasis. Only one of 12 treated children showed no improvement and in this patient, in contrast to four other patients, plasma levels of UDCA did not increase during treatment.

Conclusions

Ursodeoxycholic acid was effective in controlling parenteral nutrition-associated cholestasis. The efficacy of UDCA also in children with short bowel is related to intestinal absorption.

Aliment Pharmacol Ther 24, 387–394

INTRODUCTION

Irreversible intestinal failure (IF) is associated with permanent loss of IF as a result of short bowel syndrome (SBS) or other primary intestinal conditions.¹⁻³ Children with IF need parenteral nutrition (PN) for survival and growth. The only alternative is intestinal transplantation, which entails high mortality and enormous costs.³ Although a life-saving option, PN is associated with a number of severe complications, including PN-associated cholestasis (PNAC). PNAC may progress to irreversible liver failure, which is the most frequent indication for intestinal transplantation in children.⁴ Patients with the greatest degree of anatomic or functional bowel loss are considered at increased risk of PNAC; stoma termination of small bowel and absence of enterocolonic continuity are other risk factors.^{5, 6}

Several measures have been proposed to prevent or treat PNAC. Enteral feeding protects the liver by promoting enterohepatic recirculation of bile acids, particularly if started early and if an ileum segment is left after intestinal resection.⁷ Other interventions are related to the modes, times and composition of nutrient administration, particularly of lipids.^{3, 8} Ursodeoxycholic acid (UDCA) has been proposed for the treatment of PNAC.⁹⁻¹¹ There is some doubt about the efficacy of UDCA in cases of PNAC, and conflicting results have been obtained in neonates.^{12, 13} UDCA efficacy in children with SBS-related IF has been questioned because of its main absorption occurring in the terminal ileum, even though passive non-ionic diffusion was demonstrated in the small intestine.^{14, 15}

We evaluated the incidence of PNAC in a population of children with IF receiving PN, and tested the efficacy of UDCA in the treatment of PNAC in two groups of children with IF, those with SBS and those with other primary intestinal diseases and normal intestinal length.

MATERIALS AND METHODS

Population and definitions

All children admitted to our Unit from January 1997 to June 2004 because of IF were prospectively enrolled in this open-label trial. Intestinal failure was defined as a clinical condition resulting from a primary intestinal disease, for which PN is needed and provided at

least 75% of the total caloric input for not <4 weeks, or at least 50% of calories for not <3 months.² Based on IF aetiology, children were divided in those with SBS and non-SBS related-IF (NSBS). The IF classification system is reported elsewhere together with diagnostic procedures and PN protocols.²

The following data were recorded for each child: primary aetiology of IF, age at IF onset, age at start of PN and presence of stoma. Residual small bowel length and presence of ileocecal valve were also recorded in children with SBS. The number of catheter-related sepsis (CRS) episodes was recorded for each child and expressed as CRS per 1000 catheter days, referred to the entire follow-up period. CRS was defined as a septic episode with positive central line blood culture or for which a specific antibiotic treatment for CRS was administered.

Serum levels of gamma-glutamyl transpeptidase (γ GT), alanine aminotransferase (ALT), alkaline phosphatase (ALP), conjugated bilirubinaemia and albumin were measured and coagulation tests were performed upon hospital admission and monthly thereafter. The diagnosis of PNAC was based on increased levels of hepatobiliary enzymes (γ GT, ALP and ALT, hereafter 'PNAC parameters'; normal values: γ GT < 70 U/L; ALP < 1000 U/L; ALT < 60 U/L) with or without conjugated hyperbilirubinaemia.^{11, 16} Cholestasis was related to PN when it occurred after initiation of PN and when the following causes were excluded: active liver infections, primary metabolic disorders, cystic fibrosis, autoimmune diseases, neoplastic diseases, biliary tract diseases including cholelithiasis and bacterial cholangitis, and toxic injury other than that induced by PN.¹¹

UDCA therapy for parenteral nutrition-associated cholestasis

All children with PNAC were prospectively enrolled to receive UDCA. Exclusion criteria were: (i) modifications in the parenteral/enteral ratio of nutrient intake >10% in the previous 4 weeks and (ii) clinical signs and symptoms of intestinal obstruction requiring total parenteral feeding, and not allowing drug administration through the oral route. Informed consent was obtained for each patient. UDCA was administered at 30 mg/kg/day in three equal oral doses. UDCA was administered in tablets formulation, and ground to powder. In all treated children, γ GT, ALT, ALP and conjugated bilirubin were monitored weekly for the

first 2 weeks and then monthly for at least 6 months. The following parameters were evaluated after 6 months of treatment: (i) normalization of PNAC parameters; (ii) improvement of PNAC, defined as a reduction of at least 50% of all PNAC parameters for at least 3 months; (iii) incidence of hepatic failure. Time-to-response was also evaluated. At the last follow-up, all children with PNAC were evaluated for incidence of PNAC, evidence of liver failure and continuation of, or weaning from, PN.

Serum UDCA analysis

In a subgroup of five children, fasting concentrations of UDCA were retrospectively determined in sera collected during treatment and after a period of 4 weeks withdrawal.

Serum UDCA levels were determined by a specific solid-phase enzyme immunoassay with an UDCA-specific polyclonal antibody immobilized on 96-well polystyrene microtitre plates. A horseradish peroxidase (HRP)-UDCA conjugate was used as enzymatic tracer. For the UDCA assay, 100 μ L of serum diluted 1/50 (v/v) with assay buffer and six standard UDCA solutions with a concentration ranging from 0.1 to 100 μ M was incubated in the 96-well microtitre plates tube coated with the antibody for 1 h at 37 °C with 100 μ L of the HRP-UDCA tracer. The enzymatic activity of the tracer was measured spectrophotometrically at 492 nm using H₂O₂/1,2-phenyldiamine (OPD) as a substrate chromogen. Total serum UDCA were expressed as μ M of serum. The used enzyme immunoassay fulfils all the standard requirements of accuracy and precision with coefficient of variation always below 9%. The limit of quantification is 0.1 μ M and this allows detection of serum UDCA in 1/10 (v/v) diluted serum samples.

The assay is specific for both unconjugated, glycine- and taurine-conjugated UDCA.¹⁷

Data presentation and statistical analysis

Age at IF onset and age at starting PN are expressed as mean values and ranges. Levels of γ GT, ALT and bilirubin are expressed as mean \pm S.E.M. The incidences of PNAC in SBS and NSBS, and outcome parameters in children treated or not with UDCA were comparatively analysed by the chi-squared test. Duration of PN in SBS vs. NSBS was compared by the Student's *t*-test. Multivariate and univariate analyses were used to

investigate the association between specific conditions and the risk of developing PNAC and response to UDCA treatment. For stepwise multiple logistic regression analysis, *P*-values of 0.05 and 0.1 were chosen as cut-off points, respectively, to enter and exit the stepwise procedure. Odds ratios with a 95% confidence interval (CI) were computed. Differences were considered significant at the 5% level. Statistical analyses were performed with SPSS version 11.5.

RESULTS

Incidence of parenteral nutrition-associated cholestasis

Twenty-six children with IF were prospectively enrolled in the study period. Of these, 16 needed PN because of SBS and 10 because of NSBS. The aetiologies are listed in Table 1. Age at onset of PN was 11.1 months (range: 0–134) for SBS and 5.4 months (range: 0–21) for NSBS. PNAC developed in 16 of 26 (62%) children. The incidence of PNAC did not differ between SBS nine of 16 (56%) and NSBS seven of 10 (70%) children. Multivariate and univariate analyses showed that none of the following factors were associated with an increased risk of cholestasis in our population of children with IF: aetiology of IF, number of CRS >1 or >3 per 1000 days with a central line, start of PN in neonatal age, presence of ileocecal valve, residual small bowel length <20 cm or <50 cm, presence of stoma. Precholestasis duration of PN was 1.9 months (range: 0–5.4) for SBS and 4.2 months (range: 1–13) for NSBS (*P* = N.S.).

Effect of UDCA treatment

Four of the 16 children with PNAC did not receive UDCA: two were weaned from PN shortly after its onset

Table 1. Aetiologies of 26 consecutive children with intestinal failure enrolled from January 1997 to June 2004

Aetiology	Number (%)
Short bowel syndrome	16 (61)
Chronic intestinal pseudo-obstruction	5 (19)
Microvillus inclusion disease	1 (4)
Primary bile acid malabsorption	1 (4)
Unknown	3 (12)

and cholestasis resolved spontaneously, and two presented with severe intestinal obstruction which precluded oral administration of any drug. Thus, 12 of 16 children with persistent PNAC were enrolled to be treated with UDCA (seven SBS and five NSBS). Children were in stable condition and they did not show modifications in the parenteral/enteral ratio of nutrient intake >10% during the study period. Table 2 shows the general features of children receiving UDCA therapy.

The mean level of γ GT, ALT and direct bilirubin was higher in SBS than in NSBS children, but there was no significant difference in any of these parameters. UDCA treatment was associated with a substantial decrease in γ GT, ALT and direct bilirubin serum concentration in both groups (Figure 1). The biochemical response was more pronounced and occurred sooner in the SBS group (Figure 1). Analysis of outcome parameters showed an improvement or normalization of cholestasis in 11 of 12 treated children. UDCA had no effect in one child (#6, Table 2) with 13 cm of residual small bowel, a jejunal-abdominal stoma and without the ileocecal valve and colon. A summary of the study is illustrated in Figure 2.

The mean time-to-improvement and -normalization of cholestasis in the SBS group was 2.1 months (range:

1–4) and 3.3 months (range: 2–4) respectively. The times-to-response in the NSBS group were slightly longer, being 2.0 months (range: 1–2) and 3.3 months (range: 2–5) respectively. However, the differences between SBS and NSBS were not significant.

In four children (#1, #3, #7 and #8 of Table 2), UDCA was withdrawn after normalization of PNAC. A rebound rise of cholestasis markers occurred in three of four cases, and levels returned to normal upon resumption of UDCA (Figure 3).

We investigated whether risk factors were associated with the response to UDCA treatment. Neither improvement nor normalization of PNAC was related to aetiology of IF, number of CRS, age at start of PN, duration of PN before PNAC, presence of ileocecal valve, residual small bowel length <20 cm or <50 cm, presence of stoma.

Finally, we evaluated the long-term outcome of children with IF who developed PNAC. The two children with persistent PNAC who did not receive UDCA (one with SBS and one with NSBS) eventually developed liver failure; one died and another is still on PN and on the list for combined liver-intestine transplantation. Among the 12 children who received UDCA, after a mean follow-up of 31.3 months (range: 7–105),

Table 2. Features of children with SBS-related IF and NSBS IF, and with PN-associated cholestasis treated with UDCA

Patient	Age at PN onset (months)	IF aetiology	Total follow-up (months)	Residual small bowel length (cm)	Presence of stoma	Presence of ICV	Number of CRS	Presence of PNAC at last visit on PN	Outcome
#1	0	SBS	32	20	Yes	Yes	2.1	No	Weaned
#2	8	SBS	28	20	Yes	Yes	5	No	Weaned
#3	0	SBS	32	55	No	Yes	8.9	No	Weaned
#4	6	SBS	13		No	No	1	No	Weaned
#5	0	SBS	25	85	No	No	7.7	No	Weaned
#6	0	SBS	12	15	Yes	No	19.4	Yes	PN
#7	134	SBS	11	60	Yes	No	10.9	No	PN
#8	21	Intractable diarrhoea	105				1.1	No	Died
#9	0	Microvillus inclusion disease	7				0	No	Died
#10	2	Intractable diarrhoea	21				11.1	No	Weaned
#11	6	Chronic intestinal pseudo-obstruction	16					No	Died
#12	1	Bile acid malabsorption	74				0.46	No	Weaned

SBS, short bowel syndrome; NSBS, non-short bowel syndrome; PN, parenteral nutrition; IF, intestinal failure; ICV, ileocecal valve; CRS, catheter-related sepsis (expressed as total *N*/1000 catheter days, calculated for the entire follow-up period); PNAC, parenteral nutrition-associated cholestasis; UDCA, ursodeoxycholic acid.

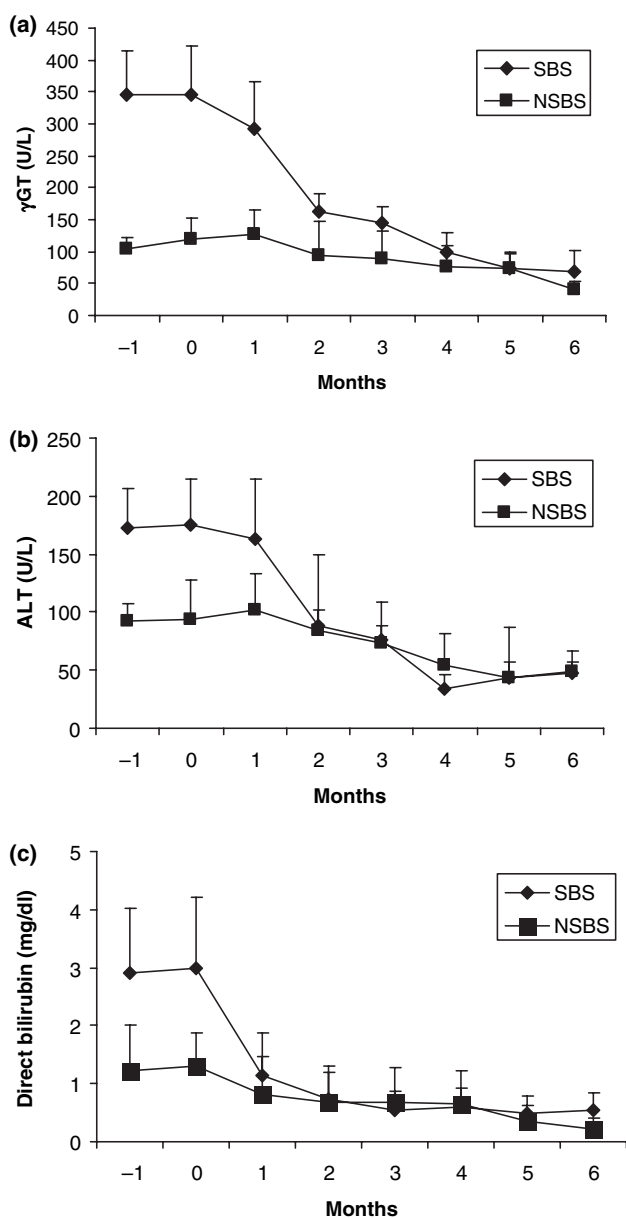


Figure 1. Serum levels of cholestasis markers in children with parenteral nutrition-associated cholestasis (PNAC) treated with ursodeoxycholic acid (UDCA). Each marker was monitored at 1-month intervals in children with short bowel (◆) and in children with other aetiologies (■). Data refer to 1 month before UDCA administration up to 6 months of therapy. Data represent mean + S.E. Mean levels of gamma-glutamyl transpeptidase (γGT; a), alanine aminotransferase (ALT; b) and direct bilirubin (c) are reported.

seven were eventually weaned from PN, two are still on PN, three died and one (#6) still shows signs of PNAC (Table 2).

Ursodeoxycholic acid therapy was well tolerated, and no serious adverse effects requiring an early drug interruption were observed.

Serum UDCA concentrations

In order to determine if the efficacy of UDCA administration was associated with its absorption, we performed serum UDCA measurements in five children, four with SBS and one with NSBS. During treatment, serum UDCA concentrations were distributed within a wide range (0.1–48 μM). Four of five patients showed substantially higher levels compared with the trace amounts found in most of them after treatment discontinuation (Table 3). Interestingly, the child with very low serum concentration during UDCA administration was the only one of this subset of patients who did not respond to therapy (#6, Tables 2 and 3).

DISCUSSION

Parenteral nutrition-associated cholestasis is a major issue in the management of children with IF receiving long-term PN. The aetiology and pathophysiology of PNAC are unknown. Early work implicated nutrient composition in PNAC, but recent evidence points to an IF-related proinflammatory state that promotes hepatic inflammation and fibrosis.¹⁸ Moreover, liver damage is considered more severe in patients with severe gut resection.⁵

We previously reported that UDCA was effective in children with IF.¹¹ The population enrolled in our previous study included five children with intractable diarrhoea and two with SBS but, differently from the present study, none of those children was receiving enteral nutrition.¹¹ In the present prospective open-label trial, we investigated the efficacy of UDCA in the treatment of PNAC in children with SBS and in children with IF due to causes other than SBS.

The incidence of PNAC in our 26 consecutively hospitalized children with IF was 62%, which is consistent with former studies.^{19–21} Although it has been suggested that stoma termination of small bowel and absence of enterocolonic continuity may place patients at further risk,⁶ we did not find a significant association between these or any other risk factors and the occurrence of PNAC. However, our sample size was not large enough to either support or rule out a link between risk factors and PNAC.

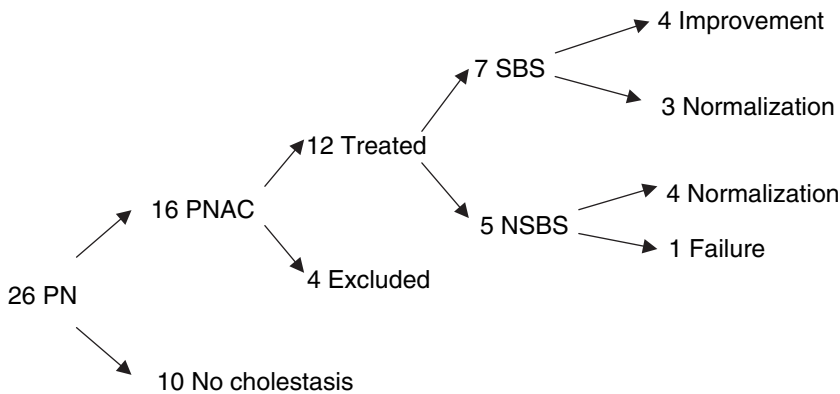


Figure 2. Flow chart of the study population and main outcomes of children treated with ursodeoxycholic acid (UDCA) according to their aetiology.

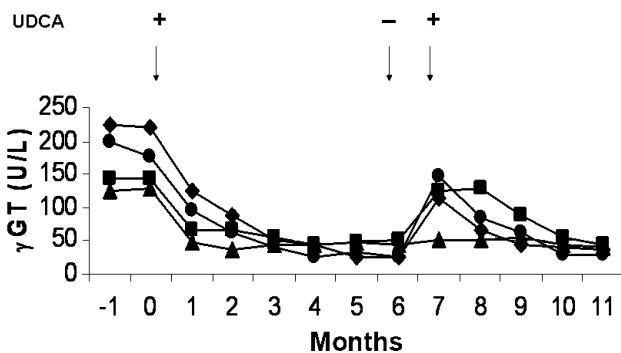


Figure 3. Rebound rise of gamma-glutamyl transpeptidase (γ GT) levels after ursodeoxycholic acid (UDCA) withdrawal. In four children receiving UDCA with normalization of γ GT levels, therapy was stopped and there was an immediate rise of cholestasis. In three of four children resumption of UDCA administration was effective on γ GT levels. +: start of UDCA administration; -: stop of UDCA administration.

Table 3. Serum UDCA determination in children with IF

Patient	IF aetiology	Serum UDCA concentration (μ M/L)		PNAC response to UDCA
		On therapy	Off therapy	
#1	SBS	5.2	0.2	Yes
#3	SBS	2.7	0.0	Yes
#6	SBS	0.1	0.0	No
#7	SBS	34	0.4	Yes
#8	NSBS	48	0.0	Yes

IF, intestinal failure; PNAC, parenteral nutrition-associated cholestasis; SBS, short bowel syndrome; NSBS, non-short bowel syndrome; UDCA, ursodeoxycholic acid.

Ursodeoxycholic acid is effective in the treatment of cholestatic liver diseases,^{22, 23} and it has been suggested for treatment of PN-related cholestasis.^{9-11, 24-26}

Heubi *et al.* reported that the taurine conjugate of UDCA is not effective against PNAC in preterm neonates receiving PN for any reason.¹² However, in a recent study, UDCA induced recovery from PNAC in very low birth weight infants receiving PN.¹⁹

We used a relatively high dose of UDCA in 12 prospectively enrolled children with PNAC. Cholestasis improved or normalized in 11 of these children; only one had no response after 6 months.

Ursodeoxycholic acid enrichment was documented in serum in four of five children, suggesting that intestinal absorption of this bile acid by passive non-ionic diffusion had occurred mainly in the small intestine and to a small extent in the colon.^{14, 15} The only child with very low serum concentration during UDCA administration was the one with the shortest residual small bowel length and absence of ileocecal valve, and the only one in this subset of patients who did not respond to therapy.

In three of four children, withdrawal of UDCA resulted in a rebound rise of cholestasis which was controlled by resuming UDCA. This observation suggests that, at least in some cases, normalization of cholestasis was not an adaptive phenomenon but rather a specific response to UDCA and dependent on continued administration of the drug.

Key issues in the debate on UDCA efficacy are the response rate and the long-term efficacy of UDCA.¹³ Considering the children enrolled in this study, UDCA lowered the final incidence of PNAC to one in 24 (3%), whereas the reported range is 7-60%.¹⁹⁻²¹ The response rate exceeded 90% and the long-term efficacy was excellent. A long-term follow-up showed persistence of PNAC in only one of 12 treated children.

The two children who were unable to receive UDCA developed severe PNAC and progressive liver failure. They also had the additional risk factor of being not fed enterally because of severely impaired motility.

Despite of the small sample population and the lack of a placebo-controlled design, we found that UDCA was almost invariably effective in children with PNAC already on some enteral nutrition, and that UDCA is as effective in children with SBS as in children with normal intestinal length. The efficacy of enterally administered UDCA is controversial because of the possibility of poorer absorption, particularly in children lacking the terminal ileum, which is the elective bile salts absorption site.^{7, 12} Here, we demonstrate that UDCA is effective also in children with extensive small bowel loss, and that absorption occurs even in children with an extreme reduction of functioning intestinal surface. It is noteworthy that we started UDCA treatment immediately we found an increase in γ GT level, the earliest and most sensitive marker of cholestasis, without waiting for an increase of bilirubin. Moreover, we used high UDCA doses because they were more effective than standard doses in the treat-

ment of primary biliary cirrhosis.²⁷⁻²⁹ Therefore, high doses of UDCA administered at an early phase of PNAC could account for the increased rate and extent of the response to UDCA vs. other studies.

Progressive liver disease with life-threatening liver failure is the most frequent indication for intestinal transplantation in children, and often requires combined organ transplantation.⁴ However, the short-term survival after combined liver and intestinal transplantation is less than that after intestinal transplantation alone.¹³ Our results show that early treatment with UDCA controls PNAC and may prevent liver failure and the need for transplantation in the vast majority of children with primary IF, including those with SBS. This suggests that early treatment with UDCA may change the natural history of IF in a substantial number of children.

ACKNOWLEDGEMENT

We are grateful to Jean Gilder for text editing. No external funding was received for this study.

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