行政院國家科學委員會專題研究計畫成果報告

早產兒肝內膽汁滯留之藥物治療及分子機轉

UDCA treatment and the molecular mechanism in TPN cholestasis in premature infants

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一、中文摘要

全靜脈營養引肝內膽汁滯留(PNALD)是長期使用全靜脈營養(TPN)的重要併發症，主要的危險因子包括早產及低出生體重，肝臟功能尚未成熟可能是重要的致病原因。許多藥物曾被嘗試用來治療及預防 PNALD 但仍未有確定結論。UDCA 是一種親水性的膽酸，已被廣泛用在兒童及成人的肝臟疾病。在豬的 PNALD 模式，UDCA 可以促進膽汁流量並降低血清及肝臟的膽紅素濃度。膽汁傳送蛋白 BSEP 是一種新近發現基因，它是肝細胞主要的膽汁傳送蛋白，BSEP 在 PNALD 的角色仍然不明瞭，我們認為 PNALD 的機轉可能與 BSEP 的功能不佳有關。

研究結果：在本研究的臨床部份中，總共有 35 名罹患 PNALD 早產兒納入研究：22 名 PNALD 病人以 UDCA 30 mg/kg/d 治療，13 名是控制組 PNALD 病人，並未以 UDCA 治療。血清膽紅素含量在治療組有 95% (21/22) 降低，但在控制組只有 46% (6/13) 降低。治療組治療後與治療前的膽紅素變化為-5.4 ± 5.6 mg/dl，而控制組變化為 1.7± 6.5 mg/dl，在 ALT 方面治療組平均變化為 -25 ± 58 U/L，控制組為 34 ± 17 U/L，另外我們以 RT-PCR 測量 BSEP 的表現，在 PNALD 的病人有 2/5 呈現陽性，而在另一組膽汁滯留(膽道閉鎖)病人有 6/7 呈陽性。免疫染色分析發現在 PNALD 的病人膽小管的 BSEP 呈現微弱的陽性反應，並有一些 BSEP 染色在細胞質內並未正確的位於膽小管上。

我們的研究顯示 UDCA 可有效的降低早產兒 PNALD 的膽紅素及 ALT 值，而 PNALD 病人之 BSEP 表現量較低，在細胞的表現位置也有異常現象，因此 PNALD 的機轉可能與 BSEP 的異常表現有相關性。

關鍵詞：膽汁滯留、早產兒、全靜脈營養

Abstract

Total parenteral nutrition associated liver disease (PNALD) is a major and potentially lethal complication in patients receiving long-term parenteral nutrition therapy. The key predisposing factors for the development of PNALD are prematurity and low birth weight. Immaturity of hepatic function appears to be important in the pathogenesis of PNALD in premature infants.

A number of pharmacologic agents have been proposed for the prevention and treatment of PNALD but no conclusive results have been documented for their beneficial effects. Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid and has been widely used in various cholestatic liver diseases in adult and in children. UDCA improves bile flow and reduces serum and liver bilirubin concentrations in piglets with PNALD. Bile salt export pump (BSEP), an ATP-dependent bile acid exporter has recently been cloned and proved to be the major canalicular bile salt transporter. The role of BSEP in PNALD has not been reported. In this report we investigate the effect of UDCA treatment in premature patients with PNALD and test the expression of BSEP in their liver samples.

Results: A total of 35 premature patients with TPN related cholestasis were recruited.

Twenty-two of them were treated with UDCA 30 mg/kg/d and 13 patients in the control group were not treated. The total bilirubin levels decreased in 95% (21/22) treated 46% (6/13) control patients. The mean change of bilirubin was -5.4 ± 5.6 mg/dl in the treatment group and 1.7± 6.5 mg/dl in the control group. The mean changes of alanine aminotransferase (ALT) levels were -25 ± 58 U/L in the treatment group and 34 ± 17 U/L in the control group. Expression of Bile salt export pump (BSEP) was tested by using RT-PCR. Two of the five premature patients of TPN liver disease and 6/7 patients of biliary atresia were tested positive for BSEP. Immunofluorescent staining showed weak canalicular staining and some cytoplasmic staining.
The results showed that UDCA treatment was effective in decreasing the bilirubin levels and ALT levels in premature patients with PNALD. Liver from patients with PNALD liver disease had lower expression levels of BSEP than biliary atresia. The mechanism of TPN cholestasis may be related to abnormal BSEP expression and bile salt excretion function.

Keywords: Cholestasis, prematurity, infants, total parenteral nutrition

二、緣由與目的

Total parenteral nutrition associated liver disease (PNALD) is a major and potentially lethal complication in patients receiving long-term parenteral nutrition therapy. The key predisposing factors for the development of PNALD are prematurity and low birth weight. Almost two-thirds of all infants weighing less than 2,000 g at birth develop cholestasis after 2 weeks of PN therapy. Immaturity of hepatic function appears to be important in the pathogenesis of PNALD in premature infants.

A number of pharmacologic agents have been proposed for the prevention and treatment of PNALD, including nonsteroidal anti-inflammatory drugs, cholecystokinin-octapetide and ursodeoxycholic acid (UDCA). No conclusive results have been documented for their beneficial effects toward PNALD.

UDCA has been widely used in cholestatic liver diseases and hepatitis in adults and in children. UDCA is a hydrophilic bile acid that is not hepatotoxic *in vitro* or in human. Long-term oral administration of UDCA improves the clinical and biochemical manifestations of chronic cholestatic liver disease in adult patients, such as primary biliary cirrhosis and sclerosing cholangitis. UDCA improves bile flow and reduces serum and liver bilirubin concentrations in piglets with PNALD.

The mechanism of PNALD is not fully understood. A number of factors have been identified, which may contribute to the development of PNALD, including effect of prematurity, early initiation of parenteral nutrition therapy, increased duration of parenteral nutrition therapy, lack of enteral feeding, sepsis, amount of energy delivered in excess of needs, imbalance in parenteral nutrition formulation, pre-existing malnutrition, and pre-existing liver dysfunction/disease. Substrates in TPN solutions have been proposed to cause direct liver damage. It is mandatory that a more effective treatment or preventive methods should be sought to reduce the morbidity and mortality in these premature infants receiving PN.

Bile salt export pump (BSEP), an ATP-dependent bile acid exporter has recently been cloned and proved to be the major canalicular bile salt transporter. In human the gene is found to cause a hereditary cholestatic liver disease, progressive familial intrahepatic cholestasis that is resulted from defective bile acid transport at the hepatocyte canalicular membrane. BSEP functions as the major bile salt export pump in hepatocyte canalicular membrane, mediating bile-salt dependent bile flow, the rate limiting-step of bile formation. Thus the regulation of BSEP in physiology and pathological conditions becomes an important issue to be elucidated.

The aims of our project were to determine the effectiveness of UDCA in treating premature infants with PNALD, and also to elucidate its molecular mechanism, by studying the expression BSEP in the liver biopsy samples of patients with PNALD.

三、結果與討論 (Results and Discussion)

1. **UDCA treatment in TPN cholestasis in premature infants.** A total of 35 premature patients with TPN related cholestasis were recruited. Twenty-two of them were treated with UDCA 30 mg/kg/d and 13 patients in the control group were not treated. The mean follow-up time was 9.2 ± 4.2 weeks and 9.5±3.6 weeks in the treatment and control group, respectively. The total bilirubin levels decreased in 95% (21/22) treated 46% (6/13) control patients. The mean change of bilirubin was -5.4 ± 5.6 mg/dl (Fig 1A) in the treatment group and 1.7± 6.5 mg/dl (Fig 1B) in the control group. The mean changes of alanine aminotransferase (ALT) levels were -25 ± 58 U/L in the treatment group and 34 ± 17 U/L in the control group.
2. **Expression of Bile salt export pump (BSEP) expression in the liver of premature patients with TPN liver disease.** We tested the expression of BSEP in needle liver biopsy samples with RT-PCR. Two of the five premature patients of TPN liver disease and 6/7 patients of biliary atresia were tested positive for BSEP (Fig 2). Immunofluorescent staining using polyclonal rabbit anti-human BSEP antibody showed weak canalicular staining and some cytoplasmic staining. Patients with biliary atresia have positive BSEP staining pattern.

The results showed that UDCA treatment was effective in decreasing the bilirubin levels and ALT levels in premature patients with PNALD. We also found that patients with PNALD had lower expression levels of BSEP than biliary atresia, another common cholestatic liver disease in infancy. The mechanism of TPN cholestasis may be related to abnormal BSEP expression and bile salt excretion function.

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四、計畫成果自評

The results provided important data on the clinical treatment of TPN liver disease in prematurity, an frequent complication of premature infants. This is the first report regarding the clinical effectiveness of UDCA treatment. Furthermore, our results are the first report on the molecular mechanisms of TPN liver disease and provided further prospect of investigation. We are now analyzing detailed results and will submit our data to the journals.

五、圖表

Fig 1A. Total bilirubin changes after UDCA treatment in premature patients with TPN liver disease

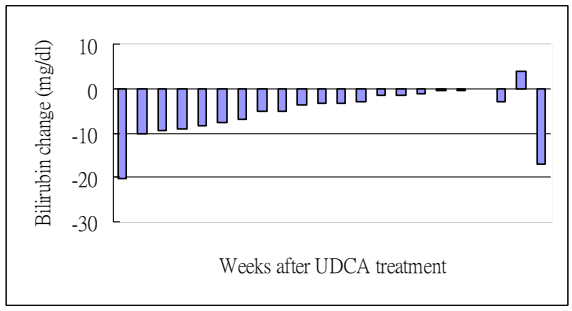


Fig 1B. Total bilirubin changes in control group with TPN liver disease

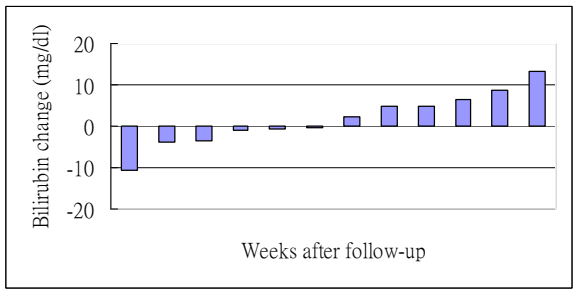
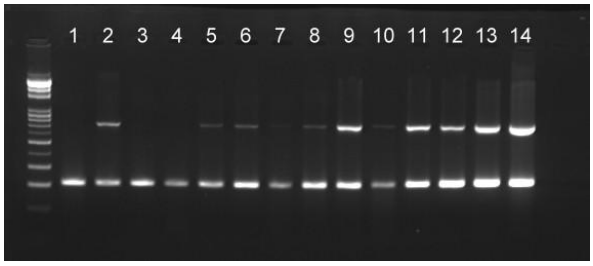


Fig 2. RT-PCR of BSEP in liver biopsy samples of premature patients with TPN liver disease. Lane 1~5, TPN liver disease; lane 6~12, biliary atresia, lane 13 and 14, positive control.



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附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

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共同主持人：

計畫參與人員： 劉有容(研究助理)

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