Biliary Atresia – Bile Acids and Prostaglandin E<sub>2</sub> in Cell Cultures of Bile Duct Epithelia

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Summary

In a cell culture model of bile duct epithelial cells, the effect of prostaglandin E<sub>2</sub>, lithocholic acid and deoxycholic acid was studied. Bile acids and prostaglandin are administered postoperatively in biliary atresia empirically as choleretics. Prostaglandin E<sub>2</sub> and the bile acids all had inhibitory effects on bile duct epithelial cells in culture. There is no clinical study proving the efficacy of either bile acids or prostaglandin E<sub>2</sub> in biliary atresia. The negative results with these substances in cell cultures warrants reserve in their routine clinical use in biliary atresia.

Key words

Biliary atresia – Bile duct epithelial cell cultures – Bile acids – Prostaglandin

Résumé

Dans une culture cellulaire expérimentale de cellules épithéliales biliaires, les effets des prostaglandine E<sub>2</sub>, de l'acide lithocholique et de l'acide deoxycholique furent étudiées. Les acides biliaires et les prostaglandines furent administrés de façon empirique, comme chélétiques en postopératoire chez des enfants souffrant d'atresie des voies biliaires. Les prostaglandines E<sub>2</sub>, ainsi que les acides biliaires ont tous un effet inhibiteur sur les cellules épithéliales des voies biliaires en culture. Il n'existe pas d'études cliniques prouvant l'efficacité des acides biliaires ou des prostaglandines E<sub>2</sub>, lors d'atresie des voies biliaires. Les résultats négatifs de ces cultures cellulaires avec ces deux substances devraient rendre prudent leur emploi de routine chez les patients souffrant d'atresie des voies biliaires.

Mots-clés

Atresie des voies biliaires – Cultures cellulaires de l'épithélium des voies biliaires – Acide biliaire – Prostaglandine

Zusammenfassung


Schlüsselwörter

Gallengangsatresie – Zellkulturen von Gallengangsepithelien – Gallensäuren – Prostaglandin

Introduction

Biliary atresia has a poor prognosis; its cause is unknown, its surgical therapy consists either of attaching a blind bowel loop to the liver hilus or substituting a healthy organ for the atretic one. Medical therapy consists of choleretics or substances believed to be choleretic.

No logical relation exists between our pathogenetic/etiologic theories and our therapeutic concepts. Not surprisingly, the results of any therapy (– surgical or medical –) remain unsatisfactory. Therefore at first the etiology should be found. Later an adequate therapy may evolve.

Numerous theories concerning the etiology and pathogenesis of biliary atresia have been put forward (17).

The authors have previously described a model of culturing bile duct epithelial cells in order to study some of the etiologic and therapeutic approaches to biliary atresia (15, 16).

Most frequently, viruses have been associated etiologically with biliary atresia. Among the viruses discussed, reovirus type 3 is most often named (3, 4, 6, 10). Rubella (8), adenoviruses (19) have been mentioned as well. The influence of these and other viruses is investigated in this study.

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Furthermore, the present study evaluates the effect of agents used therapeutically in biliary atresia. In addition to conventional agents of known choleretic effect, prostaglandin \(E_2\) and bile acids have been suggested (2, 7, 11), obviously in analogy to primary biliary cirrhosis in adults (9). These substances are studied as well.

**Material and method**

Material and method were as described earlier (14, 15, 16).

The use of \(^{3}\text{H}\)-thymidine is new to the technique and is therefore described. Secondary cells of human and bovine origin were used. The various media were changed after 24 and 72 hours after plating. To the media given at 72 hours 0.15 \(\mu\text{g}\) \(^{3}\text{H}\)-thymidine was added (activity: 2mCi). After incubation at 37 \(^{\circ}\text{C}\) with 7.1 % \(\text{CO}_2\), the media containing \(^{3}\text{H}\)-thymidine was removed. The cultures then were rinsed with PBS buffer, air dried and fixed with methanol. The cells were again dried and stained with acidic hemalum for marking the cell nuclei. This highlighted the incorporated \(^{3}\text{H}\)-thymidine. The silver granula could easily be identified. The bile acids lithocholic acid and deoxycholic acid were added to feeding media in a concentration of 0.2-1 mg/ml. This is the maximum solubility for aqueous solutions. For the virological studies primary cultures were inoculated 4-5 days after plating. The viruses used were reovirus type 3, adenovirus types 5 and 6, poxvirus types 1 and 2, herpes simplex and rubella virus. When initial results showed no cytopathic effect for reovirus type 3, viral concentration was increased to \(10^{-3.5}\) the ID\(^{50}\) as determined by virus titration on monkey kidney cells.

**Results**

Bile duct epithelial cells in culture grow better if “BDCM” is added. “Bile duct conditioned media” is prepared by incubating other bile ducts for 24 hours. This media contains unknown agents with organ-specific and species-specific characteristics (Fig. 1).

Prostaglandin \(E_2\) inhibits the growth of cultured bile duct epithelial cells (Fig. 2).

Since prostaglandin \(E_2\) is dissolved in an alcoholic solution, a second set of controls was exposed to an equivalent amount of alcohol. Again, growth was reduced compared with controls without alcohol, but less than with prostaglandin \(E_2\).

Lithocholic acid and deoxycholic acid both reduced the growth of the cells (Fig. 3). Deoxycholic acid even hindered growth.

Viral inoculation quickly lead to marked cytopathic effects (Fig. 4).

Finally all cultured cells, with one exception, died within 2-3 days. Cells inoculated with reovirus type 3 continued to grow normally. They achieved conflueny within the same time period as control cells and appeared morphologically normal (Fig. 5).

The cells were inoculated, as demonstrated by reoviruses type 3 isolated from the medium (Fig. 6).

**Discussion**

The basic mechanisms of “conditioned media” stimulating cell growth is unclear to us. It is used widely in culturing cells, especially epithelial cells. We speculate that the substance causing the improved growth of the cell cultures might be useful therapeutically in biliary atresia. Biliary atresia is histologically characterized by ductular proliferation, but still an epithelial growth stimulating factor could be useful.

Prostaglandin is used by some institutions in advanced stages of biliary atresia as a choleretic (13). In scintigraphy, prostaglandin seems to enhance bile flow (19). Our department has tried it as well, without obvious success. In order to reach the desired level the dosage has to be advanced to an amount where the child suffers additionally from abdominal cramps.

Therapeutic dosages are very expensive. In addition, in the cell culture study presented here, prostaglandin \(E_2\) had an inhibitory effect on growth. We feel that prostaglandin meets expectations in neither the clinic nor the laboratory.
It was proposed that bile acids be administered postoperatively in order to enhance bile excretion. Dehydrocholic acid is used as is ursodeoxycholic acid. Although cholestasis should represent a contraindication to the use of bile acids, in four out of six patients with biliary atresia postoperative bilirubin and total serum bile acid levels decreased (11). At Kasai's institution dehydrocholic acid is administered for several weeks and replaced later by ursodeoxycholic acid (12). Without knowing its mechanism of action, ursodeoxycholic acid has been used recently in primary biliary cirrhoses. Publications report more than 250 patients, mostly with good results (9). This substance may play an important role in the future treatment of biliary atresia.

In primary human cell cultures of hepatocytes, ursodeoxycholic acid reduces the hepatotoxicity of other bile acids. Ursodeoxycholic acid is considered hepatoprotective (5). Lithocholic acid has not yet been tried clinically. Like other bile acids, it stimulates liver cell kinetic and results in bile duct hyperplasia but also in adenomatous changes (1). As shown by $^3$H-thymidine incorporation, secondary bile acids as deoxycholic acid and lithocholic acid lead to increased proliferative activity of cultured epithelial cells.

However, the bile acids died after a few days. Cells peeled off from the plate, cell contact was interrupted and cells floated in fragments. Lithocholic acid was less cytotoxic.
Growth stimulation was observed with neither lithocholic acid nor deoxycholic acid.

Within its given limits, this bile duct epithelial cell culture model seems to work satisfactorily (14). Increased $^3$H-thymidine incorporation in defective media demonstrates the adaptability of the system. We feel, however, that the enormous waste of plastic material produced by cell culturing does not correlate well with the reproducibility and the human biological relevance of the results obtained.

More complex models might suit the goal of the study better. Unfortunately, cell cultures of bile duct epithelia alone do not seem able to contribute much to the etiology and pathogenesis of biliary atresia.

References


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