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ORIGINAL ARTICLE

Hepatobiliary effects of cholic and lithocholic acids: experimental study in hamsters

Ivonete Siviero · Saulo M. R. Ferrante · Ivens Baker Meio · Kalil Madi · Vera L. Chagas

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Abstract Etiopathogenesis of biliary atresia remains unknown. Among several theories, one proposes that the disorder may be caused by the toxic effect of monohydroxy bile acids on fetal and neonatal hepatobiliary system. In this paper we evaluated toxic effects produced by ingestion of cholic acid, a trihydroxy bile acid, and lithocholic acid, a monohydroxy bile acid in the hepatobiliary system of a hamster during gestational and perinatal periods. A diet composed by 0.5% cholic acid and 0.25% lithocholic acid was administrated to pregnant hamsters. Liver and bile ducts of the adult and newborn animals were analyzed to point out the changes induced by these acids after birth. Because hamsters and humans have a similar bile metabolism, these animals were eligible for the study. The ingestion of 0.5% lithocholic acid, during hamster's gestation, caused maternal intense ductal/ductular proliferation, inflammatory signs, hepatic cells degeneration and regeneration, hyperplasia of extra hepatic ducts epithelium, and abortion. Both 0.5% cholic acid and 0.25% lithocholic acid ingested by pregnant hamsters, caused ductal/ductular proliferation and hepatobiliary inflammatory damage in a different degree of intensity in adult animals and mild intensity in the young; and also the number of the young was reduced in the litter. We found that the ingestion of these bile acids by hamsters, during gestational period caused different degrees of toxicity on maternal and neonatal hepatobiliary systems. The histopathologic findings observed in biliary atresia patients could not be found in newborn hamsters. New experimental models are needed in the attempt to establish a correlation of these acids with neonatal cholestatic diseases.

Keywords Cholic acid · Lithocholic acid · Liver · Bile ducts · Biliary atresia · Hamster

I. Siviero (✉) · S. M. R. Ferrante · I. B. Meio · K. Madi · V. L. Chagas
Department of Surgery and Pathology,
Division of Pediatric Surgery,
Medical School of Federal University of Rio de Janeiro,
Instituto de Pediatria e Puericultura Martagão Gesteira,
Avenida Marechal Trompovsky s/n Ilha do Fundão,
Rio de Janeiro, CEP 21941-590, Brazil
e-mail: ivonetesiviero@terra.com.br

Introduction

Biliary atresia is the most common cause of obstructive jaundice, cirrhosis and liver transplantation in infancy. It is characterized by fibrous and sclerotic, total or segmental obliteration of the extra hepatic ductal system, as well as different degrees of hepatic ducts damage. Its etiopathogenesis remains unclear. To date, several theories were proposed: congenital, genetic, infectious, immunological, environmental teratogenic, common channel theory, and toxic effects of bile acids (BA). Despite controversies and comprehensive investigation, none of these theories were totally proved or refused.

Jenner and Howard have proposed that biliary atresia and neonatal hepatitis (NH) may occur through an adverse effect of monohydroxy bile acids on the fetal and neonatal system [1]. In fact, fetal and neonatal bile metabolism is different from the adult one, as comparative studies performed in bile, meconium, urine, and serum have demonstrated [2–5]. The patients with biliary atresia and NH may present "atypical" BA and changes in the BA metabolism [6]. The precise role of BA in the etiopathogenesis of liver cholestatic diseases remains unclear.

The aim of the present study is to evaluate experimentally the histological changes in the liver and biliary ducts produced by oral administration of a primary trihydroxy

bile acid, cholic acid (CA), and a secondary monohydroxy biliary acid, lithocholic acid (LCA), in adult hamsters during gestation and neonatal period.

Materials and methods

Animals

We used adult male and female hamsters from *Mesocricetus auratus* species, at the age of mating, i.e., between 30 and 40 days of age, weighing 85–110 g. They received water and a ration for laboratory rodents and were kept in a 12 h day/night cycle regime. After 15 days of conditioning and adaptation, they mated. After mating and confirmation of copulation, the male was taken out of the cage and the pregnant female received the diet prepared with BA.

The feeding of 0.5% CA began on the first day after mating; 0.25 and 0.5% LCA in the first and seventh day. The animals, mothers, and the young were killed 2 days after birth. The 0.5% CA was given to three females with 12 young ones (group 1); 0.5% LCA was given to two females since the first day of gestation (group 2); and to three females on the seventh day of gestation (group 3); 0.25%, LCA was given to three females, since the seventh day of gestation, and to their 13 young ones (group 4).

Since hamster gestation takes 16 days, CA was administrated during 18 days to three animals and LCA during 18 days to two animals and 12 days to the others. After this period the animals underwent liver and bile duct excision. The control group included two females and 20 young animals, which received the diet throughout 18 days. All the animals were sacrificed soon after being born.

This experimental study was performed in accordance with the ethical guidelines for the use of animals in scientific experiments as approved by the Brazilian College of Animal Experimentation (COBEA), affiliated to the International Council for Animal Science (ICLAS).

Diet manipulation

Diet with 99% CA (Fluka, Biochemika, Sigma-Aldrich®) and 98% LCA (Aldrich Chem®) was prepared using Carrella and Dietschy's method [7]. The CA diet was prepared with a concentration of 0.5% and LCA with 0.5 and 0.25%. The control group diet was prepared only with ether.

Liver histological analysis

After total excision, the mothers' and neonates' liver and bile ducts were fixed in 10% formaldehyde and 5 μm sections were stained in hematoxylin/eosin (HE) and analyzed through optical microscopy. Liver cholestasis, ductal and/or ductular proliferation, inflammatory infiltrate, degenerative changes (cerotic content in Kupffer cells, vacuolization, and acidophily or hepatocyte necrosis) and changes in hepatic outline and in extra medullar hematopoiesis were found. In extra hepatic bile ducts, the presence of inflammatory signs, epithelial hyperplasia and degenerative changes were investigated.

In portal area, the ductal and/or ductular proliferation was qualified by semi quantitative means as follows:

Minimum (+) until three ducts/ductulus in portal area,
Mild (++) more than three ducts/ductulus; proliferation did not surround portal area,
Moderate (+++) proliferation surrounded portal area,
Severe (++++) proliferation went beyond portal area, i.e., perilobular.

Results

Animals fed with CA

Three hamsters fed with 0.5% CA (group 1) during the gestational period presented histological hepatobiliary changes of different degrees. In the liver, ductular proliferation (moderate in one animal, minimal in another and accentuated in a third one), inflammatory infiltrate of portal area (two animals), Kupffer cells containing ceroid (three animals) and with enhanced lobulation (two animals). In the intra and/or extra hepatic bile ducts, hyperplasia or proliferation of epithelial cells was found in all animals (Fig. 1). These results may be compared to the control group (Fig. 2).

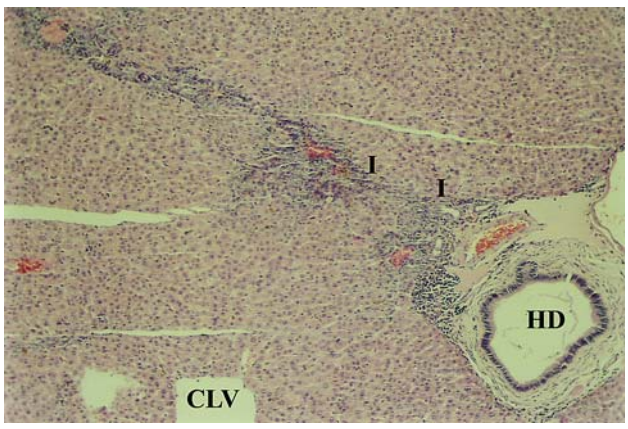

Fig. 1 Adult animal fed with 0.5% CA. Severe ductular/ductal proliferation and inflammatory infiltrate linking portal areas **I** Hepatic duct (*HD*) and centrolobular vein (*CLV*) (HE, ×100)

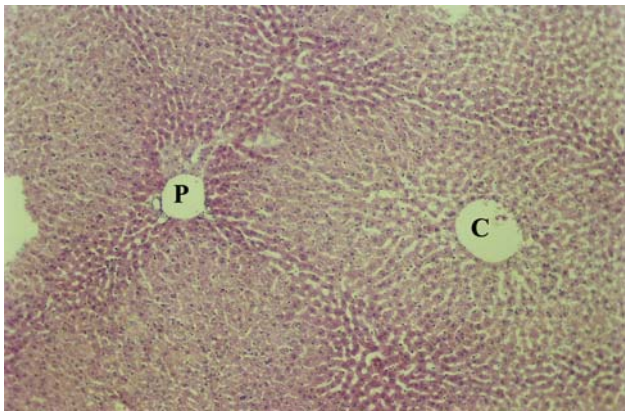

Fig. 2 Hepatic parenchyma and portal area of maternal control. Centro-lobular vein (*C*) portal área (*P*) (HE, ×100)

Liver and bile ducts histopatologic study in 12 young ones (group 1) revealed that all presented a mildly increased extra medullar hematopoiesis when compared to the control group. Three animals presented hyperplasia or proliferation of extra hepatic ductal epithelium (25%). These animals presented a minimum ductular/ductal proliferation in portal area (Figs. 3, 4, 5).

Animals fed with LCA

The administration of 0.5% LCA to two pregnant females in the first day of gestation (group 2) caused death. Both animals presented diarrhea, poor feeding and loss of weight.

One of these animals died in the tenth day of gestation presenting nine degenerated gestational sacs, only with placental debris and two fetuses, which were studied together with maternal liver. In the hepatic parenchyma, severe ductal/ductular proliferation, necrosis and vacuolization of hepatocytes with intermingled inflammatory cells, hepatic lobes atrophy and cholangitis were found. Extra hepatic bile ducts showed evidences of epithelium destruction. Gall bladder presented evidence of autolysis. In both fetuses, severe extra medullar hematopoiesis and hepatocytes vacuolization were observed.

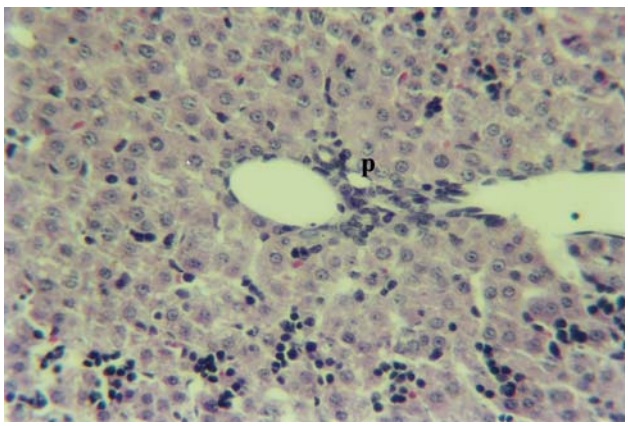

Fig. 3 Young hamster exposed during pregnancy to 0.5% CA. Details of increased extra medullar hematopoiesis, minimum ductal/ductular proliferation (*p*) (HE, ×400)

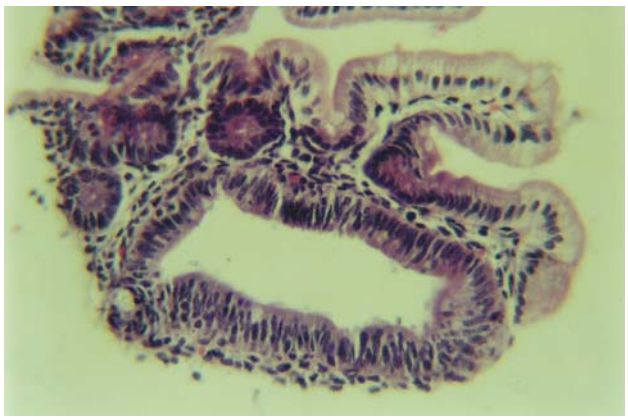

Fig. 4 Young hamster exposed during pregnancy to 0.5% CA. Choledoch lining hyperplasia (high epithelium with pseudo stratification) (HE, ×400)

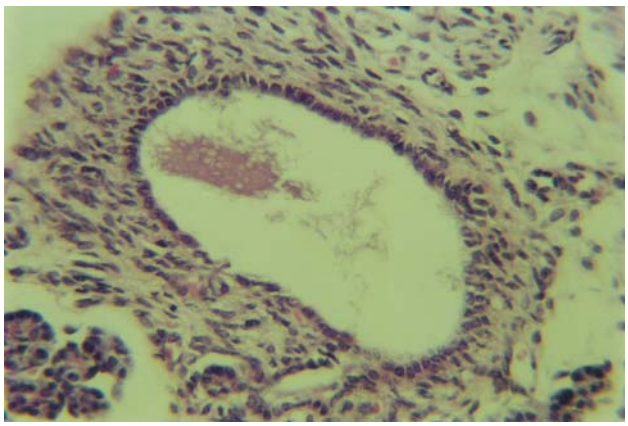

Fig. 5 Choledoch of young control. See the cuboid/columnar lining (HE, ×400)

The animal fed with 0.5% LCA (group 2) since the first day of gestation died in the 12th day, presenting ten gestational sacs without any fetus. Due to deterioration, the liver and/or bile ducts were not studied.

Even when 0.5% LCA was administrated in the seventh day of gestation to three hamsters (group 3), no young was born. Hepatic histopatological changes in the mothers' were severe and similar to these three animals, with ductal/ductular proliferation, mixed inflammatory infiltrate in portal areas and increased hepatic lobulation. One of them also presented necrosis of hepatocytes. Bile ducts showed hyperplasia, both in extra hepatic or interlobular ducts (Fig. 6) as well as in gallbladder.

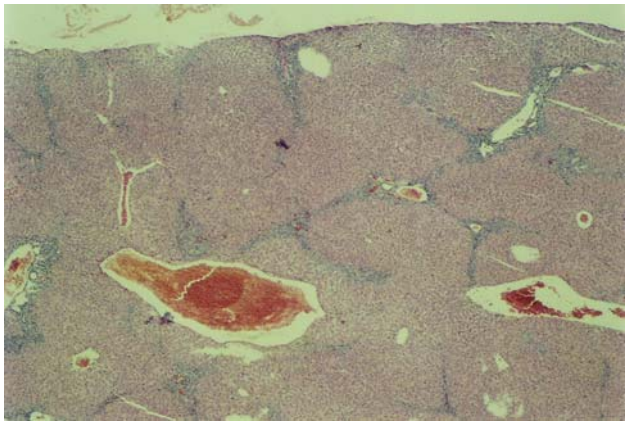

Fig. 6 Adult animal fed with 0.5% LCA. Severe ductal/ductular proliferation with incomplete surrounding of the hepatic lobe and hepatic lobulation enhancement (HE, ×40)

When 0.25% LCA was ingested by the mothers' starting from the seventh day of gestation (group 4), histological changes were seen in these three animals, although less severe than the changes in the animals fed 0.5%. All animals have shown a ductal/ductular proliferation (moderate in two cases, mild in one case) and signs of cellular degeneration. In two cases focal points of necrosis were observed, and in one case Kupffer cells were detected with ceroid material. In these three adult animals a mixed inflammatory infiltration was also observed in the portal area enhancement of the hepatic lobulation and hyperplasia of the extra-hepatic ductal epithelium. These 0.25% LCA fed mothers' (group 4) gave birth to 13 young ones, all of which presented vacuolization of hepatocytes in addition to more severe extra medullar hematopoiesis, when compared with the control group. Ductal or ductular proliferation was not observed in these animals. In extra hepatic bile ducts, gallbladder epithelium hyperplasia was seen in 13 newborns. Only one animal presented gallbladder vacuolization (Fig. 7).

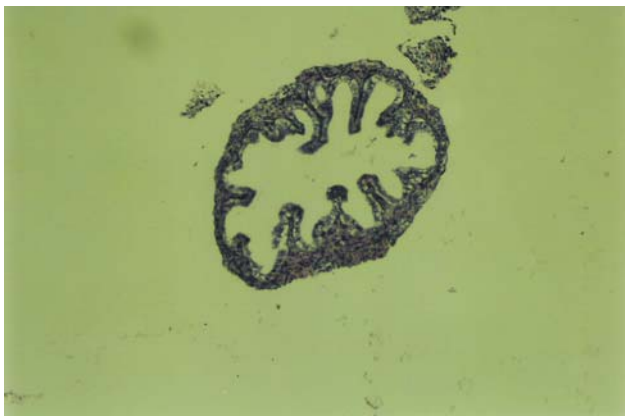

Fig. 7 Young animal fed with 0.25% LCA. Gallbladder epithelium hyperplasia and vacuolization (HE, ×100)

No cholestasis or bile plugging in the hepatic parenchyma was observed either in adult animal or in young animals following the oral administration of CA and LCA.

Discussion

Jenner and Howard have proposed that NH, biliary atresia, and choledoch cystic dilatations might be provoked by the toxic effect of BA, particularly monohydroxy acids (LCA and 3β-OH 5-cholenoic acid) on hepatobiliary system during pregnancy and/or neonatal period [1].

Several studies performed in rats, [8] pigs, [9] rabbits, [10] and dogs [11] have already proved that bile metabolism in neonatal period is different from the adult. In neonatal period, there is a decrease of synthesis, pool size, ductal flow, and BA intestinal absorption. The conjugation pattern is also different, manly taurine in neonates and glycine in adults [12–14]. There is immaturity of bile secretory function at birth, which increases after the first week of age. This fact was also confirmed in human neonate and in species phylogenetically closer to man, such as baboons [15, 16].

Despite the poor knowledge about BA metabolism during fetal life in humans, it is known that fetuses and neonates may present a primitive metabolic pattern, with individual variations. The presence in 3β-OH-cholenoic acid, LCA and DCA (dehydroxycholic acid) in meconium, was already confirmed which may be relatively toxic in vitro [17, 18].

Because of the absence of intestinal micro flora, it is possible to presume that the secondary BA cannot be formed by this via, in the beginning of life. The finding of LCA and DCA in meconium may represent the passage of these compounds through placenta [15]. However, we should not exclude the hypothesis that fetuses may produce LCA through an own endogenous via, starting from cholesterol [19, 20].

If BA play a role in the development of neonatal hepatic diseases, these disorders may be associated with a change in LCA metabolism, on account of the unmistakable evidence of cholestasis induced by this compound. It has been considered responsible for the start and permanence of hepatic cholestatic disease in humans, both in neonatal period and adult age [21].

The hamster was the animal chosen for our study because of its similarities with man's BA metabolism Studies using high-performance liquid chromatography confirm that hamsters have a conjugation pattern with taurine and/or glycine and a composition of BA in bile similar to that of man [22–26]. However, in spite of many scientific studies confirming the similarities between hamsters and human metabolism, the main BA

studied are chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) due to their therapeutic utilization [24, 26, 27].

Even when these BA, CDCA and UDCA were tested, the toxicity produced was assigned to LCA [27, 28, 29, 30]. On the other hand, despite monohydroxy bile acids being responsible as probable agents in the etiopathogenesis of biliary atresia and/or neonatal cholestasis, there are few studies about hepatic morphologic changes provoked by them. There is no study in the literature to evaluate hepatic and biliary tract morphologic changes provoked by the ingestion of monohydroxy bile acids during gestation in hamsters, although there is evidence of metabolic similarities between hamsters and man.

In 1976, in Japan, Suzuki compared the toxicity of CA, CDCA, LCA and DCA in rats and observed that DCA was the most toxic BA. Toxicity of LCA and CDCA was less than what it was supposed to be. Suzuki's hypothesis is that, in rats these BA are easily converted into a less toxic compound, the β-muricholic acid. After the ductus choledochus ligature, feeding with 1% DCA was offered and rats developed hepatic cirrhosis and pseudo ductulus proliferation [30].

In 1978, Nittono and Kato studied the effects of acids on hepatobiliary system of rabbits, observing the toxicity of CDCA, LCA, UDCA, DCA and CA, varying from the most toxic (CDCA) to the least toxic (CA). Rabbits fed with 0.1 or 0.5% BA for 20–50 days were studied. Hepatic histological changes were similar to those found in NH and biliary atresia, with pseudo tubules proliferation, fibrosis and cholestasis of portal areas, and hepatic cell infiltration. The authors concluded that these diseases may be caused by the toxic effects of BA [30]. However, it is known that rabbits present a pattern of composition and conjugation of BA totally different from that of man [31].

In the present study, 0.5% CA was administrated to pregnant hamsters in the first day of gestation and LCA (0.25 and 0.5%) in the first and seventh day, to evaluate histological changes of liver and biliary tract in these animals and to compare with the findings in patients with biliary atresia.

In animals fed with 0.5% CA since the first day of gestation, it was possible to observe the mother's liver with different degrees of ductal proliferation, mixed inflammatory infiltrate in portal area and enhancement of hepatic lobulation. Kupffer cells with ceroid pointed out the cell damage and phagocytose. Extra hepatic biliary tract of these animals showed epithelium hyperplasia. All these histological changes were severe in only one animal.

Cholic acid is a primary BA of low toxicity produced by liver as related before. In the bowel, through the action of the enzyme 7α-hydroxylase, this CA produces DCA (dehydroxycholic acid). It is more likely that hepatic changes found in the CA feeding have been due to DCA.

There were few young ones in each gestation of the hamsters that ingested CA. There was minimum ductal proliferation, hepatocytes vacuolization and extra hepatic ductal hyperplasia correlated to the severe degree of maternal liver damage. All of them showed increased medullar hematopoiesis when compared to control group. Since DCA can cross placental barrier [3], it is possible that the toxic effects caused by the ingestion of CA are due to DCA of maternal origin.

The ingestion of 0.5% LCA by pregnant hamsters prevented the birth of young and provoked severe changes in hepatic parenchyma and biliary tract of these animals. When 0.5% LCA was ingested since the first day of gestation, the animal died. When it was ingested on the seventh day by pregnant hamsters, it provoked abortion. The histological changes of liver and biliary tract were severe in the studied adult animal that died with a picture of acute hepatic disease, mimicking an acute hepatitis, including cholangitis. Severe ductal proliferation, necrosis and hepatocytes vacuolization, inflammatory cells in portal area and atrophy of hepatic lobules were found pointing out the probable progression to cirrhosis. The ductal extra hepatic system of this animal showed inflammatory infiltrate and signs of epithelial destruction, including gallbladder.

Although this animal has presented 11 gestational sacs, it had only two fetuses with hepatocytes vacuolization. The other animal which died had 10 gestational sacs without any fetus. These data suggested that with this dose and this time of gestation, LCA can provoke abortion and the death of the animal in case it is ingested since the start of gestation.

The remaining adult animals, which ingested 0.5% LCA since the seventh day of gestation, also presented severe hepatic and biliary tract changes, highlighting a picture of acute hepatic disease with inflammation and signs of cellular degeneration, necrosis and regeneration. They also presented ductal/ductular hyperplasia and epithelial hyperplasia of the extra hepatic ducts. In spite of a late start of LCA ingestion during gestation, no young was born, indicating that with this concentration and administration in this period of gestation, LCA provokes abortion. Hepatic and biliary changes were similar to the group of animals which received this acid since the start of gestation. However, the changes were less severe.

The administration of 0.25% LCA in adult hamster also produced milder acute inflammatory damage in the liver and biliary tract. The number of young ones was reduced in this group when compared to the control group. These neonate animals showed minimum hepatic changes, although they have presented epithelium hyperplasia of the extra hepatic ducts.

The bile ducts inflammation occurs in all obstructive cholangiopathies in infancy. This inflammation is a process

in which manifestations are influenced by the time of initial injury, the duration of the time and progression or remission.

The ingestion of LCA by hamsters caused acute inflammatory lesion and confirmed its toxicity to the liver and biliary ducts of adult animals and a lesser degree of toxicity in their offspring. A follow-up of the animals for a longer time should be necessary to evaluate the progression of these lesions and establish a relation with cholestatic diseases found in humans.

Although it is not possible to state that BA are involved in the etiopathogenesis of biliary atresia, the present study shows the need of LCA feeding experimental evaluation during gestation for a longer period of time and using lower concentrations than those we used. Accordingly, other BA such as 3β-OH-5cholenoic acid, should be studied. The utilization of a maternal model added to further studies in neonates, such as the assessment of the BA damage mechanism in neonates, passing through placenta or breast feeding.

Conclusions

In adult hamster, the LCA in the concentration of 0.5% may produce severe hepatobiliary changes mimicking hepatitis and cholangitis, abortion and death. The oral administration of LCA at 0.25% and CA at 0.5%, in the same animals, may produce milder inflammatory lesions in the hepatic parenchyma and biliary ducts. In the offspring of the pregnant hamsters, however, within the mentioned concentrations and timeframe, it was not possible to establish a correlation between the hepatobiliary histopathologic findings caused by the placentary passage of biliary acid and those found in biliary duct atresia cases.

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References

1. Jenner RE, Howard ER (1975) Unsaturated monohydroxy bile acids as a cause of idiopathic obstructive cholangiopathy. Lancet 23:1073–1075
2. Challacombe DN, Edkins E, Brown GA (1975) Duodenal bile acid in infancy. Arch Dis Child 50(11):837–843
3. Back P, Walter K (1980) Developmental pattern of bile acid metabolism as revealed by bile acid analysis of meconium. Gastroenterology 78(4):671–676
4. Strandvik B, Wahlen E, Wikstrom SA (1994) The urinary bile acid excretion in healthy premature and full-term infants during the neonatal period. Scand J Clin Lab Invest 54(1):1–10
5. Suchy FJ, Balistreri WF, Heubi JE et al (1981) Physiologic cholestasis: elevation of the primary serum bile acid concentrations in normal infants. Gastroenterology 80(5): 1037–1041
6. Makino I, Sjovall J, Strandvik B (1971) Excretion of 3 β-hydroxy-5-cholenoic and 3 α-hidroxy-5α-cholanoic acids in urine of infants with biliary atresia. FEBS Lett 15(2):161–164
7. Carrella M, Dietschy JM (1977) Comparation of effects of cholic acid and chenic acid feeding on rates of cholesterol synthesis in liver of the rat. Dig Dis 22(4):318–326E
8. Suchy FJ, Balistreri WF (1980) Uptake of taurocholate by hepatocytes isolated from developing rats. Pediatr Res 6:282–285
9. Tuchweber B, Ducruet N, Perea A et al (1990) Development of bile secretory function in the neonatal guinea pig. Biol Neonate 58:279–290
10. Shaffer EA, Zahavi I, Gall DG (1985) Postnatal development of hepatic bile formation in the rabbit. Dig Dis Sci 30:558–563
11. Tavoloni N, Jones MJ, Berk PD (1985) Postnatal development of bile secretory physiology in the dog. Pediatr Gastroenterol Nutr 4:256–267
12. Setchell KD, Dumaswala R, Colombo C et al (1988) Hepatic bile acid metabolism during early development revealed from the analysis of human fetal gallbladder bile. J Biol Chem 263(32): 1637–1644
13. Strandvik B (1982) Bile acid metabolism in low birthweight infants. Acta Paediatr Scand Suppl 296:71–74
14. Itoh S, Onishi S (2000) Hepatic taurine, glycine and individual bile acids in early human fetus. Early Hum Dev 57(1):71–77
15. Setchell KD, Dumaswala R, Colombo C et al (1988) Hepatic bile acid metabolism during early development revealed from the analysis of human fetal gallbladder bile. J Biol Chem 263(32): 1637–1644
16. Bhat R, Chari G, Meller J et al (1997) Bile flow and composition in preterm, term and infant baboons. Biol Neonate 72(4):235–242
17. Back P, Ross K (1973) Identification of 3 β-hydroxy-5-cholenoic acid in human meconium. Hoppe Seylers Z Physiol Chem 354(1):83–89
18. Nittono H (1980) Bile acid metabolism in infants and children and its implications for hepatobiliary diseases. Indian J Pediatr 47(389):549–554
19. Javitt NB (1974) Bile salts and liver disease in childhood. Postgrad Med J 50(584):354–361
20. Gustafsson J, Anderson S, Sjovall J (1987) Bile acid metabolism during development of lithocholic acid in human fetal liver. Pediatr Res 21(1):99–103
21. Balistreri WF, Suchy FJ, Farrell MK et al (1981) Pathologic versus physiologic cholestasis: Elevated serum concentration of a secondary bile acid in the presence of hepatobiliary disease. J Pediatr 98(3):399–402
22. Galleazzi R, Javitt NB (1977) Bile acid excretion: the alternate pathway in the hamster. J Clin Invest 60:693–701
23. Jordan JT, Killenberg PG (1980) Development of enzymatic conjugation and sulfatation of bile acids in hamster liver. Am J Physiol 238(5):429–433
24. Tateyama T, Katayama K (1976) Metabolism of chenodeoxycholic acid in hamsters. Lipids 11(12):845–847
25. Carducci CN, Matejka M, Erguven H et al (1985) High-performance liquid chromatographic analysis of bile acids in hamster bile. J Chromatogr 337(1):91–97
26. Kihira K, Yamauchi T, Kuramoto T et al (1994) Comparative studies of metabolism of simultaneously administered cheonodeoxycholic acid and ursodeoxycholic acid in hamsters. Steroids 59(7):431–435
27. Mamianetti A, Konopka HF, Lago N et al (1994) Morphologic changes in livers of hamsters treated with high doses of ursodeoxycholic acid; correlation with bile acids in bile. Pharmacol Res 29(2):187–195

28. Mcsherry CK, Morrissey KP, Sworm RL et al (1976) Chenodeoxycholic acid induced liver injury in pregnant and neonatal baboons. Ann Surg 184(4):490–499
29. Sprinkle DJ, Hassan AS, Subbiah MT (1984) Effect of chenodeoxycholic acid feeding during gestation in the rat on bile acid metabolism and liver morphology. Proc Soc Exp Biol Med 175(3):386–397
30. Suzuki T (1976) Effects of bile acids on the liver and bile acid metabolism of rats. Jpn J Pediatr 29:829 (in Japanese)
31. Kato H, Nittono H (1978) Toxic effects of bile acids on the hepato-biliary system. In: International symposium on cholestasis in infancy, Sendai, Japan, pp 43–55, 7–9 June 1978