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Effect of Iron Intake on Chronic Cholestatic Osteoporosis by Surgical CBD (Common Bile Duct) Partial Ligation

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ABSTRACT

Osteoporosis is an important type of metabolic bone disease in chronic cholangiogenic liver disease, with an incidence of up to maximum 72%. Osteoporosis and osteomalacia in patients with chronic liver disease are collectively referred to as "hepatic osteodystrophy," which is commonly found in patients with intestinal malabsorption and progressive primary biliary cirrhosis. To deal with the induction of osteoporosis by chronic cholestasis, we aimed to investigate the following: The aim of the study was to investigate the effect of iron intake on chronic cholestatic osteoporosis by surgical CBD partial ligation. Forty rats including weanling male rats weighing 70-80g and adult male rats weighing 150-160g were used. First, the rats were anesthetized with intraperitoneal ketamine injection, and then the CBD (common bile duct) was exposed by surgical procedures, complete and partial ligation was performed, and then the abdomen was closed. Partial ligation was performed by ligating different thicknesses of the suture needles with the common bile duct and then removing the suture needle. In the case of using suture needle 5-0, 85-90% of the common bile duct is considered to be obstructed and 75-80% for 4-0. After ligation of the common bile duct, naturally proceeding rats were included as contrast group and rats receiving oral administration of 6 mg/kg/day of folate iron were included as experimental group. After 8 weeks, liver function tests (ALT, AST, ZTT) and histopathological observation of the femur (H-E stained samples) were performed in the contrast and experimental groups, respectively. The significance test for viability and prevalence of osteoporosis following CBD ligation was performed by using the chi-square test, and the significance test for liver function test values was performed by using the t-test. Experimental osteoporosis is induced at 8 weeks after surgical CBD partial ligation (85-90% occlusion). Iron intake significantly suppressed the development of osteoporosis by chronic cholestasis.

Keywords: Chronic Cholestatic Osteoporosis, Common Bile Duct, Partial Ligation, Iron Intake.

Article Information

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INTRODUCTION

Osteoporosis is a typical complication of chronic cholestasis associated with primary biliary cirrhosis or primary sclerosing cholangitis, and its development is related to the older age, lower BMI(body mass index), and the severity of liver disease [6, 7, 8, 9, 10]. In a retrospective study comparing total

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cancellous bone volume and osteoid volume in tibial biopsies from patients with chronic cholestatic liver disease, primary biliary cirrhosis and primary sclerosing cholangitis, osteoporosis is an important type of metabolic bone disease in chronic cholangiogenic liver disease, with an incidence of up to maximum 72%. Osteoporosis and osteomalacia in patients with chronic liver disease are collectively referred to as "hepatic osteodystrophy," which is commonly found in patients with intestinal malabsorption and progressive primary biliary cirrhosis [3, 5, 7, 8, 10]. Currently, researchers believe that the mechanism of osteoporosis in chronic cholestasis is associated with decreased bone formation by osteoblast cells which are affected by cholestatic reservoirs such as bilirubin and bile acids and increased bone absorption [1, 2, 3].

Although there is no specific treatment, increased bone mass is reporeted in patients with chronic cholestasis, with supplementation of calcium and vitamin D. On the other hand, assessing intestinal iron absorption by the serum iron increase test after oral administration of 1 mg/kg of iron to children with or without cholestasis or iron deficiency anemia between the age of 6 and 12, the outcomes showed that children with cholestasis had lower intestinal iron absorption than those with iron deficiency anemia, and that the degree of iron deficiency was correlated with age-specific stature and BMI. The children without cholestasis had similar intestinal iron absorption as those with iron deficiency anemia. The children with cholestasis responded effectively to oral iron therapy despite their low intestinal iron absorption, which researchers believe is due to the comorbidity of iron deficiency. According to this study, the scientists suggested that a reduction in nutritional intake, malnutrition and anemia by intestinal malabsorption, which occur in approximately 60% of children with chronic liver disease, may lead to osteodystrophy [4, 5, 11, 12]. To deal with the induction of osteoporosis by chronic cholestasis, we aimed to investigate the following: The aim of the study was to investigate the effect of iron intake on chronic cholestatic osteoporosis by surgical CBD partial ligation.

SUBJECTS AND METHODS

Forty rats including weanling male rats weighing 70-80g and adult male rats weighing 150-160g were used. The Rats were reared by experimental animal shed in Pyongyang University of Medical Sciences.First, the rats were anesthetized with intraperitoneal ketamine injection, and then the CBD (common bile duct) was exposed by surgical procedures, complete and partial ligation was performed, and then the abdomen was closed. Partial ligation was performed by ligating different thicknesses of the suture needles with the common bile duct and then removing the suture needle. In the case of using suture needle 5-0, 85-90% of the common bile duct is considered to be obstructed and 75-80% for 4-0.

After ligation of the common bile duct, naturally proceeding rats were included as contrast group and rats receiving oral administration of 6 mg/kg/day of folate iron were included as experimental group. After 8 weeks, liver function tests (ALT, AST, ZTT) and histopathological observation of the femur (H-E stained samples) were performed in the contrast and experimental groups, respectively. The significance test for viability and prevalence of osteoporosis following CBD ligation was performed by using the chi-square test, and the significance test for liver function test values was performed by using the t-test. Liver function tests and pathomorphological evaluation were performed according to the diagnosis and treatment guidelines published by the Ministry of Public Health, DPRK.

RESULTS

As shown in **(Table 1)**, the mortality was low and the prevalence of osteoporosis was high 8 weeks after surgical partial ligation of CBD (85-90% occlusion).

Table1. Mortality and prevalence of osteoporosis depending on the degree of surgical CBD partial ligation.

Group			number	Survival			Osteoporosis	
				Survived for 2 weeks	Survived for 8 weeks	mortality (%)	Induced number	prevalence (%)
Complete ligation	100% Occlusion	Weanling rat	5	0	0	100	0	0
		Adult rat	5	0	0	100	0	0
Partial ligation	85~90% Occlusion	Weanling rat	10	10	8	20	8	100
		Adult rat	10	10	10	0	8	80
	75~80% Occlusion	Weanling rat	5	5	5	0	1	20
		Adult rat	5	5	5	0	0	0

As shown in **(Table 2)**, 8 weeks after surgical CBD partial ligation (85-90% occlusion) all had significantly higher values of liver function tests compared to pre-ligation, and the difference was more significant in the weanling rats compared to the adult rats.

Table 2. Changes in liver function test values (±SE, unit) in case of surgical CBD partial ligation (85-90% occlusion).

Group	number	Pre-ligation			8 weeks after partial ligation of CBD (85-90% occlusion)		
		ALT	AST	ZTT	ALT	AST	ZTT
Weanling rat	10	44.5±3.2	70.4±4.8	1.9±0.2	148.5±8.1*△	159.9±3.7*	5.3±0.5*△
Adult rat	10	57.6±7.7	81.3±9.8	2.0±0.2	112.7±7.8*	137.4±9.9*	3.7±0.2*

; p<0.05 (compared with pre-ligation); △p<0.05 (compared with adult rats).

As shown in **(Table 3)**, 8 weeks after surgical CBD ligation (85-90% occlusion) had significantly less histopathological changes of the femur in both the weanling and adult rats in experimental group compared to the control group.

Table 3. Histopathological findings of the femur in the case of surgical CBD partial ligation (85-90% occlusion).

Group		Number	8 weeks after partial ligation of CBD (85-90% occlusion)			
			Continuous rupture of periosteum	Porosity of periosteum	Reduction in bone mass	Osteoid finding
Contrast group	Weanling rat	8	8(100.0)	8(100.0)	8(100.0)	8(100.0)
	Adult rat	10	8(80.0)	8(80.0)	10(100.0)	9(90.0)
Experimental group	Weanling rat	8	-	1(10.0)*	1(10.0)*	2(20.0)*
	Adult rat	10	1(10.0)*	2(20.0)*	2(20.0)*	2(20.0)*

Incidence (%) *; p<0.05 (compared with control group).

As shown in **(Table 4)** , 8 weeks after surgical CBD partial ligation (85-90% occlusion) resulted in significantly lower prevalence of osteoporosis in both the weanling and adult rats in the experimental group compared to the control group.

Table 4. Mortality and prevalence of osteoporosis after iron administration in case of surgical CBD partial ligation (85-90% occlusion).

Group		Number	Survival		Osteoporosis	
			Survived number for 8 weeks	mortality (%)	Induced number	prevalence (%)
Contrast group	Weanling rat	10	8	20	8	100
	Adult rat	10	10	0	8	80
Experimental group	Weanling rat	10	10	0	1	10*
	Adult rat	10	10	0	2	20*

; p<0.05 (compared with control group).

DISCUSSION

According to the documents, osteoporosis and osteomalacia in patients with chronic liver disease are the so-called hepatic osteodystrophy, which is a metabolic bone disease condition due to chronic cholestasis, accounting for up to 72% of the prevalence. We first investigated the induction of experimental osteoporosis by chronic cholestasis by surgical CBD partial ligation. For this purpose, CBD exposure by surgical procedures, and 100% occlusion of the CBD was achieved. Both weanling and adult rats were sacrificed within 2 weeks when the CBD was completely ligated.

To experimentally induce hepatic osteoporosis, the experimental animals should not be sacrificed while preventing its bile from excreting for a period of time. The experiment showed that complete ligation of the CBD only sacrifices the experimental animals with acute biliary obstruction but does not result in chronic biliary excretion disorders that can lead to osteoporosis. So we performed partial ligation of the CBD to induce different degrees of biliary occlusion, by ligating suture needle of different thicknesses with the CBD and removing it to create a certain space within the bile duct. Thus, 75-80% of the CBD occlusion made all animals survive for the entire period without sacrifice, but histopathological observation result of the femur showed that no osteoporosis was induced.This suggested that occlusion of less than 80% of the CBD does not result in bone metabolic disorders as a result of excreting a certain amount of bile. However, in 85-90% occlusion of the CBD, osteoporosis was induced in 80% of adult rats and 100% of weanling rats at 8 weeks, whereas in weanling rats, 20% died due to impaired biliary excretion.

This suggests that surgical CBD partial ligation in the range of 85-90%, can increase the prevalence of osteoporosis as well as viability. In this case, changes in liver function tests showed that ALT, AST and ZTT levels were significantly elevated 8 weeks after ligation compared to pre-ligation in both the weanling and adult rats. This means chronic liver dysfunction, which was also confirmed by histopathological test such as hepatocyte degeneration, necrosis, and disorganization of hepatocyte cord. According to the documents, chronic cholestasis can result in impaired intestinal iron absorption, malnutrition, and anemia leading to osteodystrophy. We conducted a study to investigate the effect of iron intake on chronic cholestatic osteoporosis.

In our case of 85-90% occlusion with surgical CBD partial ligation as an optimal condition of experimental osteoporosis induction , we only performed partial ligation in the control group and in our experimental group and folate iron was administered for 8 weeks of after partial ligation. The control group showed continuous rupture of periosteum, periosteal porosity, reduction in bone mass and osteoid findings pathomorphologically but pathological findings like above were hardly observed in experimental group. To sum up, osteoporosis was induced in more than 80% of the control group, but in less than 20% of the experimental group. This shows that iron intake may prevent induction of osteoporosis by chronic cholestasis.

CONCLUSION

1) Experimental osteoporosis is induced at 8 weeks after surgical CBD partial ligation (85-90% occlusion).

2) Iron intake significantly suppressed the development of osteoporosis by chronic cholestasis.

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