Journal of Korean Medical Science
Vol. 11, No. 5, 397-401 Oct. 1996

Effect of Cholestyramine on the Formation of Pigment Gallstone in High Carbohydrate Diet-Fed Hamsters

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This study was designed to investigate the effect of cholestyramine on the formation of pigment gallstones in high carbohydrate diet-fed hamsters and whether that effect occurred because of cholecystokinin action. Forty seven hamsters were divided into three groups : group I(n=16) was fed on normal rodent chow(43% carbohydrate), group II(n=14) was fed on a high CHO diet(65% carbohydrate), group III(n=17) was fed on a high CHO diet containing 4% cholestyramine. Gallstones developed in 0% of group I, 42.9% of group II and 5.9% of group III(P<0.05, group II vs III). To evaluate the chronic status of cholecystokinin level, the wet weight of pancreas and the average area of pancreatic acinar in microscopic high power field were measured. There was no significant difference between group II and group III in pancreatic weight and average area of pancreatic acinar(P>0.05). In gallbladder bile analysis, there was also no significant difference between group II and group III in cholesterol, phospholipid, total calcium, total bilirubin and bile acid levels. In conclusion, cholestyramine decreases the frequency of pigment gallstone formation in high CHO diet-fed hamsters, but it is not clear whether the mechanism of cholestyramine decreasing the gallstone formation is due to the action of cholecystokinin.

Key Words : *Cholestyramine, Pigment gallstone, Hamster*

INTRODUCTION

There is a high incidence of pigment gallstone formation that occurs in high carbohydrate diet-fed hamsters, but the exact mechanism has not yet been clarified. Cholecystokinin(CCK) induces gallbladder contraction and relaxation of sphincter of Oddi. It also stimulates pancreatic growth and secretion of pancreatic enzymes(Mainz et al.,1973 ; Folsh et al.,1978 ; Solomon et al.,1978). It was postulated that a high carbohydrate diet was a relatively weak stimulator of CCK(Walsh, 1987), caused relative bile stasis and increased the formation of pigment gallstone. The release of CCK is controlled by negative feedback, intraduodenal bile salts (Gomez et al.,1988) and trypsin(Owyang et al.,1986) inhibits CCK secretion. Many authors have suggested that cholestyramine, bile salt sequestrant, increased CCK level and gallbladder emptying(Brand and Morgan, 1982 ; Koop et al.,1988 ; Gomez et al.,1989 ; Palasciano et al.,1992 ; Portincasa et al.,1994), but there have only been a few reports about whether it actually decreases the formation of gallstones(Bergman and Linden,1967 ;

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• This study was supported by a grant from Hallym Medical Center(1994).

Trautwein et al.,1993). Therefore this study was designed to investigate the effect of cholestyramine on the formation of pigment gallstone and whether that effect occurred because of cholecystokinin action.

MATERIALS AND METHODS

Forty seven syrian golden hamsters weighing 66 to 167gm were used in this study. The hamsters were divided three groups by sex and weight : Group I(n=16) was fed normal rodent chow, Group II(n=14) was fed a high carbohydrate diet and Group III(n=17) was fed a high carbohydrate diet containing 4%(wt/wt) cholestyramine powder. The diet compositions of each group are shown in Table 1. The changes in body weight and the amount of food consumed were checked weekly. After a period of 6 weeks, the hamsters were fasted overnight and sacrificed. Gallbladder bile was taken by 1cc insulin syringe and stored at -70℃ for bile analysis. The presence or absence of gallstones was checked grossly and the pancreas and left kidney(as a control) were removed. To know the chronic status of CCK, the pancreatic weight and the average area of acinar in each group were measured(Image Analyser : IBAS, Zeiss Co.). We chose three mid-weight pancreas specimens in each group and made three slides. A pathologist who did not know the intent of this study, measured the sizes of 50 round-shaped pancreatic acinars from the area where the largest acinars were gathered in the high power field of each slide. Gallbladder biles from two to three hamsters(0.1 to 0.2ml per each) in the same group were pooled and analysed by kits. Cholesterol levels were measured by the method of Roeschlau(1974). Phospholipids were measured by the method of Takayama(1977). The calcium level was measured by the method of Aderegg(1954). The total bilirubin was measured by the method of Michaelsson(1981) and the total bile acid was measured by the method of Turley(1977). Fisher's exact test and the one way ANOVA test were used for statistical analysis. $p<0.05$ was considered significant.

RESULTS

The body weight after 6 weeks and diet consumption during experimental period were not significantly different among the three groups(Table 2, Fig. 1). Gallstones were found in zero %(0/16) of Group I, 42.9% (6/14) of Group II and 5.9%(1/17) of Group III($p<0.05$: Group II vs I, III ; Table 3). The high carbohydrate diet

Table 1. Diet composition of control and high carbohydrate group

	Control chow (%)	High carbohydrate chow (%)
Carbohydrate	42.6	65.1
Corn	29.8	0
Wheat	12.8	0
Rice	0	58.5
Sucrose	0	6.6
Protein	24.2	12.7
Fat	6.4	3.1
Fiber	4.1	1.1
Water	8.9	8.7
Micellaneous	13.8	9.3

Table 2. Diet consumption(gm/kg/day) during experimental period

	Group I (N=16)	Group II (N=14)	Group III (N=17)
1 week	63.1	72.4	58.7
2 week	59.7	56.5	60.2
3 week	68.3	61.9	74.7
4 week	61.9	64.0	71.6
5 week	69.9	62.5	67.6
6 week	57.9	52.1	56.8
Average	63.5±4.7	61.6±6.9	64.9±7.4

Values are means±SD. $p>0.05$

Table 3. Frequency of gallstone formation

	Group I (N=16)	group II (N=14)	Group III (N=17)
No. of stone(+)	0	6	1
Percentage(%)	0	42.9*	5.9

* $p<0.05$ vs Group I and III

Table 4. Pancreas weight and kidney weight per body weight

	Group I	Group II	Group III
Pancreas weight(mg/gm)	2.5±0.45	2.9±1.11	2.9+0.5
Left kidney weight(mg/gm)	4.1±0.6	4.1±0.68	4.5+0.59

Values are means±SD. $p>0.05$

Table 5. Average area of pancreatic acinar in high power field(N=150)

	Group I	Group II	Group III
Average area of acinar(pixels)	11848.8 ± 4862.3*	16153.0 ± 6729.6	16652.0 ± 6746.7

Values are means±SD. * $p<0.05$ vs Group II and III

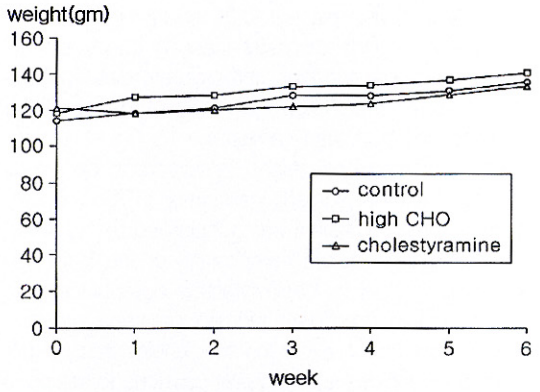

Fig. 1. Weight change during experimental period. There was no difference of body weight among three groups($p>0.05$)

Fig. 2. Gross feature of gallstones in hamster gallbladder

increased the incidence of gallstone formation and cholestyramine appeared to reverse the effect of lithogenic diet. All stones were black-colored, and their sizes were 0.1 to 1 mm in diameter making them easily visible after aspiration of gallbladder bile(Fig. 2). The pancreatic wet weight was not significantly different among the three groups($p>0.05$; Table 4). Average acinar area under microscopic high power field showed no difference between Group II and III, but that of Group I was significantly smaller than Group II and III($P<0.05$; Table 5). There were no significant differences between Group II and III in all measured components of gallbladder bile. Concentrations of cholesterol, phospholipid and total bilirubin in Group I tended to be higher than Group II and III. There were no differences among the three groups in the levels of total calcium and bile acid($p>0.05$; Table 6).

DISCUSSION

The secretion of bile salts exerts a physiologic negative feedback control on cholecystokinin and the decreased secretion of CCK by endogenous bile salts results in inhibition of pancreatic growth and gallbladder contraction(Gomez et al.,1988). Cholestyramine is a well-known bile salts sequestrant and the administration of it results in pancreatic growth and gallbladder contraction through the action of increased CCK level (Gomez et al.,1989). Therefore our study was designed to show that cholestyramine could decrease the incidence of gallstone and that the mechanism of action was through CCK. In the hamster model of cholesterol gallstones, cholestyramine decreased the incidence of gallstone formation but the mechanism of action was via a decreased lithogenic index(Trautwein et al.,1993). A similar study in animal models of pigment gallstone and in human beings has not yet been reported. In our study we found that cholestyramine also decreased the pigment gallstone formation in the high cabohydrate diet fed hamsters. Because the mechanism of pigment gallstone formaton is different from that of cholesterol gallstone formation, it is probable that the lithogenic index will not contribute to the formation of pigment gallstones. In our study of bile composition there was also no difference between two groups(lithogenic diet

Table 6. Gallbladder bile composition

	Group I (N=8)	Group II (N=6)	Group III (N=6)
Cholesterol (mg/dl)	101.2±38.3	63.3±12.8	48.1±14.0*
Phospholipid (mg/dl)	822.9±228.2	531.4±227.2	485.7±87.9*
Total calcium (mg/dl)	17.3±2.5	18.0±2.6	18.0±2.7
Total bilirubin (mg/dl)	18.9±6.6	10.4±1.8*	12.7±4.9
Bile acid (mmol/L)	38.6±31.2	51.9±38.4	34.3±23.3

Values are means±SD. * $p<0.05$ vs Group I

group vs. lithogenic diet containing 4% cholestyramine group). This finding suggested that the change of bile composition might not be the main cause of decreasing pigment gallstone formation in cholestyramine-treated hamsters. Is the decreasing effect of cholestyramine on the pigment gallstone formation due to CCK? Many previous authors have demonstrated that cholestyramine increases the CCK level and/or pancreatic growth(Brand and Morgan,1982; Koop et al.,1988; Gomez et al.,1989; Koop et al.,1991), but our study did not show that. Since we had not yet established the CCK assay, we could only show the CCK effect indirectly by measuring the pancreatic wet weight and average area of pancreatic acinar. These measurements showed no difference between Group II and Group III with the exception of the pancreatic acinar size of Group I which was smaller than Group II and III. In our previous study there was no difference between group of normal rodent chow and that of high carbohydrate diet. Theoretically, because pancreatic atrophy was anticipated in high carbohydrate group, this phenomenon was hard to interpret. In similar studies using camostat mesilate, antiproteinase, we confirmed the pancreatic hypertrophy(Kim et al.,1993; Lee et al.,1994). Camostat stimulates pancreatic growth through the CCK-mediated mechanism(Niederau et al.,1990). Therefore we thought that the pancreatic wet weight and the average area of pancreatic acinar would reflect the chronic status of CCK. In a study by Koop et al(1988), during 4 weeks of treatment with cholestyramine, the plasma CCK levels were increased for 3 weeks and then normalized at 4 weeks. But in a study of Brand and Morgan(1982), pancreatic wet weight and total protein and DNA content were increased after 4 or 8 weeks and the additive effect of cholestyramine on pancreatic growth was similar whether 2% or 6% cholestyramine was fed. In our study it was not clear whether no change in pancreatic wet weight and the average area of pancreatic acinar in cholestyramine-treated group was related to normalization of CCK after the 4 weeks of feeding, despite the fact that the results were different from the report of Brand and Morgan. This difference between our study and Brand & Morgan's might be ascribed to the difference in diet composition. The diet composition of Brand and Morgan's experiment was similar to our normal rodent chow. The proportion of carbohydrate, protein and fat in Brand and Morgan's study and ours was 52%, 22%, 6% vs. 64.9%, 12.7%, 3.1% respectively. The protein and fat proportions of our high carbohydrate diet were about half of Brand and Morgan's diet, and plasma CCK levels might also be relatively low. Since camostat induced pancreatic hypertrophy in even the high carbohydrate diet-fed hamsters, it appears that cholestyramine may be an weaker stimulator for CCK than camostat.

The mechanism by which cholestyramine decreases the formation of pigment gallstones in high carbohydrate diet-fed hamsters was not clarified by our study, and the change of bile composition or the increased plasma CCK level by cholestyramine does not explain the mechanism of action in pigment gallstone formation fully. In conclusion, we found that cholestyramine decreased the incidence of pigment gallstone formation in high carbohydrare diet-fed hamsters. Although it was expected that the increase of plasma CCK level by cholestyramine exerted on the decreased frequency of the pigment gallstone formation, the effect of cholestyramine that decrease the incidence of pigment gallstone in high carbohydrate diet-fed hamsters might not be due to CCK only. Therefore it is suggested that the decreasing effect of cholestyramine on the formation of pigment gallstones needs further study.

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