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Effect of Glucagon on Bile Acid Metabolism after Resection of Liver Cancer in Patients with Cirrhosis

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Abstract

After hepatectomy patients with cirrhosis and liver cancer may develop progressive hepatic dysfunction and eventually hepatic failure. Insulin and glucagon are often used to treat certain kinds of hepatic dysfunction and hepatic insufficiency. We investigated the effect of glucagon on bile acid metabolism and pancreatic endocrine function. In 7 patients with severe cirrhosis and cancer of the liver, 1 mg of glucagon was injected intravenously pre- and post-operatively, and total bile acids, C-AMP, and bile acid fractions were determined.

In the pre-operative glucagon tolerance test, the C-AMP level rose from a baseline of 14 ± 0.8 PMol/ml to 362 ± 94 PMol/ml 30 min after the injection of glucagon ($p < 0.01$); and the level of total bile acids decreased from a baseline of 28 ± 9 μMol/ml to 11 ± 3 μMol/ml 60 min after the injection of glucagon. The post-operative C-AMP level increased from a baseline of 13 ± 1 PMol/ml to 192 ± 58 PMol/ml level of 30 min after the injection of glucagon ($p < 0.01$), and the post-operative level of total bile acids decreased from a baseline of 64 ± 20 μMol/ml to 26 ± 7 μMol/ml 60 min after the injection of glucagon. There was a significant correlation between the 5-min increment ratio of C-AMP and the decrement ratio of total bile acids ($p < 0.01$). Analysis of bile acids showed that the pre-operative GCDCA level decreased from a baseline of $5,846 \pm 2,853$ ng/ml to $1,205 \pm 436$ ng/ml ($p < 0.05$), and the pre-operative TCDCA level from $3,169 \pm 1,384$ ng/ml to $1,576 \pm 866$ ng/ml 60 min after the injection of glucagon. The post-operative levels of GCDCA and TCDCA decreased from $18,487 \pm 4,257$ and $7,142 \pm 2,237$ ng/ml to $4,652 \pm 1,544$ and $2,061 \pm 811$ ng/ml, respectively; 60 min after the injection of glucagon ($p < 0.05$). These results reveal the important role of glucagon in serum bile acid metabolism.

Introduction

It is well known that insulin and glucagon are essential hormones in the metabolism of glucose,

Key words: Bile acid, Hepatectomy, Glucagon, Liver cirrhosis, C-AMP.
索引語：胆汁酸，肝切除，グルコガン，肝硬変，サイクリック AMP.
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protein, amino acids, lipids, electrolytes and bile acids, and in the regeneration of the liver. Insulin and glucagon are often used to treat hepatic insufficiency[3,6,12], but little is known about the role of glucagon in bile acid metabolism after hepatectomy in patients with severe cirrhosis and cancer of the liver. In this study, we investigated the effect of glucagon administration on bile acid metabolism and pancreatic endocrine function before and after hepatectomy in patients with liver cancer associated with advanced cirrhosis.

Table 1

Patient No.	R_{15} (%)	Rmax	HPT (%)	T. Bil	Ch-E	O-GTT	Alb	GOT
1	65	0.341	74	0.9	64	linear	2.3	118
2	18	0.300	51	0.7	66	linear	3.5	60
3	30	0.330	89	1.5	117	parabolic	3.3	274
4	32	0.301	51	2.6	44	parabolic	2.8	118
5	50	0.54	46	2.9	41	linear	3.1	79
6	50	0.42	59	1.2	51	linear	3.3	73
7	17	0.64	53	0.8	118	linear	3.4	93

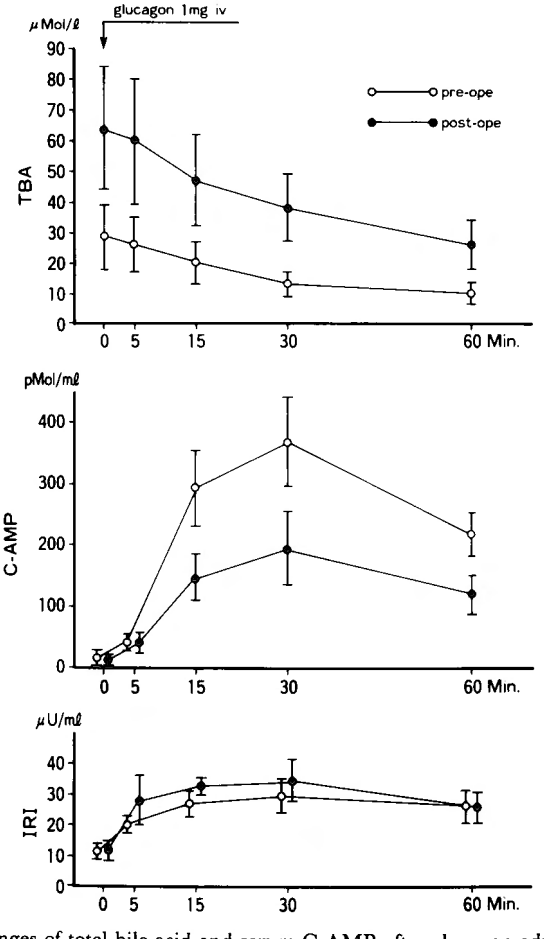

Fig. 1 Changes of total bile acid and serum C-AMP after glucagon administration.

Materials and Methods

The subjects were 7 patients with liver cancer and advanced cirrhosis in whom resection of less than one segment was possible as determined by the results of tests such as ICG-Rmax, Hepaplastin, O-GTT and serum albumin (Table 1).

Glucagon tolerance test (Fig. 1).

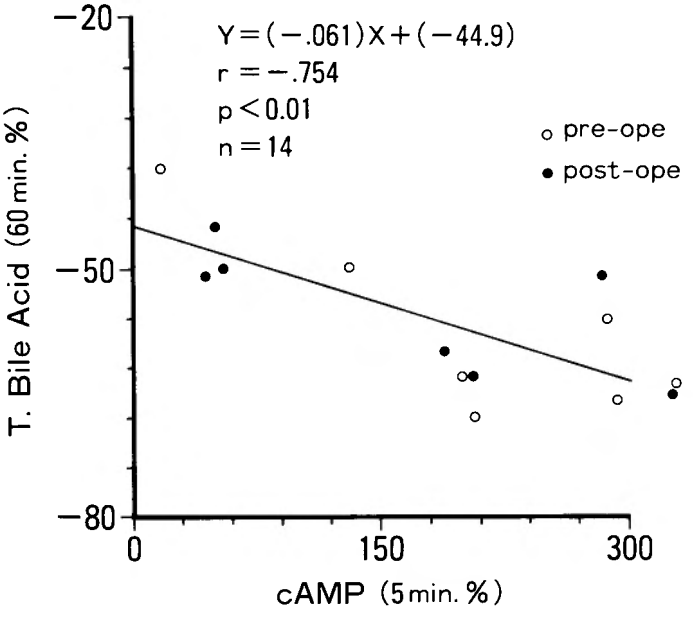

Fig. 2 Correlation between the 60-min value of total bile acids and the 5-min value of C-AMP before and after surgery ($p<0.01$).

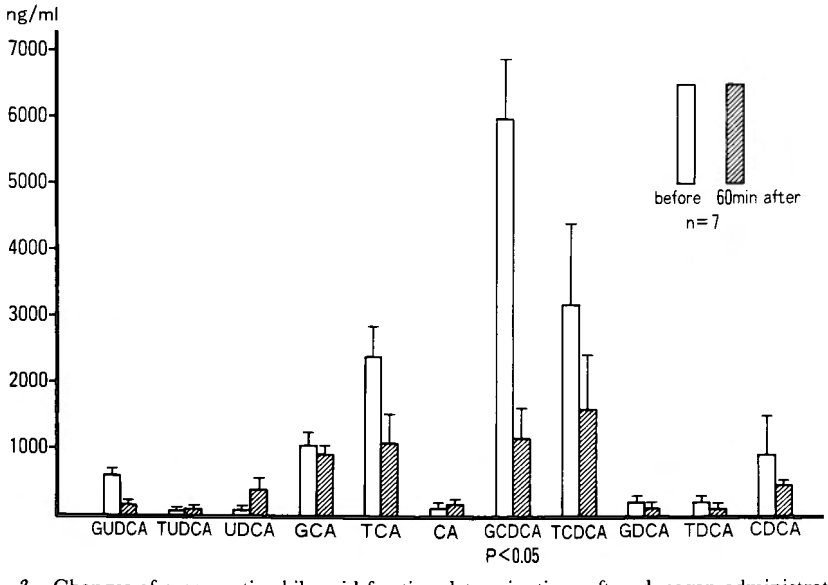

Fig. 3 Changes of preoperative bile acid fraction determinations after glucagon administration.

One month before and after hepatectomy, fasting blood was drawn early in the morning, 1 mg of glucagon (Novo) was injected intravenously, and blood samples were taken at 5, 15, 30, and 60 min, for the determination of total bile acids, C-AMP, insulin and bile acid fractions.

Blood sampling and method of measurement

Blood samples were placed immediately in wells cooled below −4°C. Serum was separated in a freezing centrifuge below −4°C and stored at −20°C. Total bile acid concentrations were deter-

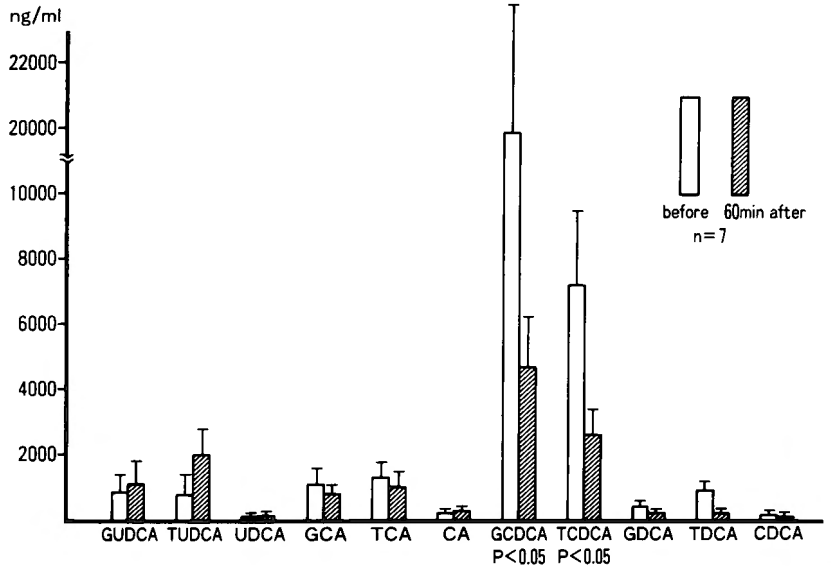

Fig. 4 Changes of postoperative bile acid fraction determinations after glucagon administration.

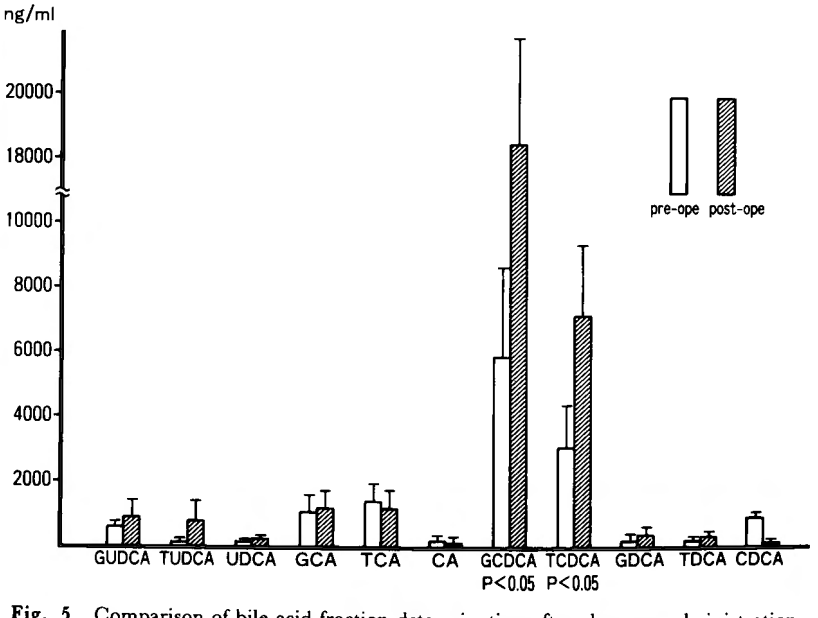

Fig. 5 Comparison of bile acid fraction determination after glucagon administration.

mined with an Enzabile kit[10], C-AMP with a RIA kit (Yamasa)[8]. Bile acid fractions were measured with HPLC in a column (3α-hydroxysteroid dehydrogenase immobilized on aminopropyl CPG-glass) by the method of Okuyama et al.[13]

The results were expressed as means±SEM. Student's t test was used for statistical analysis.

Results

1. Glucagon tolerance test (Fig. 1)

The preoperative fasting level of total bile acids in the blood was 28±9 μMol/ml, and it decreased gradually after the intravenous injection of 1 mg of glucagon: 26±9, 20±7, 13±4, and 11±3 μMol/ml at 5, 15, 30, and 60 min, respectively. On the contrary, the serum C-AMP level, which was 14±1 PMol/ml, fasting, before surgery, rose to 45±5, 289±63, 362±94, and 211±36 pMol/ml, 5, 15, 30, and 60 min, respectively, after the injection of glucagon ($p<0.01$). The preoperative level of fasting blood insulin was 11±1 μU/ml, and the level increased to 20±2, 26±4, 29±6, and 26±6 μU/ml 5, 15, 30, and 60 min, respectively, after the injection of glucagon ($p<0.05$).

The postoperative level of total bile acids in fasting blood was 66±20 μMol/ml. The level decreased gradually to 60±21, 47±16, 38±12, and 26±7 μMol/ml 5, 15, 30, and 60 min, respectively, after the injection of glucagon. On the contrary, the serum C-AMP level, which was 13±1 pMol/ml, fasting, after surgery, increased to 34±6, 124±39, 192±58, and 113±30 pMol/ml 5, 15, 30, and 60 min, respectively, after the glucagon injection ($p<0.01$). The blood insulin level, which was 12±2 μU/ml, fasting, after surgery, increased to 28±9, 33±3, 34±6, 25±6 μU/ml, respectively, after the glucagon injection.

There was a negative correlation between the 60-min value of total bile acids and the 5-min value of C-AMP before and after surgery ($p<0.01$) (Fig. 2).

The bile acid fraction determinations showed preoperative fasting GUDCA levels of 519±163 ng/ml for GUDCA, 81±38 ng/ml for TUDCA, 81±43 ng/ml for UDCA, 1,008±494 ng/ml for GCA, 1,224±547 ng/ml for TCA, 190±126 ng/ml for CA, 5,846±2,853 ng/ml for GCDCA, 3,169±1,384 ng/ml for TCDCA, 187±87 ng/ml for GDCA, 199±63 ng/ml for TDCA, and 838±676 ng/ml for CDCA. These values 60 min after the injection of glucagon were: GUDCA, 282±141 ng/ml; TUDCA, 23±36 ng/ml; UDCA, 120±41 ng/ml; GCA, 731±361 ng/ml; TCA, 673±283 ng/ml; CA, 275±144 ng/ml; GCDCA, 1,205±436 ng/ml; TCDCA, 1,576±866 ng/ml; GDCA, 91±59 ng/ml; TDCA, 101±48 ng/ml; and CDCA, 348±135 ng/ml (Fig. 3). The postoperative fasting levels were: GUDCA, 860±408 ng/ml; TUDCA, 730±574 ng/ml; UDCA, 93±33 ng/ml; GCA, 1,124±462 ng/ml; TCA, 1,312±531 ng/ml; CA, 123±60 ng/ml; GCDCA, 18,487±4,257 ng/ml; TCDCA, 7,142±2,237 ng/ml; GDCA, 368±178 ng/ml; TDCA, 244±90 ng/min; and CDCA, 83±15 ng/ml; 60 min after the glucagon injection they were: GUDCA, 1,071±778 ng/ml; TUDCA, 968±820 ng/ml; UDCA, 66±56 ng/ml; GCA, 736±275 ng/ml; TCA, 1,009±491 ng/ml; CA, 180±129 ng/ml; GCDCA, 4,652±1,544 ng/ml; TCDCA, 2,061±811 ng/ml; GDCA, 160±52 ng/ml; TDCA, 155±102 ng/ml; and CDCA, 93±47 ng/ml (Fig. 4). The postoperative fasting levels of GCDCA and TCDCA were much higher than the preoperative ($p<0.05$) (Fig. 5).

Discussion

Bile acids are synthesized from cholesterol in the liver, conjugated with glycine and taurine, and secreted into the bile (primary bile acids). In the intestines, bile acids undergo deconjugation and dehydroxylation by enterobacteria (secondary bile acids), and most of the bile acids are reabsorbed from the terminal ileum and returned to the liver via the portal circulation[7,16]. Hepatic disorders cause increased levels of serum bile acids[9,15]. Tanaka et al.[17] ascribed the increase in serum bile acids to the following factors: 1) impaired uptake by hepatic cell membranes, 2) impaired pathway within hepatic cells through which bile acids are transported by a specific carrier protein resulting in a return of bile acids to the blood stream, 3) appearance of bile acids in the blood stream through damaged junctional complexes in bile capillaries, and 4) collateral circulation. Ohkubo et al.[11] suggested that a factor responsible for the increase in serum bile acids in hepatic cirrhosis might be significant correlation between the portal vein index and serum bile acid levels. Bloomer et al.[1] found that serum bile acids were high in patients with severe hepatocellular injury, presumably because of impaired uptake of bile acids by hepatic cells. In their study of abnormal metabolic pathways of bile acids, Valhcevic et al.[18] reported that the pools of cholic acid and deoxycholic acid were reduced by a lack of 12α-hydroxylation. In the present study of patients with cirrhosis and liver cancer, the levels of GCDCA and TCDCA were higher than normal (Fig. 3), and they increased further after surgery (Fig. 5), but there were no significant changes in the other bile acid fractions. Dietmaier[5] and Reichen et al.[14] investigated the uptake of bile acids by hepatic cells using the rat hepatic perfusion system and demonstrated that bile acid uptake occurs through carrier transport and is Na^+-dependent. Glucagon activates adenyl cyclase in hepatic cell membranes, and the C-AMP produced acts as a second messenger[2,4]. It is considered that glucagon increases Na^+-dependent, active transport mediated by C-AMP in the hepatic cells, thus promoting bile acid uptake[14]. Glucagon also has an insulin secretion-stimulating action, and in our study insulin secretion was actually increased after glucagon administration; this seems to indicate that glucagon can stimulate insulin for ATP production, which activates hepatic cells.

Conclusion

When liver cancer is associated with severe cirrhosis, bile acid metabolism is disturbed and GCDCA and TCDCA are increased. After hepatectomy, this abnormality of bile acid metabolism progresses. Glucagon administration, through the elevation of C-AMP, was found to improve postoperative bile acid metabolism. The administration of glucagon seemed, therefore, to be useful after the resection of liver cancer in patients with severe cirrhosis.

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和文抄録

硬変合併肝癌切除後における胆汁酸代謝
—とくにグルカゴンの血清胆汁酸におよぼす影響について—

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肝硬変合併肝癌術後においては，肝機能障害の程度が進行し，肝機能不全への危険性をはらんでいる．ある種の肝機能障害肝機能不全に対して，インスリン，グルカゴン療法が行なわれているが，グルカゴン投与の血清胆汁酸に及ぼす影響について検討した．高度硬変合併肝癌においては胆汁酸代謝に異常を来し，総胆汁酸，胆汁酸分画 GCDCA, TCDCA が上昇しており，肝切除後には，さらにこれらの胆汁酸が増加したが，グルカゴン投与は C-AMP の上昇を介して血清胆汁酸，特に GCDCA, TCDCA を減少させる事が判明した．