World Journal of Gastroenterology

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DOI: 10.3748/wjg.v3.i4.255

World J Gastroenterol 1997 December 15; 3(4): 255-256
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL RESEARCH

Analysis of amino acid constituents of gallstones

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This project won The third-class award by the Beijing Hospital for research achievements.

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Received: October 26, 1996
Revised: December 15, 1996
Accepted: April 28, 1997
Published online: December 15, 1997

Abstract

AIM: To seek drugs that will efficaciously dissolve bilirubin, glycoprotein and black stones and that will represent improved lithotriptic agents to resolve cholesterol stones, and to study the amino acid constituents of gallstones.

METHODS: According to characteristics determined by infrared spectroscopy and to the contents of bilirubin determined by semi-quantitative chemical analysis, 30 of 148 cases of gallstones were selected and divided into 5 groups. Amino acids of the 30 cases were detected by high-speed chromatography.

RESULTS: The quantity of amino acids was highest in black stones (226.9 mg/g) and lowest in pure cholesterol stones (1.4 mg/g). In the 5 groups of gallstones, the quantity of amino acids followed the hierarchy of black stone > mixed bilirubin stone and glucoprotein stone > mixed cholesterol stone > pure cholesterol stone. The proportions were: 95.95:29.02 and 28.05:5.78:1. Aliphatic amino acids accounted for ~ 50% of the total amino acids in the gallstones, with glycine accounting for 15.3% of the total amount of the 17 kinds of amino acids.

CONCLUSION: For mixed stones, the higher level of bilirubin, the higher content of amino acids. Acidic amino acids were relatively higher in bilirubin stones than in cholesterol stones.

Key words: Gallstones; Amino acids/analysis; Bilirubin; Glycine

Chen Y, Wang LL, Xiao YX, Ni JH, Yu Y. Analysis of amino acid constituents of gallstones. *World J Gastroenterol* 1997; 3(4): 255-256 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i4/255.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i4.255

INTRODUCTION

Dissolution of cholesterol stones can be achieved by oral ursodeoxycholic acid or chenodehydrocholic acid, and by perfusion with methyl tertiary butyl ether as well. However, no effective means of dissolving bilirubin, glycoprotein and black stones has been reported. In order to identify better lithotriptic agents, the contents of amino acids in gallstones needs to be studied.

MATERIALS AND METHODS

Sample selection

Gallstones were obtained from 148 patients who were treated by surgery in our hospital during the years of 1988 to 1992. The specimens were subjected to qualitative assessment by infrared spectroscopy, and 48 cases were also subjected to semi-quantitative chemical analysis. According to the characteristics corresponding to the infrared spectrum and the constituents of bilirubin, 30 cases were selected and divided into the following 5 groups: Pure cholesterol stones (n = 10), mixed cholesterol stones (n = 7), mixed bilirubin gallstones (n = 10), glycoprotein stones (n = 2), and black stone (n = 1).

Sample treatment[1]

After pulverizing by agate mortar and drying, 20 mg of powder from each gallstone was added to 6 mL of HCl (6 mol), nitrogen sealed and baked at 110 ℃ for 24 h. The volume was brought to 25 mL with distilled water. After filtration, 4 mL was collected, dried in a rotary evaporator, and washed twice with distilled water. The remaining sample was dissolved in 2 mL distilled water, of which 50 μL or 100 μL was used to measure the 17 amino acids, and taurine and ammonia concentrations. A trace of tryptophane was detected in 2 cases.

Analytical methods

Amino acids were detected by high-speed chromatography (L-8500; Hitachi Corp, Japan). The column was 4.6 mm × 60 mm, and 5 buffer solutions were used for the stepwise wash-off, with resin of 2622 s.c. (Hitachi ion exchange resin was used). The standard amino acid samples were provided by Sodium Glutamate Corp. (Japan). The quantitative analysis was conducted with extensional calculation. The coefficient of variation (c.v.) was 1.5% in this experiment.

Table 1 Content of various amino acids in 5 groups (30 cases) of gallstones ($\bar{x} \pm s$, mg/g)

	Pure cholesterol		Mixed cholesterol		Mixed bilirubin		Glycoprotein		Black
	n	$\bar{x} \pm s$	*n*	$\bar{x} \pm s$	*n*	$\bar{x} \pm s$	*n*	$\bar{x} \pm s$	
Gly.	10	0.635 ± 0.498	7	2.333 ± 2.890	10	10.459 ± 4.451	2	19.185 ± 0.955	16.552
Ala.	8	0.109 ± 0.084	7	0.794 ± 0.622	10	4.048 ± 2.409	2	1.925 ± 2.298	13.551
Val.	9	0.133 ± 0.089	7	1.043 ± 0.674	10	4.202 ± 2.709	2	3.500 ± 0.948	15.383
Ile.	5	0.080 ± 0.044	7	0.496 ± 0.330	10	2.407 ± 1.374	2	1.590 ± 0.523	4.921
Leu.	9	0.150 ± 0.093	7	1.113 ± 0.671	10	5.914 ± 3.983	2	4.510 ± 0.410	16.834
Thr.	10	0.112 ± 0.077	7	0.743 ± 0.499	10	3.784 ± 2.874	2	3.080 ± 0.820	11.642
Ser.	10	0.117 ± 0.077	7	0.790 ± 0.565	10	2.836 ± 1.799	2	3.085 ± 0.870	11.643
Pro.	4	0.116 ± 0.054	5	0.621 ± 0.203	10	2.953 ± 2.167	2	2.560 ± 0.968	12.421
Sys.	2	0.060 ± 0.014	6	0.422 ± 0.211	10	2.018 ± 1.199	2	2.235 ± 0.813	9.943
Met.	4	0.038 ± 0.013	4	0.398 ± 0.479	10	1.080 ± 0.670	2	0.800 ± 0.070	3.309
Phe.	10	0.160 ± 0.089	7	0.797 ± 0.451	10	3.499 ± 2.397	2	3.060 ± 0.509	9.928
Thr.	8	0.108 ± 0.055	7	0.596 ± 0.351	10	2.483 ± 1.713	2	1.710 ± 0.453	6.907
Asp.	10	0.220 ± 0.162	7	1.374 ± 0.869	9	6.066 ± 4.039	2	5.250 ± 1.796	24.103
Glu.	10	0.293 ± 0.194	7	1.723 ± 1.088	10	8.595 ± 5.468	2	7.460 ± 1.950	34.358
Lys.	9	0.126 ± 0.076	7	0.680 ± 0.405	10	4.058 ± 3.195	2	2.405 ± 1.124	17.582
His.	9	0.071 ± 0.039	7	0.401 ± 0.258	10	2.041 ± 1.983	2	0.945 ± 0.247	4.316
Arg.	9	0.012 ± 0.078	7	0.716 ± 0.418	10	4.012 ± 2.492	2	3.010 ± 1.923	13.517
Tau.	5	0.052 ± 0.028	9	1.695 ± 1.697	9	1.695 ± 1.697	1	1.540	1.608
Ammon.	10	0.662 ± 0.232	10	5.332 ± 1.590	10	5.332 ± 1.590	2	6.130 ± 1.174	6.435

RESULTS

The various contents of amino acids for the 30 gallstone cases in the 5 groups are presented in Table 1. All 30 had glycine, glutamic acid, threonine, phenylalanine and ammonia; among these, glycine content was the highest, accounting for 15.34% of the total amount, followed by glutamic acid, accounting for 13.01%. Asparagine, serine, valine, leucine, lysine, histidine and arginine were detected in 29 of the gallstone cases. There was a strong correlation ($P < 0.01$) between the above-mentioned amino acids. In 29 gallstone cases, there were more acidic amino acids than alkaline amino acids (1.39-2.73:1), except for a single bilirubin mixed stone (1:1.77) which had the appearance of black mud and came from a patient with malignant changes in the gallbladder and liver metastasis detected in postoperative pathologic examinations. The content of amino acids in one sample of pure cholesterol stones was the lowest for the 30 cases of gallstones examined (1.37 mg/g). Six amino acids (glycine, glutamic acid, aspartic acid, serine, threonine, and phenylalanine), taurine and ammonia were detected in this case. In the 5 groups of gallstones, the constituents of amino acids of one case of black gallstone were the most complete and the total amount of amino acids also was the highest (226.93 mg/g), only the content of glycine was slightly lower than that of glycoprotein.

In 10 cases of pure cholesterol stones, the content of glycine was higher than that of glutamic acid and aspartic acid, and that of glutamic acid was higher than that of aspartic acid, with the differences being statistically significant ($P < 0.005$).

In 7 cases of mixed cholesterol stone cases, the content of glutamic acid was significantly higher than that of aspartic acid ($P < 0.01$), but the content of glycine was higher, but not significantly, than that of glutamic acid and aspartic acid ($P > 0.05$).

In 10 cases of mixed bilirubin stones, although the content of glycine was higher than that of glutamic acid and aspartic acid, and that of glutamic acid was higher than that of aspartic acid, there was no significant differences ($P > 0.05$).

DISCUSSION

The amino acid is the fundamental unit of protein constitution. Nowadays, it is known that there are 20 kinds of amino acids[2], which are controlled by genetic code in protein molecules. They are called living proteinic amino acids, and consist of glycine, alanine, valine, leucine, isoleucine, methionine, proline, phenylalanine, tryptophan, tyrosine, serine, threonine, cysteine, glutamic acid, aspartic acid, histidine, lysine, arginine, asparagine and glutamine. Of the 20 living proteinic amino acids, 17 kinds were detected in this study; asparagine and glutamine were not detected because during this experiment[3] they would be completely hydrolyzed to free aspartic acid and glutamic acid, leading to artifactually higher amounts. Moreover, the proteins in this study were heated and hydrolyzed with 6 mol HCl, and tryptophan was damaged; additionally, cystine, methionine, threonine and serine were also influenced by the method, so the results for each were expected to be low. It has been reported that cysteine only exists in bilirubin gallstones, but in our experiment cysteine was not detected and only a small amount of the bisulfide compound of cysteine cystine and taurine produced during the conversion of cysteine in liver was detected.

Our results indicate that protein amino acids generally exist in gallstones. The components of amino acids detected in the gallstones were: Aliphatic amino acid > Acidic amino acid > Alkaline amino acid > Aromatic amino acid > Sulfur amino acid. In the 5 groups of gallstones of this study, the contents of amino acids were: Black gallstone > Mixed bilirubin stones and glucoprotein stone > Mixed cholesterol stone > Pure cholesterol stone. Their proportions were: 95.95:29.02 and 28.05:5.78:1. Glycine was the most abundant among the 8 kinds of aliphatic amino acids or the 17 kinds of amino acids in gallstones. For mixed stones, the higher the content of bilirubin, the higher the content of amino acids. With the exception of the black gallstones, all gallstones showed higher amount of glycine than that of aspartic acid and glutamic acid. However, in the pure cholesterol stones, glycine content was higher than aspartic acid and glutamic acid content ($P < 0.005$); thus, it was clear that proportion of acidic amino acids in bilirubin stones was relatively higher than that in cholesterol gallstones. In 30 cases of gallstones, the components of acidic amino acids were higher than those of alkaline amino acids, except for the bilirubin mixed stones from a patient with malignant changes in the gallbladder and liver metastasis that was determined by postoperative pathologic examination. It is likely that the metabolic product of malignant tissue is not favorable for the stable existence of acidic amino acids. The mechanism underlying this observation, however, remains unknown.

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L- Editor: Filipodia **E- Editor:** Liu WX

Published by **Baishideng Publishing Group Inc**
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ISSN 1007-9327