Expression of pituitary adenylate cyclase-activating polypeptide 1 and 2 receptor mRNA in gallbladder tissue of patients with gallstone or gallbladder polyps

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Abstract

AIM: To detect the expression of pituitary adenylate cyclase-activating polypeptide receptor 1 (VPCAP-1-R) and VPCAP-2-R mRNA in gallbladder tissues of patients with gallstone or gallbladder polyps.

METHODS: The expression of VPCAP-1-R and VPCAP-2-R mRNA in gallbladder tissues was detected in 25 patients with gallstone, 8 patients with gallbladder polyps and 7 donors of liver transplantation by reverse transcription polymerase chain reaction (RT-PCR).

RESULTS: The VPCAP-2-R mRNA expression level in the control group (1.09±0.58) was lower than that in the gallbladder polyp group (1.64±0.56) and the gallstone group (1.55±0.45) (P<0.05) while the VPCAP-1-R mRNA expression level in the control group (1.15±0.23) was not apparently different from that in the gallbladder polyp group (1.28±0.56) and the gallstone group (1.27±0.38).

CONCLUSION: The abnormal expression of VPCAP-2-R mRNA in gallbladder tissue may play a role in the formation of gallstone and gallbladder polyps.

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Key words: VPCAP-1-R; VPCAP-2-R; RT-PCR; Gallbladder disease

INTRODUCTION

Gallbladder motility and bile delivery to the duodenum involve a complex interplay between neural and hormonal factors. Acetylcholine, cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) in the nerve endings function as neurotransmitters, leading to contraction and relaxation of the gallbladder musculature[1-3]. VIP can relax the gallbladder, reduce gallbladder tone and inhibit CCK-stimulated contraction in a dose-dependent manner [4]. VIP exerts its action through receptors on the gallbladder wall and binds to two subtypes of VIP receptors, previously called VIP₁ and VIP₂ receptors. Because these receptors also have a high affinity for pituitary adenylate cyclase-activating polypeptide (PACAP), they have recently been named VPCAP₁ and VPCAP₂ receptors. The purpose of this study was to detect the expression of VPCAP₁-R and VPCAP₂-R mRNA in gallbladder tissue and to define their role in the formation of gallstone and gallbladder polyps.

MATERIALS AND METHODS

Patients

Gallbladder tissue from 25 patients with gallbladder cholesterol stone (12 men, 13 women, mean age 59.6 years, range 34-5 years) and 8 patients with gallbladder cholesterol polyps (2 men, 6 women, mean age 46.8 years, range 26-64 years) was obtained during surgery. Patients who had a history of acute cholecystitis were excluded. Gallbladder tissue from 7 donors of liver transplantation (all men, mean age 41.4 years, range 25-63 years) was used as control. The tissues were frozen in liquid nitrogen and stored at -80 °C.

Extraction of RNA

Total RNA was extracted from 100 mg gallbladder tissue samples using TRIzol reagent according to the manufacturer’s instructions. The concentration and purity of RNA were determined by a spectrophotometer at 260 and 280 nm. All RNA isolates had an OD₂₆₀/OD₂₈₀ value of 1.8±2.0, indicating clean RNA isolates.
Reverse transcription-polymerase chain reaction (RT-PCR)
The primers for amplifying VPCAP-R and VPCAP-B mRNA were designed using the published Homo sapiens VPCAP-R mRNA (NM 004624) and VPCAP-B mRNA (NM 003382) sequences. The sequences of primers for VPCAP-R mRNA were: forward 5'- AGATGCAGCTCACTACCTAT -3' and reverse 5'- TTCAGAGTCCCTCAGTCCCTT-3', which generated a 179-bp amplification product. The sequences of primers for VPCAP-B mRNA were: forward 5'- TGCTGCAACAAGCTCATCCCT -3' and reverse 5'- GACCCAACACTTCAGTTACCAC -3', which generated a 380-bp amplification product.

Two micrograms of total RNA was used as a template for subsequent RT-PCR. The total RNA was mixed with 1 µL oligo(dT)18, 1 µL dNTPs and H2O and preheated at 65°C for 1 min to denature the secondary structure. The mixture was then cooled rapidly to 30°C for 1 min, 10 µL 2X RT buffer, 4 µL 25% MgSO4, 1 µL 22 u/µL AMV, 0.5 µL 40 u/µL RNase-inhibitor were added. Reverse transcriptase was added for a total volume of 20 µL. The RT mixture was incubated at 65°C for 30 min and then stopped by heating at 98°C for 5 min and cooling at 5°C for 5 min.

PCR was performed on a PTC-200 PCR machine using 3 µL of cDNA, 0.1 µL of each oligonucleotide primer, 2 µL of each dNTP, 0.2 µL Taq polymerase and 10 X Taq polymerase buffer in a total volume of 25 µL. The PCR conditions were denaturation at 94°C for 3 min, then a 94°C for 45 s, followed by 35 cycles of annealing of VPCAP-R mRNA at 52.5°C for 1 min and VPCAP-B mRNA at 57.3°C for 1 min, extension at 72°C for 1 min, a final extension at 72°C for 7 min.

The PCR products were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide. The gels were photographed on top of a 280 nm UV light box. The gel images were captured with a digital camera and analyzed with the ID Kodak Imager analysis program. RT-PCR values were presented as a ratio of the receptor mRNA signal divided by the β-actin signal.

Statistical analysis
Data were expressed as mean ± SD. Statistical analyses were performed by the independent two-tailed t test. P<0.05 was considered statistically significant. The SPSS11.5 software was used for statistical analysis.

RESULTS
Total RNA isolated from gallbladder tissues was subjected to reverse transcription-PCR analysis for the expression of VPCAP-R and VPCAP-B mRNA. A 179-bp band and a 380-bp band, specific for VPCAP-R and VPCAP-B mRNA, were found in gallbladder tissue of all the three groups (Figures 1A and 1B). Furthermore, expression of VPCAP-R and VPAC1 P-R mRNA was detected by RT-PCR assay. The levels of PCR amplified VPCAP-R and RT-PCR amplified VPCAP-B mRNA and β-actin mRNA in three groups were compared.

Expression of VPCAP-R mRNA in gallbladder tissue
The VPCAP-R mRNA level in control group (1.15 ± 0.23) was not significantly different from that in gallbladder polyps group (1.28 ± 0.56) and gallstone group (1.27 ± 0.38) (Table 1).

Expression of VPCAP-B mRNA in gallbladder tissue
The VPCAP-B mRNA level in control group (1.09 ± 0.58) was lower than that in gallbladder polyps group (1.64 ± 0.56) and gallstone group (1.55 ± 0.45) (P <0.05) while no difference in the expression of VPCAP-B mRNA was found between these two groups (Table 1).

DISCUSSION
Vasoactive intestinal peptide (VIP), a 28-amino acid peptide capable of inducing vasodilation, was first isolated from porcine intestine[5]. It has many other actions as a neuroendocrine hormone and neurotransmitter. It may play an important role in the central nervous system (CNS)[8]. VIP can stimulate prolactin secretion from the pituitary[1], regulate noncholinergic trans-synaptic functions of the adrenal medulla[9], and inhibits proliferation of T cells in the immune system[9]. Other functions of VIP include protection against oxidant injury[10], stimulation of
electrolyte secretion\textsuperscript{10}, relaxation of smooth muscle\textsuperscript{11,12}. Intrinsnic nerves modulate gallbladder function. Nitric oxide synthase (NOS) and VIP are present in gallbladder neurons and nitric oxide and VIP modulate its epithelial functions\textsuperscript{13}. Intravenous infusion of VIP is associated with the secretion of bicarbonate from the gallbladder mucosa\textsuperscript{14}. Relaxation of canine gallbladder depends on nerve stimulation by adrenergic and non-adrenergic as well as non-cholinergic (NANC) nerves. Nitric oxide and VIP contribute to relaxation of NANC nerves in canine gallbladder\textsuperscript{15}. The effect of VIP on guinea pig gallbladder in vitro suggests that VIP has no effect on basal tone, but produces a 26.7 ± 6.6\% relaxation of CCK-contracted strips\textsuperscript{16}.

The first recombinant receptor for VIP is isolated from rat lung by Ishihara et al\textsuperscript{17}. This receptor is originally described as the VIP receptor and subsequently designated as the VIP\textsubscript{1} receptor\textsuperscript{18}. Messenger RNA encoding the VPCAP\textsubscript{1} receptor is widely distributed in CNS\textsuperscript{19,20}, peripheral tissues of liver\textsuperscript{21}, lung\textsuperscript{22,23} and intestine\textsuperscript{24} as well as in T lymphocytes\textsuperscript{25}. The second receptor that responds to VIP and PACAP with comparable affinity has been cloned from the rat olfactory bulb by Lutz et al\textsuperscript{17}. The highest concentration of messenger RNA is found in CNS\textsuperscript{26}. The receptor is also present in several peripheral tissues of pancreas, skeletal muscle, heart, kidney, adipose tissue, testis and stomach\textsuperscript{27,28}.

Researches about the distribution of VIP receptor in the gallbladder tissues are relatively few. Guo et al\textsuperscript{29} studied VIP receptor expression in patients with gallstones using immunohistochemical technique and found that positive VIP receptor expression level is higher in patients with abnormal fasting gallbladder volume than in patients with normal fasting gallbladder volume. Fu et al\textsuperscript{30} studied values of the max bind content (Bmax) of VIP receptor in gallbladder wall tissue of guinea pigs by radioisogand binding assay and found that the values of Bmax are obviously increased during formation of gallstone. Dupont et al\textsuperscript{31} found that there are specific binding sites for VIP in isolated epithelial cells of human gallbladder measured by radioimmunoassay. Their results indicate two functionally independent classes of receptor sites and VIP strongly stimulates adenosine 3':5' monophosphate (cyclic AMP) production.

In our study, the VPCAP\textsubscript{1} receptor mRNA level in gallstone group was significantly different from that in control group; the VPCAP\textsubscript{2} receptor mRNA level in gallstone group was higher than that in control group; predominant VPCAP\textsubscript{2} receptor was found in smooth muscle (in blood vessels and smooth muscle layer of the gastrointestinal and reproductive systems). The main hormonal regulator of gallbladder contraction is CCK. Recent studies suggest that CCK receptor mRNA level is down-regulated in patients with gallstone and animal\textsuperscript{32,33}. Previous studies have shown that human gallbladders with cholesterol stone reduce their contractions in response to agonists such as cholecystokinin, acetylcholine and muscle defects responsible for impaired gallbladder muscle contraction in plasma membranes of smooth muscle cells because of excessive incorporation of cholesterol\textsuperscript{34,35}. The diffuse membrane defect caused by cholesterol may also affect other transmembrane proteins that mediate muscle relaxation. It was reported that gallbladder relaxation is significantly reduced in gallbladders with cholesterol stones\textsuperscript{36}. Up-regulation of VPCAP\textsubscript{2} receptor mRNA may compensate for the abnormal receptor function of cholesterol. But the down-regulation of CCK receptor mRNA cannot compensate for the abnormal receptor function of membranes. Therefore contraction function of gallbladder is greatly affected rather than the relaxation function. Since up-regulation of VPCAP\textsubscript{2} receptor mRNA in epithelial cells can affect their secreting function, the abnormal expression of VPCAP\textsubscript{2} receptor mRNA may play a role in gallstone formation.

Excess cholesterol is the main cause of gallbladder polyps and may reduce the membrane fluidity, which in turn affects receptor function or receptor G-protein interaction. There are two specific binding sites for VIP in isolated epithelial cells of human gallbladder. In our study, VPCAP\textsubscript{2} receptor mRNA was over-expressed in patients with gallbladder polyps, which may be due to the abnormal receptor functions of cholesterol. Over-expression of VPCAP\textsubscript{2} receptor mRNA may occur in epithelial cells, leading to abnormal secretion and absorption of epithelial cells. This disorder may play a role in formation of gallbladder polyps.

A large number of factors, such as genetics, cholesterol saturation, sphincter of Oddi pressure, bacterial contamination of biliary tree, can induce formation of gallbladder stone and gallbladder polyps. The motility disturbances related to up-regulation of VPCAP\textsubscript{2} receptor mRNA may play a role in formation of gallbladder stones and gallbladder polyps. However, what cell membranes does the over-expression of VPCAP\textsubscript{2} receptor mRNA occur needs to be further studied.

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