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Effect of emodin on mobility signal transduction system of gallbladder smooth muscle in Guinea pig with cholelithiasis

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ABSTRACT

Objective: To study the effect of emodin on protein and gene expressions of the massagers in mobility signal transduction system of cholecyst smooth muscle cells in guinea pig with cholesterol calculus.

Methods: The guinea pigs were randomly divided into 4 groups, such as control group, gall-stone (GS) group, emodin group and ursodeoxycholic acid (UA) group. Cholesterol calculus models were induced in guinea pigs of GS, emodin and UA groups by lithogenic diet, while emodin or UA were given to the corresponding group for 7 weeks. The histomorphological and ultrastructure change of gallbladder were detected by microscope and electron microscope, the content of plasma cholecystokinin (CCK) and $[Ca^{2+}]_i$ were analyzed successively by radioimmunoassay and flow cytometry. The protein and mRNA of Gsα, Giα and Cap in cholecyst cells were determined by western blotting and real time polymerase chain reaction (RT-PCR).

Results: Emodin or UA can relieve pathogenic changes in epithelial cells and muscle cells in gallbladder of guinea pig with cholesterol calculus by microscope and transmission electron microscope. In the cholecyst cells of GS group, CCK levels in plasma and $[Ca^{2+}]_i$ decreased, the protein and mRNA of GS were down-regulated, the protein and mRNA of Gi and Cap were up-regulated. Emodin significantly decreased the formative rate of gallstone, improved the pathogenic change in epithelial cells and muscle cells, increased CCK levels in plasma and $[Ca^{2+}]_i$ in cholecyst cells, enhanced the protein and mRNA of Gs in cholecyst cells, reduced the protein and mRNA of Gi and Cap in cholecyst cells in guinea pig with cholesterol calculus.

Conclusion: The dysfunction of gallbladder contraction gives rise to the disorders of mobility signal transduction system in cholecyst smooth muscle cells, including low content of plasma CCK and $[Ca^{2+}]_i$ in cholecyst cells, abnormal protein and mRNA of Gs, Gi and Cap. Emodin can enhance the contractibility of gallbladder and alleviate cholestasis by regulating plasma CCK levels, $[Ca^{2+}]_i$ in cholecyst cells and the protein and mRNA of Gs, Gi and Cap.

1. Introduction

Cholelithiasis represents a significant burden on healthcare systems throughout the world, affecting approximately 22.87% of the Han Chinese [1]. Cholelithiasis is also an important public health problem in some Asian countries including China, where cholecystectomy was executed for 11.5% of all hospitalizations between 1985 and 1995 [2–4]. Several mechanisms contribute to gallbladder stone formation, including gallbladder hypo motility, cholesterol super-saturation of bile, destabilization of bile by kinetic protein factors, and abnormal mucin products [2,5,6]. Among them gallbladder motility disorders is more important for cholelithiasis that is triggered by the abnormality of gallbladders smooth muscle movement [7,8]. Impaired muscle contraction and relaxation in gallbladders may be due to an abnormal

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cholecystokinin (CCK) receptor-binding capacity and a difficult signal-transduction cascade distal to the activation of G proteins [9,10]. The mechanism for abnormal CCK receptor-binding capacity gives rise to decreased membrane fluidity, an excessive cholesterol content and high cholesterol-to-phospholipid ratio, which may affect the functions of G protein signal-transduction cascade, including inhibitory adenylate cyclase G protein (Gi), stimulating adenylate cyclase G protein (Gs), second messengers inositol 1,4,5-trisphosphate (IP_3) and the enzyme Calponin (Cap) [11].

Emodin (1, 3, 8 – trihydroxy – 6 – methyl -anthraquinone) is a biologically active natural anthraquinone extracted from the roots and rhizomes of *Rheum palmatum* (Chinese name DaHuang), which is one of the most effective traditional Chinese medicines for constipation and has now been officially listed in the Chinese Pharmacopoeia [12]. Some possible mechanisms could be involved in the emodin-induced smooth muscle contraction in the gastrointestinal tract. Firstly, emodin enhances the function of small intestinal peristalsis through inhibiting the secretion of somatostatin and triggering the release of motilin [13]. Secondly, emodin directly contracts the colon smooth muscle by elevating the intracellular Ca^{2+} concentration [14]. It has been reported that emodin has been applied in cholesterol stones and cholecystitis for enhancing the contraction of gallbladder smooth muscle [15]. However, the mechanisms have not been fully elucidated.

This study intends to explore the mechanism of emodin regulating the motility of gallbladder signal transduction function and improving the gallbladder contraction ability, including histological analysis and of cholesterol gallstone in guinea pig, plasma CCK levels and $[Ca^{2+}]_i$ concentration detection, Gs, Gi, Cap protein and mRNA determination in the gallbladder smooth muscle cells.

2. Materials and methods

The antibodies of Gs, Gi, Cap, β-actin were purchased from Abcam. Ursodeoxycholic acid (UDCA') was purchased from Sanwei Pharmaceuticals (Shanghai, China). Benzyl sulphonic acid fluorine (PMSF) was from Shanghai Shengneng gaming biological technology co., Ltd; Acrylamide was from Gibco; Tris-base was from Boehringer; Emodin, sodium dodecyl sulfate (SDS), protease inhibitor cocktail, glycine, ammonium persulfate, DEPC and TEMED were from Sigma; Chemiluminescence chromogenic reagent kit and protein assay kit were from (Thermo Scientific). Hematoxylin and eosin were purchased from Sinopharm Chemical Reagent Co., Ltd. Cholecystokinin (CCK) radiation immunity test kit was provided by the second military medical university neurobiology teaching and research section.

2.1. Animals and grouping

White guinea pig with red eye [200–240 g/body weight, half were male and half were female], were obtained from the Experimental Animals Center of the Shanghai University of Traditional Chinese Medicine (Shanghai, China). Guinea pigs maintained under pathogen-free conditions at a room temperature of (23 ± 3) °C and air humidity of (55 ± 15)% in a 12 h light/12 h dark cycle.

A total of 60 guinea pigs were provided free access to water and were divided into four groups (15 guinea pigs for each group) according to their body weight: control group, gall-stone (GS) group, emodin group and ursodeoxycholic acid (UA) group. Guinea pigs in GS group were fed by lithogenous diet containing 1% cholesterol, 0.5% cholic acid and 15% butter fat for 8 weeks. For gallstone prevention studies, guinea pigs in emodin group were given a lithogenic diet supplemented with emodin (Sigma, USA) at 100 mg/kg every 12 h for 8 weeks. Those in UA group were administered with a lithogenic diet supplemented with UA (80 mg/kg/d) (Shanghai Sanwei Pharmaceuticals, China, No.H31021950) for 8 weeks [16]. The gallbladder samples were taken for detection at the eighth weekend. The experimental protocols were approved by the Committee of Animal Experimentation of the Shanghai University of Traditional Chinese Medicine.

2.2. Histological analysis

Gallbladder specimens were fixed in 10% buffered formalin, processed by standard techniques and embedded in paraffin. Cross-sectional cuts 3 μm thick were taken from the middle zones of the gallbladder. The sections were stained with hematoxylin and eosin for histopathology, and examined with a light microscope (Zeiss Axioscop 40, Zeiss) by a pathologist who was blinded to the experimental groups. Ten high power fields were observed (×200), and digital images were obtained with a digital camera (Nikon 4500, Japan) and archived.

2.3. Electron microscopy

Obtain the gallbladder specimen fast. The gallbladder slices were prepared for electron microscopy by fixation, dehydration, and embedment in araldite in situ. Portions of the embedment containing suitable cells selected by light microscopy were cut out and affixed to a small metal dowel, sectioned in a Porter-Blum microtome with glass knives, mounted on bare grids, stained with 1.0% $KMnO_4$, and studied in a Siemens Elmiskop I.

2.4. Radioimmunoassay

2 mL blood was obtained from guinea pig heart accurately and injected into precooling, 20 μL 1% heparin anticoagulant. Serum (100 μL was diluted with 400 μL of saline and extracted twice with 2 mL of diethyl ether). The water phase was left frozen in solid carbon dioxide bath and ether extracts were combined and evaporated to dryness. The buffer or standard solution, radio ligand and antiserum, 100 μL, each were added. The tubes for unspecific binding determination contained radio ligand and buffer final volume in each tube was 300 μL The content of tubes was mixed in vortex, and after overnight incubation at 4 °C, 500 μL of dextran coated charcoal (0.25 and 0.025 g/100 mL) was added to each tube. The content was vortexed briefly and after 10 min staying at 4 °C, the tubes were centrifuged at 4 °C and 3000 rpm for 10 min. The supernatants were decanted into another set of tubes in which the radioactivity of ^{125}I was measured on Berthold gamma counter. The concentration of CCK was calculated from log–logit plot.

2.5. RNA extraction and real-time reverse transcription (RT)-PCR analysis

Total RNA in gallbladder was isolated by using Trizol reagent (Invitrogen, USA) and reverse transcribed into cDNAs by using a First-strand cDNA Synthesis kit (Roche Applied Science). Real-time PCR was performed for quantitative estimation of Gi, Gs and CAP mRNA. The primers for Gi were sense 5′-CAGAGGATGCATTTTGAGCA-3′ and antisense 5′-

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