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Albiflorin Granule significantly decreased the cholesterol gallstone formation by the regulation of insulin transduction signal

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ABSTRACT

Objective: To study the mechanism of insulin resistance in the cholesterol gallstone formation from insulin signal transduction pathway so as to reveal the possible mechanism and the effective role of Albiflorin Granule on preventing the cholesterol gallstones.
Methods: Serum triglycerides (TG), free fatty acid (FFA), and total cholesterol (TC) from different groups were measured and liver cells InsR, PKB, IKK-β protein expression levels were detected by western blotting.
Results: Albiflorin significantly decreased the cholesterol gallstone formation rate, increased glucose infusion rate in gallstone guinea pigs and improved insulin resistance. Compared with the normal group, insulin receptor and PKB protein expression in GS group were significantly reduced. IKK-β protein in the GS group increased significantly and Albiflorin could reduce IKK-β protein expression in guinea pig liver cells.
Conclusions: The model of insulin resistance in cholesterol gallstone guinea pig was successfully established, which plays an important role in the cholesterol gallstone formation. All aspects of insulin signaling pathway are involved in gallstone formation. Albiflorin can regulate various aspects of insulin signal transduction pathway to prevent the formation of gallbladder.

1. Introduction

Gallstone disease has become one of the most common digestive disorders in the world. With the development of economic conditions in recent years, cholesterol gallstone (CGS) has become the main type of gallstones [1]. Gallbladder stone may induce pancreatitis, severe biliary infection, and malignant biliary tumor. The super saturation of biliary cholesterol is very important for the formation of cholesterol gallstones. The main components of bile are the products of hepatic synthesis, intake, and secretion. Thus, hepatic metabolism is related to the super saturation of biliary cholesterol. Triglycerides (TG), free fatty acid (FFA), and total cholesterol (TC) are main hepatic metabolism indicators which associated with CGS formation. Insulin resistance (IR) is a pathological condition in which cells fail to respond to the normal actions of the hormone insulin. When insulin under conditions of insulin resistance, the cells in the body is resistant to the insulin and is unable to use it effectively, leading to high blood sugar. Beta cells in the pancreas subsequently increase their production of insulin, further contributing to a high blood insulin level. This often remains undetected and can contribute to a diagnosis of Type 2 diabetes or latent autoimmune diabetes of adults [2]. Rare type 2 diabetes cases sometimes use high levels of exogenous insulin. At the cellular level, much of the variance in insulin sensitivity between untrained, non-diabetic humans may be explained by two mechanisms: differences in phospholipid profiles of skeletal muscle cell membranes, and in intramyocellular lipid (ICML) stores within these cells [3]. High levels of lipids in the bloodstream have the potential to result

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in accumulation of triglycerides and their derivatives within muscle cells, which activate proteins Kinase, ultimately reducing the glucose uptake at any given level of insulin [4,5]. This mechanism is quite fast-acting and may induce insulin resistance within days or even hours in response to a large lipid influx [6]. As short-term overdosing of insulin causes short-term insulin resistance, it has been hypothesized that chronic high dosing contributes to more permanent insulin resistance. More researches show that IR is the important start factor for the formation of gallstones [7–9]. Only insulin bind to the cell membrane insulin receptor (InsR) and it can play their physiological effect. Therefore, the number of InsR anomalies and function defects may affect insulin signal transduction and lead to IR. PKB is key signaling proteins in PI3K way on insulin regulation, so in the process of insulin resistance the PKB expression is abnormal. PKB is the important factor to maintain blood sugar stable. In the PKB knockout mouse models, the blood glucose reduction ability of insulin is significantly reduced and the mouse characterized with insulin resistance and diabetes symptoms. Recent research shows that in the process of insulin signal transduction except for tyrosine phosphorylation a variety of signaling proteins regulates Ser/Thr phosphorylation. Ser/Thr protein kinase I kappa B predominate-beta kinase (IKβ) plays an important role in the development of IR [10]. In this study, the model guinea pig gallstones were established by feeding the high fat method. The model was administered by Albiflorin. Then, the serum TG, TC, FFA level and expression level of InsR, PKB, IKK-β were observed in guinea pig liver tissue.

2. Material and method

2.1. Materials

The antibodies of InsR, PKB, IKKβ were purchased from Abcam. Ursodeoxycholic acid (UDCA) was purchased from Sanwei Pharmaceuticals (Shanghai, China). Albiflorin was purchased from Alfa Chemistry (Stony Brook, NY 11790, USA); Acrylamide was from sigma (St. Louis, MO USA); Tris-base was from Boehringer; sodium dodecyl sulfate (SDS), glycine, ammonium persulfate and TEMED was from Sigma (St. Louis, MO USA); Chemiluminescence chromogenic reagent kit and protein assay kit were from Pierce (Shanghai, China).

2.2. Animals and grouping

White guinea pig with red eye, weighing (200–240) g, half male and half 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. All guinea pigs were provided free access to water and divided into four groups: control group, gall-stone (GS) group, Albiflorin group and ursodeoxycholic acid (UDCA) group. Guinea pigs in GC 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 Albiflorin group with a lithogenic diet supplemented with Albiflorin at 10 mg/kg every 12 h for 8 weeks. Those in UDCA group were administered with a lithogenic diet supplemented with UDCA (80 mg/kg/d) for 8 weeks. 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.

2.3. Gallstone identification by infrared spectrum

The infrared spectroscopy method was used to identify the gallstones. Mix 2 mg gallstones powder and 200 mg dry potassium bromide powder, load to the molding, vacuum (1–2) min, plus 10 T pressure (vacuum) at the same time, and then take out the sheeting for inspection by Magna FTIR-750 infrared spectrometer (Nicolet).

2.4. The detection of FFA

FFA detection referred to Nanjing Jiancheng FFA detection kits. Free fatty acid is a result of adipose decomposes; determine the content of free fatty acids that can be used to measure the fat decomposition. FFA can conjugate with copper ions to form fatty acid and copper salt soluble in chloroform. The content of copper salt is proportional to the content of free fatty acids. To detect the copper ions in the by copper reagent can calculate, the content of FFA.

2.5. The detection of TG

TG test was referred to the Nanjing Jiancheng triglycerides detection kits. Briefly, the 3 μL serum was added into 300 μL reagent and incubated at 37 °C for 5 min, then detected optical density value on 546 nm by Thermo Scientific Microplate Reader (Multiskan MK3). The blank TG and the standard TG was implemented as the same procedure.

2.6. The detection of TC

TC test was referred to the Nanjing Jiancheng TC detection kits by COD-PAP colorimetric method. Briefly, cholesterol was oxidized by COD to produce hydrogen peroxide, and then react with PAP to generate red quinone imine pigment. The concentration of TC was detected by on 520 nm by Thermo Scientific Microplate Reader (Multiskan MK3). The blank TC and the standard TC was implemented as the same procedure.

2.7. Western blot analysis

The liver proteins were homogenized in PBS with protease inhibitor cocktail. The homogenates were centrifuged for 15 min at 14000 rpm in 4 °C. Supernatants of the tissues were collected and protein concentration was measured with a bicinchoninic acid assay kit. An equal amount of protein from each sample (150 μg) was resolved in 10% Tris-glycine SDS polyacrylamide gel. Protein bands were blotted to nitrocellulose membranes. After incubation for 1 h in blocking solution at room temperature, the membrane was incubated for 24 h with anti-InsR, PKB, IKKβ antibodies at 4 °C. The secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin) was added and incubated at room temperature for 1 h. Peroxidase labeling was detected with the Western blotting detection system and analyzed by a densitometry system. The relative protein levels of InsR and PKB were normalized to β-actin.