Screening methods for antihyperlipidemic activity: A review

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ABSTRACT

Hyperlipidemia is characterized by increased level of cholesterol in the form of low-density lipoprotein (LDL), chylomicrons, very LDL (VLDL), etc. Development of new antihyperlipidemic drugs is a challenging job of researchers because most of the screening procedures are tedious and time-consuming. The present techniques for screening methods for antihyperlipidemic activity could be classified under in vitro and in vivo methods. The in vitro methods include Triton-, PTU-, and high-fat diet-induced hyperlipidemic models. In contrast, the reported in vitro methods are limited and include assay using Caco-2 cell lines and inhibitory activity on 3-Hydroxy-3-methylglutaryl-coenzyme A reductase. There is a lack of simple but sufficiently reproducible and sensitive in vitro technique for screening of antihyperlipidemic activity.

KEY WORDS: Caco-2 cells, 3-Hydroxy-3-methylglutaryl-coenzyme A reductase, NADPH, Propylthiouracil, Thincert plate, Triton

INTRODUCTION

Plasma cholesterol and triglycerides are clinically important because they are major treatable risk factors for atherosclerosis and cardiovascular diseases. Many diseases such as diabetes, obesity, hyperlipidemia, and hypertension are associated with abnormal lipid metabolism. Today, cardiovascular disease is one of the major causes of death.1 Even if stains remain the major hypolipidemic agent currently in use, an increasing number of patients who are treated with stains suffer from side effects or they do not respond well to therapy. Thus, the development of novel antihyperlipidemic agents is worthy in the present scenario. Cholesterol acts as a precursor for steroid and hormones. In addition, cholesterol has many roles in the body as an important constituent in cell membranes. Hyperlipidemia is characterized by increased level of cholesterol in the form of low-density lipoprotein (LDL), chylomicrons, VLDL, etc. This can be estimated using various enzyme kits, and nowadays, automatic cholesterol analyzers are also available. Drugs used to treat hyperlipidemia are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor or statin derivatives (lovastatin, simvastatin, pravastatin, and atorvastatin), bile acid sequestrants (cholestramine and colestipol), lipoprotein lipase activators (clofibrate and fenofibrate), and inhibitors of lipolysis and triglyceride synthesis (nicotinic acid). Cardiovascular diseases are more common in young people, also thus reflecting the changed lifestyle. Lack of exercise is the other reason for the elevation of cholesterol in blood. Elevated cholesterol may deposit in arteries, especially coronary artery. This results in the development of conditions such as atherosclerosis. The antihyperlipidemic drugs act mainly by inhibiting certain enzyme responsible for cholesterol biosynthesis, and others may reduce the absorption fats and fatty acid from gastrointestinal tract. Development of new antihyperlipidemic drugs is a challenging job of researchers because most of the screening procedures are tedious and time-consuming. The experiments usually employed for screening of antihyperlipidemic activity of new chemical entity could be classified under in vitro methods and in vivo methods.
IN VIVO METHODS

Triton-induced Hyperlipidemic Rat Model

In vivo screening technique of antihyperlipidemic activity is reported using Triton-induced hyperlipidemic rat model. Here, albino Wistar rats (160–200 g) can be used for in vivo screening of hyperlipidemic activity. The animals are maintained in polypropylene cages in a well-ventilated room, at temperature of 25 ± 1°C with 12:12 h light/dark cycle. Standard pellet feed and filtered tap water should be provided throughout the experimentation period. The rats are randomly divided into three groups of 12 rats in each group. Triton is dissolved in normal saline to get 5% concentration and is injected at a dose of 300 mg/kg to all the rats except the control group. Blood samples are collected from rats by retro-orbital plexus. Finally, serum levels of LDL, VLDL, total cholesterol, and triglycerides are assayed using standard diagnostic kits.[2]

PTU-induced Hyperlipidemic Rat Model

This method requires short time. Hyperlipidemia is induced by propylthiouracil (PTU). PTU is a drug used for hyperthyroidism. It produces hypothyroid state associated with increased total cholesterol, LDL, VLDL, and triglycerides. Here, 32 rats are required and divided into 5 groups, and a high dose of cholesterol is injected to all groups 6 h before evaluation of total cholesterol, VLDL, and LDL. PTU at a dose of 10 mg/kg body weight of rat is given to all groups except control group for 7 days. PTU at a concentration of 0.01% is given for 7 days. Standard drug should be given to Groups 4 and 5. Measurement of total cholesterol in serum, feces, and liver extract and comparison with control group measurements is carried out finally.[3]

High-Fat Diet (HFD)-Induced Hyperlipidemia (Chronic Model)

High intake of cholesterol and fats diet leads to an increase in the triglycerides and fatty acid level in serum and induce the hyperlipidemia. This results in the generation of atherosclerosis and various cardiovascular disease.[4–6] This response produced in rodent is very similar to symptoms produced in human during hyperlipidemia conditions. Hence, high-fat-induced rat model is widely used for screening hypolipidemic agents. This model mimics human hyperlipidemia. In this method, high amount of cholesterol is mixed with vegetable oil and treated with all group except the control group. After the chronic treatment with high fat, 2nd group receives standard drug, 3rd group receive test sample, and 4th group receives only normal diet as considered as control group. At the end of 30th day, collect the blood sample by a suitable method under slight anesthesia. Sacrifice the animals and isolate the organs such as heart, liver, aorta, pancreas, spleen, and kidney, weigh and are subjected to histopathological studies.

IN VITRO METHODS

In vitro Assay using Caco-2 Cell Lines

This method is based on the measurement of lipid profile secreted from human intestinal epithelium-like cells from the colon cancer cell line, Caco-2. In this method, Caco-2 cells are seeded in well plates in the medium containing 10% fetal bovine serum penicillin and streptomycin for 2 days. The differentiation of Caco-2 cells are initiated by adding 0–5 mM sodium butyrate for 4 days. After an incubation period, Caco-2 cells will be converted into intestinal epithelium-like cells and plenty of microvilli can be observed on the apical side of cell when viewed under electron microscope. Sodium oleate is then added into the medium containing culturing cell and then differentiated Caco-2 cell secrete lipoprotein profile to the medium through microporous membrane. This secreted four class fractions such as chylomicron, VLDL, LDL, and high-density lipoproteins are determined.[7–10]

Inhibition of HMG CoA Reductase

HMG CoA reductase is an important regulating enzyme involved in the cholesterol biosynthesis from acetyl CoA. This enzyme reduces 3-hydroxy, 3-methyl glutacyl CoA (HMG CoA) to mevalonate. Due to inhibition of this enzyme, cholesterol is not synthesized. Hence, this in vitro model is issued to evaluate the hypolipidemic activity of a chemical moiety. The existing statin derivatives exhibit their hypolipidemic activity through this enzyme inhibiting mechanism. Inhibition of HMG CoA reductase induces expression of LDL receptors in the liver, which lowers plasma concentration of cholesterol. This is an NADPH-dependent reaction. HMG CoA assay kits are commercially available. This kit consists of HMG CoA reductase assay buffer, HMG CoA reductase, NADPH, and an inhibitor (atorvastatin). Assay kit measures the utilization of NADPH, which can be measured by the decrease of absorbance at 340 nm. By using this type of kit, measurement of activity of purified enzyme is possible in addition to testing of HMG CoA reductase inhibitors.[11,12] Calculate the percentage of inhibition using the following equation:

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\text{Percentage inhibition} = \frac{\text{absorbance of enzyme}}{\text{absorbance of enzyme with inhibitor}} \times 100
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CONCLUSION

Development of a hypolipidemic agent is much desirable and of utmost importance in the present
scenario. However, the major hurdle toward this goal is the requirement of a suitable screening technique for the hypolipidemic activity. The present techniques for screening methods for antihyperlipidemic activity could be classified under in vitro and in vivo methods. The in vivo methods include Triton-, PTU-, and HFD-induced hyperlipidemic models. In contrast, the reported in vitro methods are limited and include assay using Caco-2 cell lines and inhibitory activity on HMG CoA reductase. There is a lack of simple but sufficiently reproducible and sensitive in vitro technique for screening of antihyperlipidemic activity.

REFERENCES


Source of support: Nil; Conflict of interest: None Declared