International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 7 (1): 74-78, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.74.78

A Simple Method for Screening Antihyperlipidemic Agents

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Abstract: Screening of antihyperlipidemic agents generally takes 1-2 month using hyperlipidemia-animal model induced by high cholesterol diet. The aim of this research was to develop a method for screening of antihyperlipidemic agents in relatively short time. This method takes only 8 days, yet it gives similar result compared to conventional method. Animals were orally induced with propylthiouracil of 10 mg kg^{-1} b.wt. dosage and 0.01% PTU in drinking water for 7 days. On day 8 test drugs were given to animals orally. One hour after test drugs administration, animals were given a solution of high dosage cholesterol in vegetable oil of 400 mg kg^{-1} b.wt. Serum total cholesterol level was measured in every 1 h after administration of cholesterol for 6 h. After 6 h, a level of total cholesterol in the liver and feces were measured. Result showed that administration of cholesterol in hypothyroid animals significantly raised the serum total cholesterol level in 6 h compared to normal animals induced by cholesterol without previous PTU administration. Administration of low dose of propylthiouracil and 0.01% PTU in drinking water for 7 days before the cholesterol played importance role in increasing the serum total cholesterol level in blood. This method is simpler and requires less time to get hyperlipidemia animal model. Serial measurement of serum total cholesterol level in every hour for 6 h gave a cholesterol profile that can explain different drug mechanisms in cholesterol homeostasis.

Key words: Antihyperlipidemic agents, cholesterol, propylthiouracil, screening, total cholesterol level

INTRODUCTION

Hyperlipidemia is one of the major risk factors for cardiovascular disease (Brown and Goldstein, 1986). Hyperlipidemia prevalence continued to increase annually, requiring the development of drugs capable of lowering blood lipids to reduce mortality and morbidity due to cardiovascular complications. Currently, statin is the first choice for lowering cholesterol especially LDL cholesterol levels. But there are many hyperlipidemic patients under statin therapy that unsuccessfully gained the target cholesterol level (Wang, 2007). Therefore, the risk of cardiovascular diseases remains high in these patients.

Natural medicines have been used empirically by many people to lowered cholesterol level. However, to ensure the effectivity of natural medicine, they require testing on antihyperlipidemic activity *in vivo*. Nowadays, *in vivo* screening for antihyperlipidemic agents used the method of high diet cholesterol induction for at least 1-2 month (Zuraini *et al*., 2006; Samir Bashandy, 2007; Kim *et al*., 1995; Santo *et al*., 2004). This becomes ineffective, because it takes a long time to identify the potential of a drug as cholesterol lowering agent. Here, we developed a simple method for screening antihyperlipidemic agents that require only one week of induction by propylthiouracil (PTU). The PTU is a drug used for treatment of hyperthyroidism. It is known that the hypothyroid state is accompanied by hypercholesterolemia, an increase of total cholesterol, LDL and triglycerides in blood serum (Kutty *et al*., 1978).

The proposed method will accelerate and facilitate the screening of antihyperlipidemic agents by observe the homeostasis of cholesterol in serum, liver and feces by antihyperlipidemic agents in a relatively short time. Moreover, this method can observe the effects and action mechanisms of antihyperlipidemic agents on the process of absorption, metabolism and excretion of cholesterol via feces.

MATERIALS AND METHODS

Animals: Thirty male wistar rats of 3 month age and weighed about 160-200 g were used in this study. Animals were divided into five groups of six animals in each group. Before treatment, animals were adapted in a cage room temperature (±25°C) and were given access to food and drink for a week. This study was conducted from March-June 2010, in the Laboratory of Pharmacology and Toxicology, School of Pharmacy, Institute of Technology Bandung (ITB), Indonesia. All experimental procedures were approved by animal ethics committee of Hasan Sadikin Hospital, Bandung, Indonesia.

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Table 1: Experimental procedures applied to five groups of animals to obtain the best hypercholesterolemia animal model

Group	PTU, 10 mg kg^{-1} b.wt. daily 7 days before cholesterol sol	0.01% PTU drinking water 7 days before cholesterol sol	Simvastatin 5 mg kg^{-1} b.wt. 1 h before cholesterol sol	Ezetimibe 3 mg kg^{-1} b.wt. 1 h before cholesterol sol	Cholesterol solution 1 h before measurement
1					√
2	√				√
3	√	√			√
4	√	√	√		√
5	√	√		√	√

Experimental design: The profiles of total cholesterol level in serum from five different experimental procedures were compared to obtain the best hypercholesterolemia animal model. The profiles were measured every one h for 6 h. Table 1 showed the procedures applied to each group. Group 1 was used as control. Group 2-3 were used to show the role of PTU treatment before given the cholesterol (purchased from Sigma) dissolved in vegetable oil (Bimoli®) obtained from local market. Group 4-5 were used to test the model against anti-hyperlipidemic agents such as simvastatin (from Kimia Farma, Indonesia) and Ezetimibe (Ezetrol®). During observation animals were placed in metabolic cages and feces were collected for analysis of total cholesterol. After observation, the animals were sacrificed and the liver were taken for analysis of cholesterol.

Measurement of total cholesterol: Total cholesterol of serum, liver and feces were measured using commercial enzymatic kits. Liver and feces were homogenize in 10% Tris HCl buffer solution 10 mM (pH 7.4) followed by extraction using organic solvents in accordance with the Folch method (Folch *et al*., 1957). The extract was then dried in a vacuum and re-suspended in saline containing sodium lauryl sulphate 0.1% in accordance with previous methods (Yang and Koo, 1997; Rodriguez-Sureda and Peinado-Onsurbe, 2005). Sodium lauryl sulphate 0.1% in saline was used to improve the recovery of cholesterol in the extract of homogenate (Rodriguez-Sureda and Peinado-Onsurbe, 2005). The data obtained were analyzed using ANOVA ($p<0.05$).

RESULTS AND DISCUSSION

Total cholesterol level in serum over a period of 6 h after induction with a solution of cholesterol in the vegetable oil can be seen in Fig. 1. The PTU administration by both oral and drinking (group 3) significantly increase total cholesterol level compared to group that received only the induction of cholesterol (group 1). Significant increase of total cholesterol level also showed by group 3 in the first 2 h after administration of cholesterol compared to group 2.

The new proposed model was tested using antihyperlipidemic agents (simvastatin and ezetimibe).

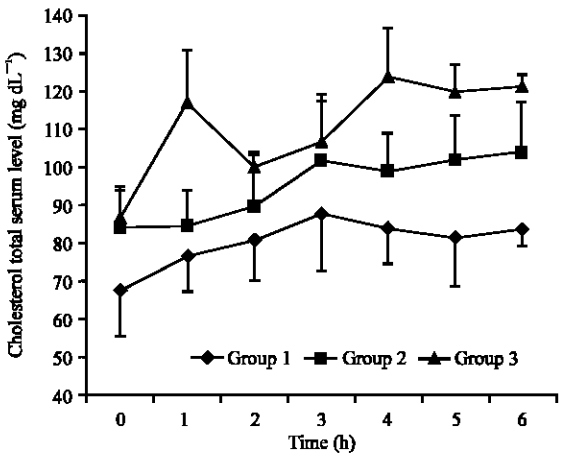

Fig. 1: Profile of total cholesterol in serum for different treatment groups. Group 1: Normal rats received cholesterol solution in vegetable oil, Group 2: Received PTU 10 mg kg^{-1} b.wt. orally for 7 days before cholesterol administration, Group 3: Received PTU both 10 mg kg^{-1} b.wt. orally and in drinking water 0.01% for 7 days before cholesterol administration

Treatment by antihyperlipidemic agents resulted in different total cholesterol level in serum, liver and feces compared to group 3. Antihyperlipidemic agents also lowered the total cholesterol in serum and liver while increased cholesterol excretion via feces (Fig. 2, 3).

Cholesterol is a component of cell membranes that play an important role in maintaining normal cell function (Maxfield and Tabas, 2005). Cholesterol is also an important precursor for the synthesis of steroid hormones and bile acids. Cholesterol has an important role in regulating metabolism because it serves as ligand for cell nuclear receptor that regulates expression of certain genes (Chawla *et al*., 2001). Cholesterol homeostasis controlled through a coordinated regulation by the three main routes in the liver that include supply of cholesterol controlled by regulate synthesis *de novo* from acetate, uptake of plasma cholesterol via LDL and SR-BI receptors and elimination of cholesterol through bile acid synthesis (Princen *et al*., 1997).

Body responds to the excessive intake of cholesterol by keeping cholesterol levels in the blood to normal level by different mechanisms such as inhibiting endogenous

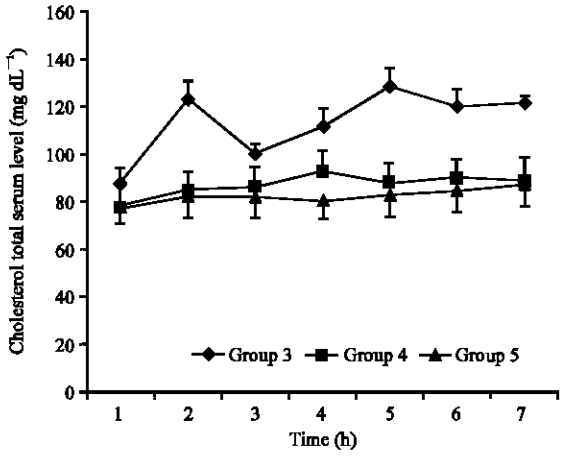

Fig. 2: Profile of total cholesterol in serum for antihyperlipidemic agents compared to group 3. Group 3: Received PTU both 10 mg kg^{-1} b.wt. orally and in drinking water 0.01% for 7 days before cholesterol administration, Group 4: As group 3, received simvastatin 5 mg kg^{-1} b.wt., Group 5: As group 3, received ezetimibe 3 mg kg^{-1} b.wt.

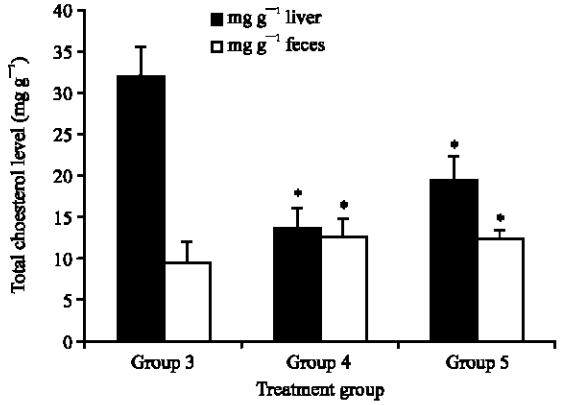

Fig. 3: Profile of total cholesterol in liver and feces for antihyperlipidemic agents compared to group 3 (* = significant compared to Group 3) Group 3: Received PTU both 10 mg kg^{-1} b.wt. orally and in drinking water 0.01% for 7 days before cholesterol administration, Group 4: As group 3, Received simvastatin 5 mg kg^{-1} b.wt. before cholesterol administration,Group 5: As group 3, received ezetimibe 3 mg kg^{-1} b.wt. before cholesterol administration

cholesterol synthesis, limiting cholesterol absorption from intestine, or increasing cholesterol excretion via feces (Dietschy and Siperstein, 1967). Liver regulates cholesterol balance in the body by controlling the endogenous cholesterol synthesis in response to dietary cholesterol. Dietary intake of high cholesterol would inhibit endogenous cholesterol synthesis up to 90% (Morris and Chaikoff, 1959). Our study showed that induction of high-dose cholesterol 400 mg kg^{-1} b.wt. without propyl thiouracil (PTU) did not alter the total cholesterol level in serum up to 6 h of observation.

Thyroid hormones play important role in regulating the synthesis, metabolism and lipid mobilization. Disruption of lipid profile occurs in hypothyroid condition (Pearce, 2004). In most cases, lipid abnormalities in hypothyroidism increase the level of lipoprotein containing apo B 100 including VLDL and LDL (Bar *et al.*, 2007). That situation led to longer cholesterol turn over time (Abrams and Grundy, 1981).

Propyl thiouracil (PTU) is a drug used to treat hyperthyroidism. Animal model with impaired cholesterol metabolism produced by giving a low dose of PTU 10 mg kg^{-1} b.wt. per oral for one week. Hypothyroid animals under normal diet showed normal cholesterol levels in serum (Fig. 1). High doses of cholesterol in hypothyroid animals (group 2 and 3) significantly increased cholesterol levels in serum compared with normal animal (group 1). Group 3 that received the PTU diet both oral and drinking showed the most increase of cholesterol. The increased of cholesterol level was caused by decreased expression of LDL receptors and metabolic disorders of apolipoprotein in the liver including Apo A-I and Apo A-IV (Apostolopoulos *et al.*, 1987; Shin and Osborne, 2003). Apo A-I and Apo A-IV are two HDL components that efflux cholesterol from tissue into the liver (Steinmetz *et al.*, 1990).

The increased of total serum cholesterol levels in group 3 was higher than group 2. This is due to PTU administration in drinking water may help to reach an equilibrium state in serum levels of PTU to produce a hypothyroid state. The increased of serum cholesterol level during the 6 h of observation showed the inability of the animal body to maintain normal levels of total cholesterol in serum as a result of disturbances in cholesterol metabolism in hypothyroidism. There was reported previously, that cholesterol absorption was increase in hypothyroid state (Story *et al.* 1974). It is showed in this study that in hypothyroid animals increased absorption of cholesterol in response to high cholesterol intake was marked by an increase in serum cholesterol and liver cholesterol while less conversion of cholesterol into feces. In this animal model, there was inability of the animal body to maintain homeostasis of cholesterol in serum, liver and feces.

Antihyperlipidemics agents were able to maintain homeostasis of cholesterol by increasing the conversion of cholesterol through feces so that cholesterol levels in serum and liver were relatively normal. We also observed a decrease in liver cholesterol levels in the group that received antihyperlipidemic treatment compared to group 3. An increase in liver choles terol level is caused by an interruption in the process of VLDL catabolism causing accumulation of cholesterol in the liver (Dory and Roheim, 1981).

In conclusion, the proposed method of induction with PTU per oral 10 mg kg^{-1} b.wt. and 0.01% PTU in drinking water for one week followed by induction of cholesterol solution in vegetable oil (Bimoli®) of 400 mg kg^{-1} b.wt. dose produced hyperlipidemic animal models that have cholesterol metabolism disruption. It is considered that in the proposed method most of cholesterol in the body came from diet. Therefore, this method can be used to observe the profile of cholesterol absorption originating from the diet and cholesterol metabolism. Profiles of total cholesterol were observed for 6 h after administration of cholesterol solution in vegetable oil. Application of this method can be used to screen antihyperlipidemic agents in short time. The effect of drugs that inhibit the absorption of cholesterol can be observed on the first two h, whereas drugs that have a mechanism of increasing cholesterol metabolism can be observed afterward.

ACKNOWLEDGMENT

This research was supported in part by IMHERE grants, DIKTI, Indonesia 2010. We thank to Dr. rer. nat. J.I. Sigit for his valuable contribution.

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