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Polysorbates as novel lipid-modulating candidates for reducing serum total cholesterol and low-density lipoprotein levels in hyperlipidemic C57BL/6J mice and rats

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ABSTRACT

Polysorbates are amphiphilic, non-ionic surfactants composed of fatty acid esters of polyoxyethylene sorbitan which are widely used in the cosmetic, food and pharmaceutical industries owing to these special characteristics and their low toxicity profiles. In the present study, polysorbates were investigated for their hypolipidemic activity. C57BL/6J mice and Sprague–Dawley rats were fed a high-fat diet for four weeks, then were divided into several groups, normal saline, polysorbates and positive control drugs such as lovastatin and colestyramine were administered orally to the animals for another four weeks. Complete lipid profiles of the experimental animals were determined by assessing the serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. The results indicate that polysorbates significantly lowered the lipid components. Polysorbates are potential candidates for preventing intestinal absorption of redundant lipid from daily intake and subsequently for preventing hyperlipidemia as well as atherosclerosis.

1. Introduction

Hyperlipidemia is the major cause of atherosclerosis and atherosclerosis-associated conditions such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease (Grundy, 1986). Although the incidence of atherosclerosis-related events has improved because of advances in drug therapy and prevention, these conditions still account for the majority of morbidity and mortality among middle-aged and older adults (Barzi et al., 2005; Heron, 2007). The incidence and absolute number of annual events will likely increase over the next decade because of the epidemic of obesity and the aging of the world population (Yusuf, 2002). Dyslipidemia is characterized by abnormalities in lipid metabolism such as increases in serum total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and a decrease in high-density lipoprotein cholesterol (HDL-C) level (Koruk et al., 2003). Specially, increased concentrations of LDL-C have been shown to play a key role in the pathogenesis of atherosclerosis and have been strongly associated with a greater prevalence of cardiovascular disease (Gordon et al., 1981). Reduction of LDL-C by dietary and/or pharmaceutical means leads to an important reduction in the incidence of cardiovascular events (LaRosa et al., 2005). The fact that dyslipidemia is a risk factor of atherosclerosis has led to the development of drugs that reduce cholesterol levels. These drugs provide benefits in patients, primarily by reducing levels of total cholesterol and LDL-C. It is very necessary to discover and develop novel drugs for reducing vascular disease risk. Preferable would be a novel lipid-modification candidate with strong lipid lowering effects and low toxicity.

Polysorbates are amphiphilic, non-ionic surfactants composed of fatty acid esters of polyoxyethylene sorbitan widely used as solubilizing agents, emulsifiers, dispersants and wetting agents in the chemical, cosmetic, food and pharmaceutical industries (National Toxicology Program, 1992; Chinese Pharmacopeia, 2010). Until now, there have been no reports regarding the anti-hyperlipidemic effects of polysorbate compounds. In this study, we aimed to evaluate the antihypercholesterolemic activities of polysorbates. The hypercholesterol models induced by a high-fat diet were established in C57BL/6J mice and Sprague–Dawley rats. The results showed that polysorbate-80 and its homologues such as polysorbate-20, polysorbate-40 and polysorbate-60 had significant anti-hyperlipidemic effects primarily by downregulation of serum LDL-C and total cholesterol levels. These results make important contributions towards the discovery of a novel safe and effective hypolipidemic drug.

2. Materials and methods

2.1. Chemicals and reagents

Polysorbate-80 (polyoxyethylene sorbitan monolaurate, PS-80) and homologues, polysorbate-20, polysorbate-40 and polysorbate-60, were all of chemical grade, purchased from Guangdong Xilong Chemical Reagent Co. Ltd. (Guangdong, China). The purities of these compounds

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were higher than 99%. Lovastatin was obtained from Beijing Winsunny Pharmaceutical Co. Ltd. (Beijing, China) and colestyramine was obtained from Nanjing Housheng Pharmaceutical Co. Ltd. (Nanjing, China). Polysorbate-80, polysorbate-20, polysorbate-40 and polysorbate-60 were dissolved in 0.9% normal saline. Lovastatin and colestyramine were suspended in the same solvent. Determination kits for total cholesterol, LDL, HDL, and triglyceride were purchased from the Beijing Center of Shouyi Clinical Medicine (Beijing, China).

2.2. Animals

C57BL/6J mice (both sexes, 9 weeks old, 18–22 g) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). Sprague–Dawley rats (both sexes, 200–230 g) were supplied by the Animal Center of Capital Medical University (Beijing, China). All of the animals were pathogen-free. Prior to the experiments, all animals were housed for 5 days for acclimatization to animal room conditions, and maintained in a 12 h light/12 h dark cycle at room temperature (24 ± 1 °C) and humidity (55% air humidity), fed a rodent standard diet and water was provided ad libitum. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals (Zimmermann, 1983). In addition, the protocols employed were approved by the Animal Care and Use Committee of Capital Medical University.

2.3. High-fat diet

The normal diet prescription was prepared and used with some modifications (Shigeru et al., 1999; Mariarosaria et al., 1999; Hisae et al., 2006). The normal food was composed of 50% corn, 20% wheat bran, 15% soybean, 10% wheat flour and 5% fish meal and an appropriate amount of sodium chloride (g/g). Based on the normal food mentioned, the high-fat diet used in this study contained 3% cholesterol, 10% fat, 0.5% sodium cholate and 0.2% propylthiouracil (g/g).

2.4. Experimental protocols

Mice of both sexes were allocated randomly into seven groups (6 male and 6 female for each group) with food and water freely available. The first group of animals received the standard diet as the negative control group (control), while the other groups (groups 2−7) were fed a high-fat diet for 28 days. At the end of 4 weeks, lipid levels were detected. If serum lipids increased about three times, then it was believed that the hyperlipidemic model was established successfully and dosing of the test drugs and reagents was initiated for a period of 4 weeks. The negative control group animals were maintained on a rodent standard pellet diet and water ad libitum without any drug administration. Group 2 received 0.2 ml/10 g normal saline per day as vehicle (hypercholesterolemic group, HC), group 3 received lovastatin (30 mg/kg/day; HC + LS) as a positive control, group 4 received colestyramine (2000 mg/kg/day; HC + CA) also as a positive control, and groups 5, 6 and 7 received 400, 1600, and 6400 mg/kg/day of polysorbate-80 (HC + PS), respectively. Lovastatin, colestyramine and polysorbate-80 were given orally for the second 28 days of the experimental period. The body weight of all animals was recorded weekly throughout the entire experimental period. At the end of the experiments, the mice were fasted for 16 h and anesthetized by intraperitoneal (i.p) injection of barbital. Blood samples were collected from the eye socket vein, transferred into the centrifuge tubes and centrifuged to obtain serum. The tissues were removed immediately by dissection, washed in ice-cold saline, blotted between two filter papers, and fixed in paraformaldehyde for histological study.

Rats of both sexes were also divided randomly into seven groups (4 male and 4 female for each group). The treatment methods and procedures were the same as described earlier except the doses were as follows: lovastatin 7 mg/kg, colestyramine 1300 mg/kg, and polysorbate-80 3700 mg/kg, 740 mg/kg and 148 mg/kg.

Effects of the polysorbate-80 analogues, polysorbate-20, polysorbate-40 and polysorbate-60, on serum lipid levels were investigated using the C57BL/6J mice. The treatment methods and procedures were also the same as described earlier. The doses were as follows: lovastatin, 30 mg/kg, colestyramine, 2000 mg/kg, and polysorbate-20, polysorbate-40 and polysorbate-60 were all 1600 mg/kg.

2.5. Serum lipid measurement

The blood samples were collected from C57BL/6J mice and rats, kept for 1 h at room temperature, then centrifuged for 10 min at 3000 rpm, the supernatant serum was collected and used for determination of the serum lipid. Serum concentrations of total cholesterol, HDL, LDL and triglyceride were determined by enzymatic colorimetric methods using the assay kits commercially available (Beijing, China). The assay was performed according to the manufacturer's instructions.

The percent of total cholesterol (TC) decline (%) was calculated as described in Eq. (1). The percent of LDL decline (%) was calculated by a similar equation as for total cholesterol. The atherogenic index (AI) was calculated by HDL/total cholesterol and LDL/HDL in the rat expeiments (Dobiásová and Frohlich, 2001; Milada and Frohlich, 2001; Holmes et al., 2008).

$$\text{the percent of TC decline \%} = \frac{\text{TC content in the HC group} - \text{TC content in the treatment group}}{\text{TC content in the HC group}} \times 100\% \quad (1)$$

2.6. Histological examination

For the histological study, tissues of heart, liver, and stomach ($n = 3$) of all groups were excised and fixed in 4% paraformaldehyde for 48 h, embedded in paraffin and sectioned at 5 μm. These sections were stained with haematoxylin and eosin, and examined by light microscopy.

2.7. Statistical analysis

Data were presented as mean ± S.D. Comparison between the groups was made by one-way analysis of variance (ANOVA). If ANOVA analysis indicated significant differences, a Student Newman–Keuls post-test was performed to compare mean values between treatment groups and the control. The statistical differences between groups were considered significant at $P<0.05$.

3. Results

3.1. Effect of polysorbate-80 on serum lipid profiles in hypercholesterolemic mice

The mean effects of polysorbate-80 on serum total cholesterol, HDL, LDL and triglyceride levels in the hyperlipidemic C57BL/6J mice fed a high-fat diet are shown in Table 1. The results showed that serum total cholesterol and LDL concentrations in the high-fat diet model mice were three and eight times higher than that of the normal saline control group, respectively. These data demonstrated that the hyperlipidemic model induced by the high-fat diet in C57BL/6J mice was successfully established (Yang et al., 1996; Wang et al., 2007). After control and experimental drugs were given to the mice by intragastric administration for 4 weeks, the levels of total cholesterol and LDL were significantly decreased by more than 40% by lovastatin, colestyramine and polysorbate-80 high, middle and low doses, compared with the