Effect of Aspirin on Hyperlipidemia in Rats

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Abstract

Aspirin is a drug candidate for treatment of inflammation, pain and fever and prevention of cardiovascular diseases with less side effects. The experiment will be conducted to investigate the efficacy of Aspirin on curing hyperlipidemia in Wistar rats. The rats were fed with high fat diet (HFD), for 8 weeks to induce hyperlipemia.

Compared with the model group, the results showed that Aspirin at 20 mg/kg dosage could significantly decrease the hyperlipidemia indexes including triglyceride (TG), low density lipoprotein (LDL) and total cholesterol (TCH) (p < 0.01), for five weeks drug administration. Meanwhile, simvastatin had same effect on hyperlipidemia indexes such as TG, LDL and TC.

Aspirin was effective against hyperlipidemia and had same anti-hyperlipidemic effect like simvastatin. Under the experimental circumstance, the dose of Aspirin to cure hyperlipidemia is 20 mg/kg for five weeks in Wistar rats.

Keywords: Aspirin; Hyperlipidemia; High fat diet; Rats

Introduction

Aspirin has been widely used as a drug for treatment of inflammation, headache, fever, cardiovascular diseases for more than a century. Aspirin is well recognized as an effective anti-platelet drug for secondary prevention in subjects at high risk of cardiovascular events. However, it is well known that the side effects of aspirin have limited the using of this drug [1]. The biochemical mechanism or mode of action of aspirin has been described previously [2-4]. Also the side effect of aspirin (e.g. gastrointestinal ulcers) via inhibition of cyclooxygenase (COX) which is a key enzyme to catalyze prostaglandin formation has been reported [2]. Recently, aspirin was extended to prevention and treatment of cardiovascular diseases based on its anti-thrombotic action in platelets since inhibition of COX by blocking thromboxane A2 production which is crucial for blood clotting [5].

High dose aspirin not only lowers the inflammation mediated pathogenesis of the metabolic syndrome [6], but it also diminishes hypertriglyceridemia in obese rodents [7], and patients with type 2 diabetes mellitus [8]. Ahmed et al., reported that High dose aspirin (120mg/kg), showed maximum decrease in serum triglyceride level (42.0%), serum total cholesterol level (19.36%), and serum LDL level (6.18%) [9]. Moreover, Lin HL et al. reported that low dose aspirin (5 mg/kg) can ameliorate high fat diet induced hyperlipidemia and hyperinsulinemia in Sprague Dawley rats [10]. also suggested that low dose aspirin may have potential in the prevention of hyperlipidemia induced lymphocyte adhesion and inflammation. Hua Y et al., also reported that aspirin (5-20 mg/kg) for 4 weeks can reduce total cholesterol, triglyceride, LDL and elevate HDL in rabbits which were given with high fat diet [11].

Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the bloodstream. The concentrations of lipids, such as triglycerides (TG), cholesterol (TCH) and low density lipoprotein (LDL) increase, or the level of high density lipoprotein (HDL) decrease in the blood [12]. Hyperlipidemia is becoming a major health problem in the world recently even in human and companion animal clinic [13,14].

Materials and Methods

Chemicals and reagents

CMC-Na (carboxyl methyl cellulose sodium) and simvastatin was supplied by Tianjin Chemical Reagent Company (Tianjin, China). Aspirin were supplied by Aladdin Industrial Corporation (Shanghai China). High fat diet feed (standard rat diet 77.8 %, yolk power 10 %, lard 10 %, cholesterol 2 %, bile salts 0.2 %) consists of 41.5 % lipids, 40.2 % carbohydrates, and 18.3 % proteins (kcal) and standard rat diet consists of 12.3 % lipids, 63.3 % carbohydrates, and 24.4 % proteins (kcal). The feed was supplied from KeaoXieli Co., Ltd (Beijing, China). The TG, TC, LDL and HDL kits were provided by Ningbo Medical System Biotechnology Co., Ltd (Ningbo,China). Erba XL-640 analyzer (German) was used to measure the blood lipid level.

Animals

Wistar male rats with clean grade, aged 7 weeks and weighing 160–180 g were purchased from the animal breeding facilities of Lanzhou Army General Hospital (Lanzhou, China). They were housed in plastic cages of appropriate size (50 × 35 × 20 cm, ten rats per cage) with stainless steel wire cover and chopped bedding. Light/dark regimen was 12/12 h and living
temperature was 22 ± 2°C with relative humidity of 55 ± 10 %. Rat feed and drinking water were supplied ad libitum. The study was performed in compliance with the Guidelines for the care and use of laboratory animals as described in the US National Institutes of Health and approved by Institutional Animal Care and Use Committee of Lanzhou Institute of Husbandry and Pharmaceutical Science of CAAS. Animals were allowed a 2-week quarantine and acclimation period prior to start of the study.

**Drug preparation**

Aspirin and simvastatin suspension liquids were prepared in 0.5 % of CMC-Na.

**Group and dosing**

After their arrival for two weeks, rats were randomized into two main groups, group I as blank control (n = 10 rats), feed with basal diet and group II (n = 40 rats) feed with high fat diet (HFD) for eight weeks. After hyperlipidemias were induced successfully in rats, the HFD group was averagely divided into four groups and they were model group and three treatment groups. So it included three test groups and two control groups as blank and model groups (Table 1).

The dosage for rats was based on individual weekly body weights for five weeks. Drugs were administered intragastrically to each rat. High fat diet (HFD), continued during the experiment period.

In order to compare Aspirin and simvastatin. For the comparability of the results in the experiment, The 0.5 % of CMC-Na at the dose of 4ml · kg\(^{-1}\) was as the drug vehicle control and the dosage of CMC-Na was close to equal in comparison with Aspirin and simvastatin. Simvastatin (10mg · kg\(^{-1}\)) was choosed as positive control drug to compare with Aspirin (20 mg · kg\(^{-1}\)).

At the end of 4th, 6th, 8th, 10th and 13th week after HFD was used, the rats were fasted for 10-12h and anesthetized with 10% chloral hydrate. Approximately 1.5ml blood samples were taken from tail tip for blood lipids examination

**Blood sampling**

After fasting for 10-12 h, rats were anesthetized with 10 % chloral hydrate and blood samples were taken from tail tip for blood lipids examination. In order to make sure the volume of the blood sample, the rat tails were immersed in water bath at 45°C for 5min to make the blood vessel swelling, then approximately 1.5ml blood sample was collected from the rat tail. The serums were got through centrifuge for 15min at the speed of 4000 g at 4°C.

**Design of the experiment**

First hyperlipidemia model disease is needed to be established successfully by HFD. At the beginning of the experiment, the rats were divided into two main groups. Group I feed with standard diet and Group II feed with HFD. The blood samples were taken on 4th, 6th and 8th week to examine blood lipids. After the success of hyperlipidemia animal model, Group II was divided into four groups, model group and three treatment groups, and high fat diet was continued during the rest experiment period.

After the administration of drug, blood samples were taken to measure the change in blood lipids on 10th week and 13th week. Blood sample was analyzed with the same method.

**Statistics**

The statistical analyses were carried out using IBM SPSS 19.0 (USA). All data obtained from the experiment are expressed as mean ± standard deviation (SD). The difference between Group I and Group II were evaluated by Student’s t-Test. Statistical differences between the treatments and the control were evaluated by using one-way ANOVA with Duncan test. P-values less than 0.05 were considered statistically significant.

**Results**

**Animal disease model**

In regard to blood lipid levels, there was no significant difference between Group I and Group II in week 4 and 6 (data not shown). So the administration time of HFD was prolonged. At week 8, there were significant increase of TG, LDL and TC and decrease of HDL of model group (p < 0.01, seen in Table 2) which confirmed that the hyperlipidemia animal model was successfully established by HFD in the experiment.

**Body weight**

From the results of body weight before drug administration, blank group had a lower body weight than other groups (P < 0.01, seen in Table 3), and this indicated that HFD had a remarkable influence on body weight. There was no significant difference among other groups except blank group. After drug administration, the average values of body weight in drug treatment group were less than model group and there was still significant difference between blank and model group (P < 0.01).

**Table 1:** The experimental design in the study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of rats</th>
<th>Food</th>
<th>Drug</th>
<th>Dosage</th>
<th>Average volume per rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>Basal diet</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>HFD diet</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>HFD diet</td>
<td>ASA</td>
<td>20 mg/kg</td>
<td>0.65 ml</td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>HFD diet</td>
<td>Simvastatin</td>
<td>10 mg/kg</td>
<td>0.63 ml</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>HFD diet</td>
<td>CMC-Na</td>
<td>20 mg/kg</td>
<td>1.28 ml</td>
</tr>
</tbody>
</table>

Note: HFD: high fat diet. A: blank group, B: model group, C: Aspirin group, D: simvastatin group, E: CMC-Na group.
Hyperlipidemia, is a major modifiable risk factor for atherosclerosis and cardiovascular disease. Increased levels of LDL are related to the development of atherosclerosis [15,16]. HDL plays an important role in removing cholesterol from tissues and protecting against cardiovascular disease. Hyperlipidemia can be the result of an inherited disease in certain breeds of dogs [13,17]. In pets, hyperlipidemia most often occurs as a consequence of some disorder, hyperlipidemia even can also occur spontaneously after a meal of high-fat foods, particularly for dogs [13,17]. In pets, hyperlipidemia most often occurs as a result of an inherited disease in certain breeds of dogs [13,17].

Anti-hyperlipidemic effect

Blood samples were taken for lipids examination after the drugs were given for two weeks, but there was no significant difference among groups (data not shown). So the time was extended for five weeks. From Table 4, the difference effects of drugs on hyperlipidemia appeared after Aspirin was given for five weeks. Meanwhile, there was no statistical difference between model and CMC-Na group, which indicated that CMC-Na has no effect on hyperlipidemia indexes as a vehicle control (Figure 1).

In the blood lipid analysis (seen in Table 4), following five weeks administration of drugs, TG, TC and LDL were significantly decreased in varying degrees in comparison with model group. These changes meant that there were significant differences between the treated groups and the model group at the end of 13th week. In regard to TG index (Figure 2), the results in Aspirin and simvastatin groups were significantly reduced when compared with model group (p < 0.01). Simvastatin, Aspirin, reduced significantly levels of LDL (Figure 3) and TC (Figure 4) (0.01), when compared with model group. LDL in model group showed no significant difference from blank group at week 13. This apparently unexpected result could be explained by the light increase of HDL in model group and decrease in blank group, some studies found that the diet content can effect HDL level [20], light decrease of HDL in blank group may due to the feeding long period rich of carbohydrate in standard diet. Some studies found that the increased plasma apoE of apolipoproteins in aged hyperlipidemia rats can increase HDL after a long period [21-25]. The result in this study showed there was significant change on blood lipid indexes after administrating the drug for five weeks. The examination of hyperlipidemia indexes after two weeks of drug administration showed no significant changes in most groups. So the time was extended to five weeks. The changes in TC, TG and LDL indexes confirmed that Aspirin had influence on hyperlipidemia. Previous study on Aspirin showed that it had effect on TG and TC in Wistar rats with standard diet [26]. The changes in TC, TG and LDL indexes confirmed that Aspirin had influence on hyperlipidemia. Previous study on Aspirin showed that it had effect on TG and TC in Wistar rats with standard diet [26].

The average body weight values in CMC-Na group were similar with model group.

Discussion

Hyperlipidemia, is a major modifiable risk factor for atherosclerosis and cardiovascular disease. Increased levels of LDL are related to the development of atherosclerosis [15,16]. HDL plays an important role in removing cholesterol from tissues and protecting against cardiovascular disease. Hyperlipidemia can be the result of an inherited disease in certain breeds of dogs [13,17]. In pets, hyperlipidemia most often occurs as a consequence of some disorder, hyperlipidemia even can also occur spontaneously after a meal of high-fat foods, particularly table scraps [18,19]. In this study, the anti-hyperlipidaemic effects of Aspirin were investigated in rats with induced hyperlipidemia by high fat diet (HFD). The blood lipid indexes were observed during eight weeks. When compared the blood lipid level between blank and model group at week 8, there was a significant elevation in the levels of serum LDL, TG and TC, and decrease of HDL (Table 2). Changed levels of these parameters in serum are presumptive markers of hyperlipidemia in serum. LDL is more related to hyperlipidemia and is an important index in these parameters. On other hand, HDL in this experiment decreased at week 8 in model group. However, HDL value in model group showed no significant difference from blank group at week 13. This apparently unexpected result could be explained by the light increase of HDL in model group and decrease in blank group, some studies found that the diet content can effect HDL level [20], light decrease of HDL in blank group may due to the feeding long period rich of carbohydrate in standard diet. Some studies found that the increased plasma apoE of apolipoproteins in aged hyperlipidemia rats can increase HDL after a long period [21-25]. The result in this study showed there was significant change on blood lipid indexes after administrating the drug for five weeks. The examination of hyperlipidemia indexes after two weeks of drug administration showed no significant changes in most groups. So the time was extended to five weeks. The changes in TC, TG and LDL indexes confirmed that Aspirin had influence on hyperlipidemia. Previous study on Aspirin showed that it had effect on TG and TC in Wistar rats with standard diet [26]. The changes in TC, TG and LDL indexes confirmed that Aspirin had influence on hyperlipidemia. Previous study on Aspirin showed that it had effect on TG and TC in Wistar rats with standard diet [26].


calculated with the body weight in Group I and II at the end of 8th week after using HFD diet.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unit</th>
<th>TG</th>
<th>TCH</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>mmol/L</td>
<td>0.77 ± 0.42</td>
<td>1.01 ± 0.25</td>
<td>0.55 ± 0.07</td>
<td>0.12 ± 0.08</td>
</tr>
<tr>
<td>Group II</td>
<td>mmol/L</td>
<td>1.51 ± 0.38**</td>
<td>2.18 ± 0.31**</td>
<td>0.44 ± 0.06**</td>
<td>0.52 ± 0.08**</td>
</tr>
</tbody>
</table>

Note: TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; TCH: Total cholesterol. *=P < 0.01 significant difference from blank group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight [g ]</th>
<th>Fifth week after drug treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>276.1 ± 64**</td>
<td>330.1 ± 5.6**</td>
</tr>
<tr>
<td>B</td>
<td>295.4 ± 7.1</td>
<td>358.8 ± 7.2</td>
</tr>
<tr>
<td>C</td>
<td>305.6 ± 6.9</td>
<td>343.4 ± 5.1**</td>
</tr>
<tr>
<td>D</td>
<td>306.8 ± 5.9</td>
<td>342.4 ± 7.1**</td>
</tr>
<tr>
<td>E</td>
<td>302.7 ± 6.7</td>
<td>352.3 ± 6.6</td>
</tr>
</tbody>
</table>

Note: A: blank group, B: model group C: Aspirin group, D: simvastatin group, E: CMC-Na group. *=P < 0.01 significant difference from model group before administration. bbP < 0.01 significant difference from model group after administration. The time before drug treatment was the end of 8th week after HFD diet was used and fifth week after drug treatment was the end of 13th week after HFD diet was used.
Table 4: The blood lipids levels at the end of 13th week (after drugs administration for five weeks, n = 10).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Blank</th>
<th>Model</th>
<th>CMC-Na</th>
<th>Simvastatin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>0.77 ± 0.2**</td>
<td>1.65 ± 0.22</td>
<td>1.63 ± 0.34</td>
<td>1.12 ± 0.15**</td>
<td>1.27 ± 0.15**</td>
</tr>
<tr>
<td>HDL</td>
<td>0.46 ± 0.04</td>
<td>0.52 ± 0.05</td>
<td>0.58 ± 0.02</td>
<td>0.56 ± 0.05</td>
<td>0.61 ± 0.05</td>
</tr>
<tr>
<td>LDL</td>
<td>0.11 ± 0.03**</td>
<td>0.54 ± 0.04</td>
<td>0.51 ± 0.05</td>
<td>0.23 ± 0.04**</td>
<td>0.3 ± 0.04**</td>
</tr>
<tr>
<td>TCH</td>
<td>1.01 ± 0.18**</td>
<td>2.49 ± 0.14</td>
<td>2.11 ± 0.38</td>
<td>1.61 ± 0.23**</td>
<td>1.8 ± 0.10**</td>
</tr>
</tbody>
</table>

Note: TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; TCH: Total cholesterol. The unit of TG, HDL, LDL and TC is mmol/L. *P < 0.01 significant difference from model group.

Figure 1 Effects of different drugs on hyperlipemia indexes after drugs administration for five weeks (n = 10). TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; TCH: Total cholesterol.

Figure 2 Effects of different drugs on TG: Triglyceride (n = 10).

Figure 3 Effects of different drugs on LDL: Low density (n = 10).

Figure 4 Effects of different drugs on TCH: Total cholesterol (n = 10).

Figure 5 Effects of different drugs on HDL: High density lipoprotein (n = 10).

decreased TG, LDL and TC, while simvastatin has similar effect; this confirmed that Aspirin is benefit on curing hyperlipidemia based on the results (Table 4 P < 0.01). On the other hand, CMC-Na as a vehicle had no effect on hyperlipidemia, which confirmed that the influence on hyperlipidemia is due to Aspirin only. The study on the metabolism of Aspirin showed that Aspirin, Salicylic acid (SA), from Aspirin metabolism as a final metabolite excreted in urine was coincided with Salicylic acid from Aspirin metabolism. The body weight of the rats was increased during experiment in all groups, which indicated that the rats grew up healthy during the experiment. These mean the drugs are safety and no conflict in using on rats.

In addition, Table 4 showed significant differences in the blood lipid index of the model group compared with the blank group. The action mechanism of simvastatin depends on inhibition of enzyme 3-hydroxy-3-methyl glutaryl co enzyme A (HMG-CoA reductase inhibitors) [27,28], which is play a crucial role on cholesterol synthesis in liver endogenous pathway.
There are many chemical drugs that could ameliorate hyperlipidemia such as: statins, fibrates, ezetimibe and niacin acid, but most of them are expensive and have undesirable effect. So there are increasing interest in alternative drug for the prevention and treatment of hyperlipidemia. Currently available hyperlipidemic drugs have been associated with a number of side effects. Therefore, now it’s important to search for drug that is less toxic, less expensive, which can provide better safety and efficacy on a long term usage.

Conclusions

In summary, there were significant differences in blood lipid indexes throughout the experimental period under the present study conditions. For Wistar rats, the optimal dose for curing hyperlipidemia by Aspirin was considered to be 20 mg/kg/day administrating for five weeks, further studies should be conducted to investigate its prevention effect and the action mechanism of Aspirin on antihyperlipidemia

Author Contributions

Conceived and designed the experiments: IK NM JYL YJY. Performed the experiments: IK NM XWL YJY. Analyzed the data: IK NM JYL XWL. Contributed reagents/materials/analysis tools: IK NM JYL XWL. Performed the experiments: IK NM XWL YJY. Analyzed the data: IK NM JYL XWL. All authors read and approved the final manuscript.

Acknowledgement

The work was supported by the National Natural Science Foundation of China (No.31402254), and special project of Fundamental Scientific Research Professional Fund of Central Public Welfare Scientific Research Institutes (1610322015013).

References


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