ORIGINAL ARTICLE

Hyperlipidemia induced by Triton WR1339 (Tyloxapol) in Wistar rats

Luiz Carlos Bertges [1], Carlos Alberto Mourão Jr [2], Jonathan Batista Souza [3], Vinícius Antônio Coelho Cardoso [4]

Abstract

Pharmacological relevance: Triton WR1339 (Tyloxapol) has been used by several studies to induce hypercholesterolemia in animals. However, no works in literature were found to clarify the quality and durability of the hypercholesterolemic state. Thus, when studying hypocholesterolemic agents in rats, it is difficult to determine whether the decrease in cholesterol levels resulted from the treatment or from its metabolization throughout the days. **Study objective:** To determine the cholesterol levels in Wistar rats during the 9 days after the application of Tyloxapol. **Materials and methods:** Blood samples of 16 rats were collected by venipuncture of the caudal vein for total cholesterol level measurement. A single intraperitoneal dose of Tyloxapol at 200mg/kg was subsequently given. New samples were taken every three days and total cholesterol levels were also measured. **Results:** Drug action peaked after 72 hours of application, returning to baseline values after that. **Conclusion:** The induction of hypercholesterolemia by Tyloxapol has shown to be effective in Wistar rats within 72 hours of application of the drug, after that period the results may not reflect the action of cholesterol-lowering agents using this model.

Keywords: cholesterol, hyperlipidemias, wistar rats.

[1] PhD, Associate Professor at the Physiology Department at UFJF. Professor at SUPREMA.
[2] PhD, Assistant Professor at the Physiology Department at UFJF.
[3] Physician
[4] Physician
Institution (Affiliation): Universidade Federal de Juiz de Fora MG
Correspondence: Luiz Carlos Bertges, Departamento de Fisiologia, Instituto de Ciências Biológicas, UFJF, Campus Universitário, Bairro Martelos, Juiz de Fora, MG, CEP 36036900.
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1. INTRODUCTION

Hypercholesterolemia is a metabolic condition that determines the onset of chronic degenerative diseases such as atherosclerosis.[1,2,3] The formation of initial lesions appears to originate, more often, from the focal increase in lipoprotein content within regions of the intima, due not only to changes in the permeability of the overlying endothelium, but mainly because they bind to constituents of the cellular matrix, increasing the residence time of lipid-rich particles within the arterial wall.[4] In the extracellular space of the intima, lipoproteins may undergo changes and evidence points to a pathogenic role for such modifications.[5] Hypercholesterolemia, therefore, is an important risk factor for cardiovascular diseases[6,7,8] and reduction of plasma cholesterol and endothelial protection become important steps for the control of atherosclerotic disease and its complications such as acute myocardial infarction and systemic hypertension.[9]

Due to this, there is a constant search for new drugs to prevent or reduce damage caused by genetic problems or excess fat diets that lead to hypercholestrolemia.[10] To that end, attempts have been made to cause an increase in plasma cholesterol using laboratory animals to better understand the relationship between changes in lipid metabolism and atherogenesis and to test possible treatments for their reduction.[11] In this context, the use of Triton WR1339, also known as Tyloxapol (Sigma Aldrich), is noteworthy. It is a non-anionic detergent of polymeric[3] structure that has been successfully used in several studies to induce hypercholesterolemia.[12,13,14,15,16,17]

However, no works in literature were found to clarify the quality and durability of the hypercholesterolemic state induced by intraperitoneal administration of Tyloxapol in rats. Thus, when studying drugs, plants or foods with a hypocholesterolemic aim in rats, it is difficult to determine whether the drop in cholesterol levels resulted from treatment or from its spontaneous reduction throughout the days due to metabolism.

The aim of this study was to determine cholesterol levels in Wistar rats over 9 days after Tyloxapol use, thus obtaining the required parameters for the study of cholesterol-lowering drugs, plants or foods.

2. MATERIALS AND METHODS

Sixteen Wistar adult male rats from the vivarium of the Universidade Federal de Juiz de Fora were used. The animals were weighed, placed in individual cages at room temperature under a 12-hour light/dark regimen and offered water and food ad libitum.

After an adjustment period of five days, the animals received intraperitoneal anesthesia with ketamine and xylazine and 0.5 mL blood samples were collected by caudal venipuncture. The material was sent to a clinical laboratory for determination of total cholesterol with enzymatic kits (BioSiystems SA).

Soon after collection the rats were administered intraperitoneal Triton WR1339 (Tyloxapol), acquired from the laboratory Sigma Aldrich, dissolved in 0.9% NaCl at 200 mg/kg body weight. The animals were observed during nine days, when they were offered water and food ad libitum. No terceiro, sexto, e nono dias foram colhidas novas amostras de 0,5 mL de sangue obedecendo os mesmos padrões da primeira coleta. New 0.5 mL blood samples were collected on days 3, 6, and 9 following the same standards as the first collection.

11 Statistical analysis was performed using the program Graphpad version 5.0 using a freedman ANOVA for repeated measures with Dun'ss method for posthoc analysis. The study follows the Ethical Principles for Animal Experimentation, adopted by the Colégio Brasileiro de Experimentação Animal (COBEA - Brazilian College of Animal Experiments) and was approved by the Ethics Committee in Animal Experimentation (EAEC) at the Research Department/UFJF. Protocol 024/2007CEEA.

3. RESULTS

Ten animals died during the study, six of them three days after drug administration, one died six days after drug administration and three died nine days after drug administration.

Mean cholesterol, before Triton WR 1339 administration, was 66.0 (n = 6) (Figure 1). Three days after drug administration, there was an increase of 448.0% of the mean values to 296.0 (n = 6) (p = 0.004) (Figure 1). Six days after drug administration, there was a 64.19% decline when compared to the third day, with a mean of 106.0 (n = 6), with no difference compared to the initial mean ($p < 0.05$) (Figure 1). On the ninth day the mean was 76.5 (n = 6), also with no statistically significant difference compared to baseline values ($p < 0.05$) (Figure 1).

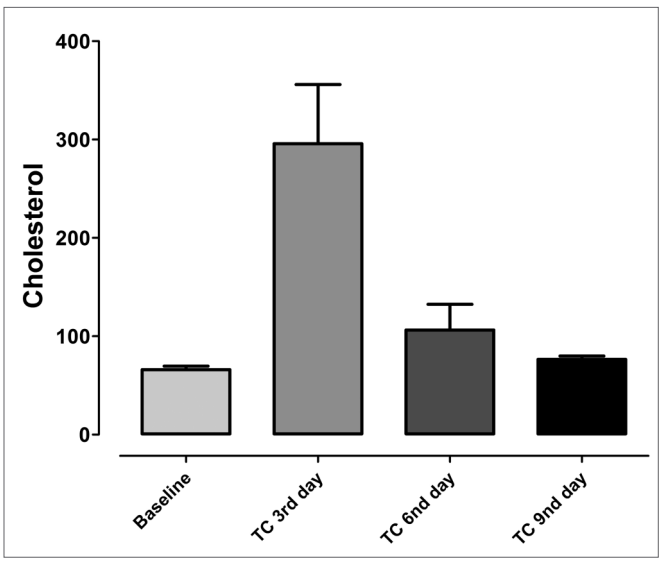

Figure 1. Average cholesterol of Wistar rats

4. DISCUSSION

The results show that Tyloxapol was effective in increasing the levels of serum total cholesterol from baseline, peaking on the third day after application. On days 6 and 9, however, there was a decrease in lipid levels, no longer with a statistically significant difference between the measured values and baseline values. This suggests that within 72 hours of Tyloxapol application at 200mg/kg, high cholesterol values can be achieved.

Several studies also showed that Tyloxapol was effective in raising plasma cholesterol levels in Wistar rats when administered by the same route and dose. However, despite the increasing lipid elevation shown over 24 hours, it is not possible to observe the durability of this effect on these papers.[18,19,20] This same conclusion was also drawn when using the drug at a intravenous dose of 400mg/kg.[21]

In another study[22] also using Triton WR1339 which assesses "dolichols" doses, a product that participates in the same process of cholesterol biosynthesis, the observed results also very close to those found in this study. The serum concentration of this product over eight days peaked on the fourth day and started to decline already on the eighth day, but with higher values compared to baseline. These data indicate that Triton WR 1339 acts on the concentrations of both cholesterol and dolichols in the same way, since both share the same biosynthesis process. In mice, peak action is observed at 24 hours and a drop of serum cholesterol is observed 48 hours after drug use.[23]

Although several studies have used Triton WR 1339 to induce hypercholesterolemia in rats, no literature was found that clearly define the variation in plasma cholesterol levels for long periods.

In this study, the induction of hypercholesterolemia by Triton WR 1339 proved to be effective in rats, peaking 72 hours after application of the drug. After this period there was a decrease to levels similar to baseline values, which can be misleading in studies with cholesterol-lowering agents for a period longer than three days after using the drug. It is noteworthy that throughout the study 10 animals died, pointing to a lower survival rate after drug application, which can be a limiting factor for future studies using this hypercholesterolemia induction model for longer periods.

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