Pharmacological Reports 67 (2015) 553–559

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Hypocholesterolemic and choleretic effects of three dimethoxycinnamic acids in relation to 2,4,5-trimethoxycinnamic acid in rats fed with a high-cholesterol/cholate diet

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ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form 30 November 2014
Accepted 16 December 2014
Available online 29 December 2014

Keywords:
2,4,5-Trimethoxycinnamic acid
Dimethoxycinnamic acids
HMG CoA reductase

ABSTRACT

Background: 2,4,5-Trimethoxycinnamic acid (2,4,5-TMC) is the major and non-toxic metabolite of α-asarone, which retains hypocholesterolemic and choleretic activities. We compared the activities of 2,4,5-TMC with those of 2,4-dimethoxycinnamic acid (2,4-DMC), 3,4-DMC and 3,5-DMC, to understand the role of the methoxyls on carbons 2, 4 and 5 on the pharmacologic properties of these compounds.
Methods: The methoxycinnamic acids were administered to high-cholesterol/cholate-fed rats. We measured bile flow, and quantified bile acids, phospholipids and cholesterol in bile, and cholesterol and cholesterol-lipoproteins in serum. The inhibition of HMG-CoA reductase by the methoxycinnamic acids was evaluated *in vitro*.
Results: The four methoxycinnamic acids decreased serum cholesterol, without affecting the concentration of HDL-cholesterol. 2,4,5-TMC produced the highest decrease in LDL-cholesterol, 73.5%, which exceeds the range of statins (20–40%), and produced the highest inhibition of the activity of HMG-CoA reductase. 3,4-DMC produced the highest increase in bile flow, bile acids and phospholipids concentrations, and reduction in bile cholesterol, which led to a decrease in the biliary cholesterol saturation index.
Conclusions: 2,4,5-TMC (which has three methoxyls) had the highest hypocholesterolemic activity, while 3,4-DMC, which lacks the methoxyl in carbon 2 but conserves the two other methoxyls in an adjacent position, had the highest choleretic activity and a probable cholelitholytic activity. In methoxycinnamic acids with two methoxyls in non-adjacent positions (2,4-DMC and 3,5-DMC), the hypocholesterolemic and choleretic activities were not as evident. 2,4,5-TMC and 3,4-DMC, which did not cause liver damage during the treatment period, should be further explored as a hypocholesterolemic and choleretic compounds in humans.

Introduction

Yumel (*Guatteria gaumeri*, Greenman, Annonaciae) is a native plant from Yucatán, Mexico, that has been used as a bark infusion in traditional medicine for the treatment of gallstones [1]. α-asarone (2,4,5-trimethoxy-1-propenylbenzene), the active substance of *G. gaumeri*, decreases rat and human cholesterol serum levels [2]. α-asarone has hypocholesterolemic [2] and hypolipidemic properties attributed to the inhibition of HMG-CoA reductase [EC 1.1.1.34 (*R*)-mevalonate:NADP$^+$ oxidoreductase (CoA-acylating)] [3], but it cannot be used in clinical trials because of its genotoxic and hepato-carcinogenic properties in rodents [4]. 2,4,5-Trimethoxycinnamic acid (TMC), the major metabolite of α-asarone, does not have the above-mentioned toxicity [4], but it retains the beneficial properties of α-asarone: it decreases total serum cholesterol and serum LDL-cholesterol, but has no effect on serum HDL-cholesterol. α-asarone and 2,4,5-TMC also increase bile flow, bile acids and bile phospholipids, and decrease bile cholesterol and biliary cholesterol saturation index (CSI) in hypercholesterolemic rats [5].

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http://dx.doi.org/10.1016/j.pharep.2014.12.009
1734-1140/

High levels of serum cholesterol and LDL-cholesterol correlate with an increased occurrence of atherosclerosis [6,7], therefore, it is important to develop efficient therapies for the treatment of hypercholesterolemia. Statins are considered as the most effective drugs for the treatment of hypercholesterolemia; however, they have some adverse effects, such as renal dysfunction, myalgia, arthralgia and cardiac failure [8,9]. In this study, we evaluated the role of the methoxyls on carbons 2, 4 and 5 of 2,4,5-TMC on its pharmacological properties. We evaluated three 2,4,5-TMC derivatives, which lack one methoxyl and have the other two methoxyls in different positions on their aromatic rings: 2,4-DMC, 3,4-DMC and 3,5-DMC (Fig. 1), in order to find out which DMC retains most of the pharmacological properties of 2,4,5-TMC. DMCs could be used as therapeutic hypocholesterolemic and choleretic agents.

Materials and methods

Reagents

Cholesterol, 2,4,5-TMC, 2,4-DMC, 3,4-DMC, 3,5-DMC, 3-α-hydroxysteroid dehydrogenase [EC 1.1.1.51 3(or 17)-beta-hydroxysteroid dehydrogenase] (from *Pseudomonas testosteroni*), sodium cholate, sodium pentobarbital, NADPH, EDTA and Tris–HCl were obtained from Sigma–Aldrich (St. Louis, MO, USA). Standard pellet diet (Rodent Lab Chow) was from PMI Nutrition International (Brentwood, MO, USA).

Diet preparation and administration of the methoxycinnamic acids to rats

Fifteen-week-old male Wistar rats were divided into six groups (10 rats per group), and were placed at random in stainless steel cages (5 rats per cage) under normal lighting conditions (12 h light/12 h darkness) with free access to food and water. Group 1 (Control I, normocholesterolemic rats) received a standard pellet diet, and the other five groups received a high-cholesterol/cholate diet (standard pellet diet supplemented with 1% cholesterol, 0.2% sodium cholate and 5% olive oil). Group 2 (Control II) was left untreated, while groups 3–6 were treated with 2,4-DMC, 3,4-DMC, 3,5-DMC or 2,4,5-TMC, respectively. The methoxycinnamic acids were dissolved in a sodium bicarbonate solution, and were injected subcutaneously at 8:00 am for 8 days, at a dose of 80 mg/kg body wt./day. The treatments with TMC and the DMCs started at the same time as the change of diet. After this treatment, the rats were weighed and anesthetized with sodium pentobarbital (5 mg/100 g body wt.). The abdomen was opened by a midline incision and bile was collected from the common bile duct cannulated with polyethylene tubing (Clay Adams No. 10). Bile was collected for 60 min and bile flow was determined as the amount of bile (μl) collected/min/g of liver. In the collected bile, the amount of bile acids, phospholipids, and cholesterol was determined. Blood was collected from all the rats by abdominal aorta puncture and allowed to clot, and serum was used to determine the activity of alanine transaminase (EC 2.6.1.2 L-alanine:2-oxoglutarate aminotransferase) and aspartate transaminase (EC 2.6.1.1 L-aspartate: 2-oxoglutarate aminotransferase), and the concentrations of cholesterol and cholesterol-lipoproteins. The rats were fasted overnight prior to these procedures, to allow a proper evaluation of cholesterol and lipoprotein levels in blood. Finally, the rats were killed and their livers were removed and weighed. The study was repeated 3 times, with a total of 180 rats. This study was approved by the Bioethics Committee of our Institution, according to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health [10].

Bile analysis and determination of cholesterol saturation index (CSI)

Bile was extracted with chloroform:methanol:water (Folch extraction) before assaying for bile acids, cholesterol and phospholipids. Bile acids concentrations were determined by an enzymatic method using 3-α-hydroxysteroid dehydrogenase [11] with less than 0.1% of 3-β-hydroxysteroid dehydrogenase activity. Cholesterol and phospholipid concentrations were measured with kits (Randox Laboratories, Antrim, UK and Spinreact, Sant Esteve de Bas, Spain, respectively). The CSI is the relation between the biliary concentration of cholesterol (mol%) and the maximum solubility of cholesterol in bile. This last parameter was calculated using both the relative concentrations of phospholipids and bile acids (mol%) and the total concentrations of bile lipids, which were interpolated in Carey's critical tables [12].

Determination of the activity of serum alanine and aspartate transaminases

The activities of serum alanine and aspartate transaminases were measured with kits (Randox Laboratories Ltd., Crumlin, CO, Antrim, UK).

Determination of the serum concentrations of cholesterol and cholesterol-lipoproteins

The levels of serum cholesterol, LDL-cholesterol and HDL-cholesterol were determined by enzymatic methods using kits (Randox Laboratories). Total cholesterol was measured by the enzymatic colorimetric cholesterol oxidase and peroxidase method. LDL-cholesterol was measured by quantifying cholesterol in the supernatant (LDL fraction) after elimination of chylomicrons,

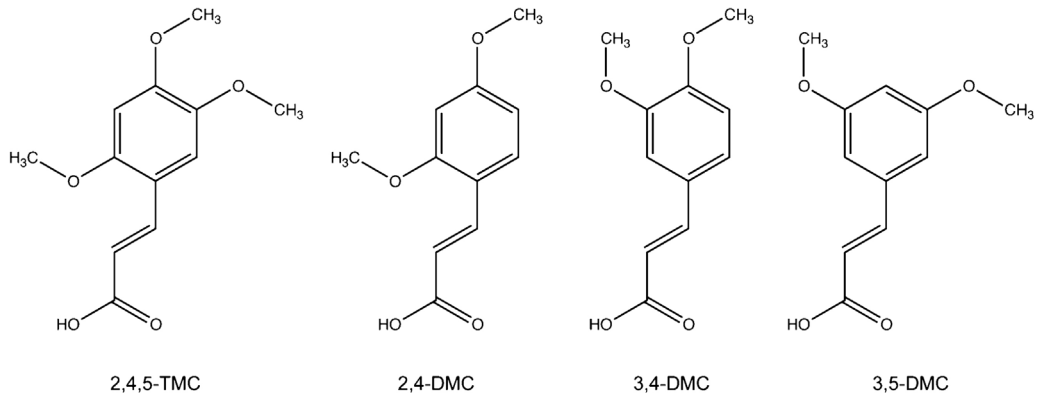

Fig. 1. Chemical structures of the dimethoxycinnamic and trimethoxycinnamic acids.