Saudi Pharmaceutical Journal (2017) **25**, 120–127

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Chitosan treatment abrogates hypercholesterolemia-induced erythrocyte's arginase activation

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Received 10 December 2015; accepted 19 May 2016
Available online 26 May 2016

KEYWORDS

Erythrocytes;
Chitosan;
Hypercholesterolemia;
Nitric oxide;
Arginase

Abstract This study aimed to evaluate the protective effect of chitosan (CS) against hypercholesterolemia (HC) induced arginase activation and disruption of nitric oxide (NO) biosynthesis using erythrocytes as cellular model. Human erythrocytes were isolated and classified into eight groups. Next, cells were treated with L-arginine (L-ARG), N^{ω}-nitro-L-arginine methyl ester (L-NAME), CS or CS + L-ARG in the presence of normal plasma or cholesterol enriches plasma. Then, erythrocytes were incubated at 37 °C for 24 h. The present results revealed that, HC induced significant increase of cholesterol inclusion into erythrocytes membrane compared to control. Moreover, HC caused significant decrease in nitric oxide synthase (NOS) activity similar to L-NAME; however, arginase activity and arginase/NOS ratio significantly increased compared to control. On contrast, treatment of HC with, L-arginine, CS or CS plus L-arginine prevents HC induced cholesterol loading into erythrocytes membrane, NOS inhibition and arginase activation. This study suggested that CS could be protective agent against HC induced disruption of erythrocyte's oxidative status and arginase activation.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2016.05.007

1. Introduction

Arginase is an important regulator of nitric oxide (NO) production, and an increase of arginase activity reduces the availability of L-arginine for NO synthase, thus reducing NO production, and leading to endothelial dysfunction (Pernow and Jung, 2013). Increased activity of arginase has been demonstrated in several pathological conditions including cardiovascular dysfunction and other vascular diseases (Pernow and Jung, 2013). Hypercholesterolemia (HC) induces lipemic stress due to extra cholesterol deposition into the membranes of vascular cells and erythrocytes, and this triggers reactive oxygen species (ROS) production, and membrane aberration (Uydu et al., 2012). Furthermore, lipemic stress is associated with disruption of L-arginine transport into cells and inactivation of nitric oxide synthase (NOS), while arginase activity is increased. Additionally, L-arginine analogues increase, particularly asymmetric dimethylarginine and symmetric dimethylarginine (Eligini et al., 2013; Yang et al., 2013; Porro et al., 2014). The net result of these events is the decrease of NO levels as a key player in the regulation of homeostasis, vasodilation, neurotransmission, free radicals scavenging and erythrocytes function (Eligini et al., 2013).

In past decades, several studies reported that vascular NO is mostly produced from endothelial cells by endothelial NO synthase (eNOS); however nowadays, erythrocytes were listed as another major source of NO in vascular lumen (Eligini et al., 2013; Ramírez-Zamora et al., 2013; Porro et al., 2014). For NO biosynthesis, NOS utilizes L-arginine as substrate; flavoproteins and tetrahydrobiopterin were used as coenzymes (Eligini et al., 2013; Porro et al., 2014). Conversely, arginase competes with NOS on L-arginine as common substrate; therefore, it NO production (Yang et al., 2013, Li and Förstermann, 2013). The proper balance between NOS and arginase is essential for maintenance of NO homeostasis (Porro et al., 2014; Yang et al., 2013). Functional erythrocytes have antioxidant machinery that neutralizes ROS generated in the vasculature; however, malfunctioned erythrocytes can act as a source of ROS (Minetti et al., 2007). Moreover, such erythrocytes release arginase that limits NO production (Porro et al., 2014; Yang et al., 2013). Therefore, oxidized erythrocytes act as prooxidant bombs to vascular endothelium. Although, several studies reported that erythrocyte's arginase activity was augmented by oxidative stress (Yang et al., 2013; Porro et al., 2014; Li and Förstermann, 2013), no enough published data address this topic, and further research are necessary to address this issue.

Most of lipid-lowering agents have many therapeutic problems with severe side effects, while dietary fibers as lipid lowering therapy are safer. Chitosan (CS) is a dietary fiber biodegradable, biocompatible and has many health benefits including wound healing, antiinflammatory, anti-cancers, immune-modulator, hemostatic agent, lipid-lowering agent and antioxidants (Xia et al., 2011; Luo and Wang, 2013; Anandan et al., 2013). The lipid-lowering effect of CS is attributed to its binding to fatty acid, cholesterol, and bile salts; this resulted in delaying the digestion and absorption of fat (Xia et al., 2011). Additionally, CS augments lipoprotein lipase activities and influences plasma adipocytokines, which significantly reduce adiposity index. Therefore, CS can regulate the level of circulating triacylglycerol and ameliorates metabolic alterations (Luo and Wang, 2013). CS can help the body maintain the antioxidant activity, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and other antioxidants, which play important roles in counteraction of oxidative stress (Xia et al., 2011; Luo and Wang, 2013).

Despite the beneficial effects of CS in hyperlipidemic conditions have been extensively studied, yet no data available about the effect of CS on arginase activity in HC condition have been explored. Therefore, the main goal of this study was to test the hypothesis that CS as innovative therapeutic agent approaches for treatment of HC induced disruption of erythrocyte NO biosynthetic pathway. NOS activity and arginase activities were investigated under effect of HC exposure in the presence and absence of CS.

2. Materials and methods

2.1. Chemicals

Low molecular weight chitosan, L-Arginine (L-ARG), N^{ω}-nitro-L-arginine methyl ester (L-NAME), and water-soluble cholesterol were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of reagent grade.

2.2. Isolation of erythrocytes and experimental design

Blood samples were drawn from healthy human volunteers by venipuncture into heparinized test tubes. Plasma sample was separated by centrifugation at 3000 rpm for 5 min; buffy coat was removed by aspiration. Then, erythrocytes were gently washed 3 times with an equal volume of phosphate buffered saline pH 7.5. Next, supernatant was discarded and erythrocytes were separated. Plasma sample was mixed with water-soluble cholesterol, after this referred to cholesterol-enriched plasma (HC) (Kanakaraj and Singh, 1989). HC was confirmed by measuring cholesterol level using cholesterol oxidase method by commercially available kit (Randox Laboratories, Crumlin, UK). The protocol for this study conformed to the guidelines of the Institutional Ethical Committee.

Washed erythrocytes were classified into eight groups as follows:

Group 1: Normal control (NC), in this group washed erythrocytes were incubated with normal plasma (cholesterol = 150 mg/dl).
Group 2: CS treated group (CS), herein, washed erythrocytes were incubated with *normal* plasma plus CS solution (1 mg/mL) (Fernandes et al., 2008).
Group 3: In this group cells were treated with L-NAME as reference group for NO inhibition, in which erythrocytes were incubated with *normal* plasma plus (10 mM) of L-NAME (Kuwai and Hayashi, 2006).
Group 4: L-arginine treated group as reference samples for NO production, in this group erythrocytes were suspended in normal plasma plus (10 mM) of L-ARG (Kuwai and Hayashi, 2006).
Group 5: HC incubated group, in this set erythrocytes were exposed to HC plasma (cholesterol 450 mg/dl) (Kanakaraj and Singh, 1989).
Group 6: HC + L-ARG, samples of this group, erythrocytes were exposed to HC and treated with (10 μM) L-arginine.