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Antiatherogenic effect of quercetin is mediated by proteasome inhibition in the aorta and circulating leukocytes

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Abstract:
Quercetin, a plant-derived flavonoid, has attracted considerable attention as promising compound for heart disease prevention and therapy. It has been linked to decreased mortality from heart disease and decreased incidence of stroke. Here, we report new data showing the angioprotective properties of quercetin mediated by its effect on proteasomal proteolysis. This study was designed to investigate the ability of quercetin to modulate proteasomal activity in a rabbit model of cholesterol-induced atherosclerosis. First, we show proteasomal trypsin-like (TL) activity increased up to 2.4-fold, chymotrypsin-like (CTL) activity increased by up to 43% and peptidyl-glutamyl peptide-hydrolyzing (PGPH) activity increased by up to 10% after 8 weeks of a cholesterol-rich diet. A single intravenous injection of the water-soluble form of quercetin (Corvitin) significantly decreased proteasomal TL activity 1.85-fold in monocytes, and decreased the CTL and PGPH activities more than 2-fold in polymorphonuclear leukocytes (PMNL) after 2 h. Prolonged administration (1 month) of Corvitin to animals following a cholesterol-rich diet significantly decreased all types of proteolytic proteasome activities both in tissues and in circulating leukocytes and was associated with the reduction of atherosclerotic lesion areas in the aorta. Additionally, the pharmacological form of quercetin (Quertin) was shown to have an antiatherogenic effect and an ability to inhibit proteasome activities.

Key words:
proteasome inhibition, atherosclerosis, flavonoids

Introduction

Proteasomal degradation of proteins contributes significantly to the regulation of the vital functions of cells. Disturbances in proteasomal proteolysis are associated with the development of various diseases, including neuropathology, aging, and cancer [2, 5, 7, 17, 20], and are believed to play pivotal roles in pathologies of the cardiovascular system, such as atherosclerosis. In fact, all principal processes involved in atherogenesis, such as lipoprotein exchange, expression of cell adhesion molecules, receptor recycling, and apoptosis of smooth muscle and endothelial cells, require proteasomal proteolysis [1, 28, 29, 31, 32].

Herrmann's study on hypercholesterolemia modeling showed that the amount of ubiquitinated proteins in the coronary artery of domestic pigs was 35% higher in samples from animals on a high-cholesterol diet compared with control animals, even though there was no difference in the proteasome proteolytic activity among the studied groups [10].

The available experimental data were further extended by reports showing alterations of proteasomal proteolysis in atherosclerotic-changed human vessels. Versari et al. [30] showed a decrease of proteasomal chymotrypsin-like (CTL) activity in atherosclerotic plaques of carotid arteries of patients with symptoms of cerebral ischemia. Additionally, the accumulation of ubiquitin conjugates in these patients increased when compared with the corresponding parts of arteries of patients without these symptoms. Overall, the lack of data about proteasome activity in intact human arteries makes it difficult to elucidate the significance of the changes in proteasome proteolysis during atherosclerosis. In addition, there are no experimental data concerning the use of specific proteasome inhibitors, which would be a helpful tool in resolving this question. In experiments conducted by Herrmann et al., the proteasome inhibitor MLN-273 injected for a period of 12 weeks into animals on a high-cholesterol diet led to an increased intima/media ratio and other negative effects [11]. The current data conclude that there is a proatherogenic effect of proteasome proteolysis inhibition.

It has been recently shown that polyphenols of green tea, especially epigallocatechin-3-gallate, are powerful specific inhibitors of proteasomal CTL activity both *in vitro* and *in vivo* [22]. Our previous experiments provide strong evidence that quercetin, one of the dietetic bioflavonoids, has the ability to decrease proteasome activity [6]. There are also several works that show that proteasome inhibitors, including quercetin and other bioflavonoids, have antiatherogenic properties [3, 8]. In particular, Juźwiak et al. [14] demonstrated that quercetin effectively reduces the formation of atherosclerotic plaques in the aorta and in the injured carotid artery in rabbits fed a high-fat diet. These authors suggest that this effect is mediated by the reduction of serum triglycerides and cholesterol levels.

In the present study, we report for the first time that quercetin inhibits proteasome activity in circulating leukocytes of control animals 2 h after a single intravenous administration and significantly prevents the increase of all three proteasome activities in aorta. The prolonged application of quercetin decreases the intensity of atherosclerotic lesions in rabbits fed a high-cholesterol diet. Moreover, the studied drugs have no effect on triglyceride, low-density lipoprotein and very low-density lipoprotein levels.

Materials and Methods

Experiments were performed on 56 rabbits with an average weight of 2.95 ± 0.35 kg. Animals were divided into four groups: control (16 rabbits); a group fed a cholesterol-rich diet (CRD, 1% cholesterol) every day over 4 weeks (15 rabbits); a group fed the same CRD with simultaneous intravenous injections of Corvitin (SIC "Borshchahivskiy chemical-pharmaceutical plant" CJSC, Kiev, Ukraine) in a dose of 5 mg/kg every second day for 4 weeks (10 rabbits); and a group fed the same CRD with Quertin (SIC "Borshchahivskiy chemical-pharmaceutical plant" CJSC, Kiev, Ukraine) application orally in a dose of 15 mg/kg daily for 8 weeks (15 rabbits). After 4 weeks, blood from the lateral vena of the ear was taken to measure proteasome activity in blood cells. Additional blood samples were obtained from 5 rabbits that received a single intravenous injection of Corvitin before and 2 h after injection.

Blood cells were fractionated by centrifugation on a Percoll gradient. Briefly, blood stabilized with Na-EDTA was diluted in a 0.9% NaCl solution in a 1:1 ratio and stratified on a Percoll gradient solution containing 4 layers with densities of 72, 63, 54, and 45%. To obtain these concentrations, 9 parts of Percoll were mixed with 1 part of 10X Hanks solution (pH 7.4) and the corresponding amount of a 0.9% NaCl solution to reach required density. The first centrifugation was performed at 400 × g for 5 min, the first supernatant layer was removed, and this volume was replaced with 0.9% sodium chloride solution. The second centrifugation was done at 800 × g for 15 min with subsequent collection of cells between the 45 and 54% density layers (monocytes), 54 and 63% (lymphocytes), and 63 and 72% (PMN leukocytes). Cells were washed from the Percoll by centrifugation at 800 × g for 5 min and the sediment was resuspended in Hanks solution (pH 7.4). The number of leukocytes was determined using a cell count chamber. Cells were then sonicated, permeabilized by saponin (0.1 mg/ml) and used for biochemical analyses.

After sonication, unlysed cells, membranes, and nuclei were removed by centrifugation at 800 × g for 10 min. The supernatant was incubated in a buffer containing 25 mM Tris-HCl (pH 7.5) and 1 mM dithiothreitol. The fluorogenic substrate Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin was used to measure the CTL activity of the proteasome; Boc-