Pharmacological Reports
2011, 63, 1009–1018
ISSN 1734-1140

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

Denis A. Pashevin, Lesya V. Tumanovska, Victor E. Dosenko, Vasyl S. Nagibin, Veronika L. Gurianova, Alexey A. Moibenko

Department of General and Molecular Pathophysiology, Bogomoletz Institute of Physiology, Bogomoletz st. 4, Kiev 01024, Ukraine

Correspondence: Victor E. Dosenko, e-mail: dosenko@biph.kiev.ua

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-

Leu-Ser-Thr-Arg-7-amido-4-methylcoumarin was used for detection of TL activity and CBZ-Leu-Leu-Glu-AMC for PGPH activity. After a 30-min (for TL activity) or 1-h (for others) incubation with 6 μM fluorogenic peptide, the fluorescence of the reaction products was monitored at 380 nm excitation and 440 nm emission using free 7-amino-4-methylcoumarin (AMC) as a standard on a Hitachi 4000 spectrofluorometer. To differentiate between nonproteasome- and proteasome-mediated peptide hydrolysis, reactions were performed in the absence and presence of selective proteasome inhibitors, clasto-lactacystin β-lactone (2.5 μM) or MG132 (5 μM). The percent inhibition of hydrolysis of the respective substrates by selective inhibitors was used to assess proteasome activity, which was expressed as nM AMC per 10^6 cells per 1 min [24].

Animals were euthanized by air embolia to investigate proteasome activity in tissues. The aorta was homogenized in a glass homogenizer in Tris-HCl buffer (pH 7.4), and the suspension was centrifuged at 900 × g for 10 min. The supernatant was used for biochemical analyses. The protein concentration in the aortic homogenate was estimated by the method of Lowry et al. [19]. Proteasomal activity was expressed as nM of AMC per 1 mg of protein per 1 min. Fluorogenic substrates, clasto-lactacystin β-lactone, MG132, dithiothreitol, and Percoll were purchased from Sigma (USA).

The levels of cholesterol, triglyceride, LDL, and VLDL were determined using Bio System A25 (BioSystems S.A., Spain).

Histological changes in aortic tissues were established as follows: the frozen aortic arch from all animals of all experimental groups was sliced at a thickness of 10–12 μm on a cryomicrotome from 3 segments at an interval of 3–4 mm. The slices were then fixed on glass. A stock solution of Oil Red was diluted with isopropanol in a 3:2 ratio, filtered and used to stain microsamples for 10 min. Then, samples were washed by distilled water, stained by hematoxylin for 3 min, and after fixation, slices were examined under an optical microscope.

All experiments were performed according to European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Statistical analyses were made using Origin 7.0 and Excel 2000. All values are presented as the mean ± SE. The Student's *t* test was used to evaluate significance; $p < 0.05$ was considered statistically significant.

Results

Intravenous injection of Corvitin

Our experiments show that after 90 min of intravenous injection of Corvitin, proteasomal activities had changed differently in isolated leukocytes. In monocytes, TL activity decreased 1.85-fold ($p < 0.05$) after Corvitin injection in comparison with the control level, but there were no significant changes in CTL and PGPH activities (Tab. 1). CTL and PGPH activities in lymphocytes decreased by 1.7-fold ($p = 0.2$) and 1.9-fold ($p = 0.2$), respectively, but TL activity increased up to 40% ($p = 0.15$). The most significant changes in proteasome activities following Corvitin injection were observed in PMN leukocytes (Tab. 1):

Tab. 1. Influence of Corvitin (5 mg/kg of body weight, 90 min after injection) on CTL, TL and PGPH activities in isolated monocytes, PMN and lymphocytes of rabbits

	TL activity (nmol AMC/10^6 cells per min)	CTL activity (nmol AMC/10^6 cells per min)	PGPH activity (nmol AMC/10^6 cells per min)
Control monocytes	36.01 ± 7.524	18.23 ± 3.732	0.22 ± 0.814
Monocytes + Corvitin	19.52 ± 4.116*	19.23 ± 3.524	0.24 ± 1.226
Control lymphocytes	12.14 ± 2.731	6.34 ± 1.289	0.06 ± 0.073
Lymphocytes + Corvitin	15.83 ± 1.623	3.84 ± 0.912	0.03 ± 0.005
Control PMN	0.21 ± 0.632	4.45 ± 0.411	0.21 ± 0.440
PMN + Corvitin	0.11 ± 0.020	2 ± 0.232*	0.08 ± 0.008*

* $p < 0.05$ compared with control. Control – n = 8; Corvitin – n = 10

all three proteolytic activities of the proteasome were reduced: TL by 2-fold ($p = 0.09$), CTL by 2.4-fold ($p = 0.038$), and PGPH by 2.6-fold ($p = 0.047$). Overall, the data provide primary evidence that intravenously injected quercetin is able to inhibit proteasomal activity *in vivo*.

Effect of a high-cholesterol diet on proteasomal activity

To control for the effectiveness of atherosclerosis modeling by CRD, we monitored the metabolism of some lipids and defined the level of indicators of cholesterol in rabbit plasma. Experimental data clearly indicate the adequacy of our model, showing the increase of cholesterol, triglycerides, LDL and VLDL levels up to 32-, 2.2-, 30-, and 5-fold, respectively (Fig. 1).

Furthermore, we discovered a significant influence of an 8-week cholesterol-rich diet (CRD) on proteasome activity in aorta: TL activity increased 2.4-fold in comparison with control ($p < 0.05$), CTL increased by 43% and PGPH activity increased by 10% (Fig. 2). The addition of Corvitin to the CRD reduced proteasomal activity in aortic tissues. In particular, both TL and PGPH activities decreased by 25% ($p < 0.05$) and 50% ($p < 0.05$), respectively. The most prominent reduction was detected for CTL (10-fold, $p < 0.05$) after Corvitin application when compared with rabbits that received only the high-cholesterol diet.

CTL activity in monocytes of animals from the CRD group decreased by 40% in comparison with those from control, and Corvitin led to a 2.5-fold decrease of this activity. CRD did not have a significant effect on PGPH activity, whereas Corvitin administration decreased it by up to 8.7-fold (Tab. 2). A similar pattern was observed in lymphocytes: an atherogenic diet increased CTL activity by 10% and augmented PGPH activity up to 4-fold ($p < 0.05$). Corvitin decreased CTL and PGPH activities by 16.5- and 7.4-fold, respectively, in comparison to animals that received only cholesterol forage diets. Both CTL (5-fold) and PGPH (40%) activities were reduced in PMN leukocytes upon Corvitin application in comparison with the CRD group (Tab. 2). These data clearly show that Corvitin administration decreases proteasomal CTL and PGPH activities in blood cells,

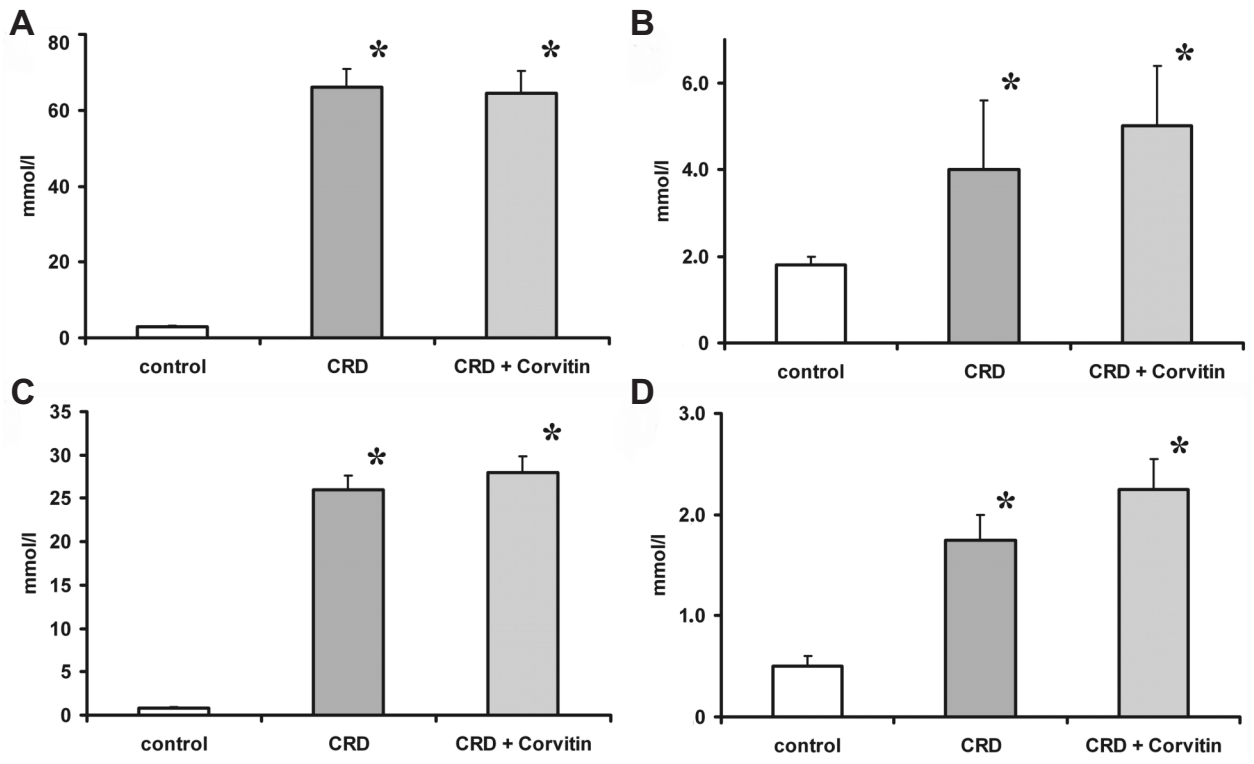

Fig. 1. Levels of cholesterol (**A**), triglycerides (**B**), LDL (**C**), and VLDL (**D**) in the blood plasma of control (n = 10), cholesterol-rich diet (CRD; n = 16), and CRD + Corvitin (n = 10) groups. * $p < 0.05$ compared with control

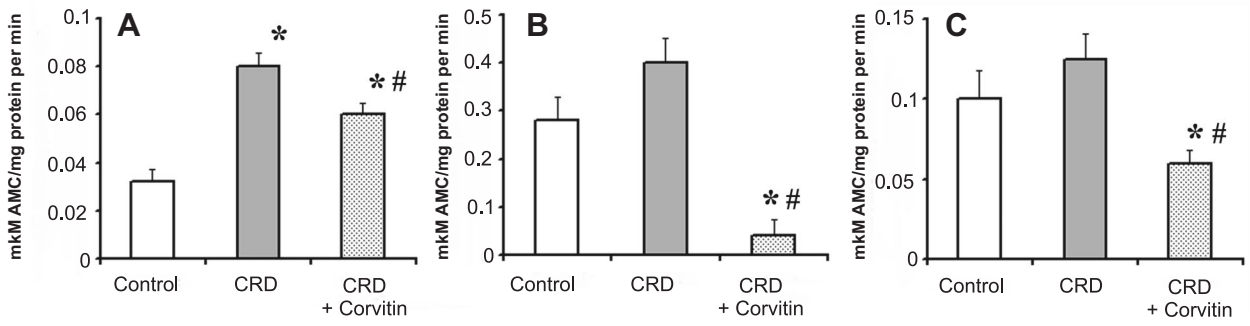

Fig. 2. Changes in proteasome activity in aortic tissues of control (n = 10), cholesterol-rich diet (CRD; n = 16), and CRD + Corvitin (n = 10) groups. (**A**) Trypsin-like; (**B**) chymotrypsin-like; and (**C**) PGPH activity. * $p < 0.05$ compared with control; # $p < 0.05$ compared with CRD

Tab. 2. Changes of CTL and PGPH activity of the proteasome in monocytes, lymphocytes and PMN in a model of cholesterol atherosclerosis due to Corvitin.

	CTL activity (nM AMC/10^6 cells per min)	PGPH activity (nM AMC/10^6 cells per min)
Monocytes of control rabbits	21.97 ± 3.525	0.059 ± 0.008
Monocytes of rabbits, 4 weeks of cholesterol-reach diet (CRD)	15.13 ± 1.841	0.05 ± 0.012
Monocytes of rabbits(4 weeks of CRD + Corvitin)	6.13 ± 2.825*†	0.01 ± 0.002*†
Lymphocytes of control rabbits	8.46 ± 1.624	0.10 ± 0.033
Lymphocytes of rabbits after 4 weeks of CRD	9.24 ± 2.876	0.44 ± 0.103*
Lymphocytes of rabbits (4 weeks of CRD + Corvitin)	0.55 ± 0.182†	0.05 ± 0.013†
PMN of control rabbits	3.83 ± 0.623	0.2 ± 0.05
PMN of rabbits after 4 weeks of CRD	3.56 ± 0.551	0.11 ± 0.125
PMN of rabbits (4 weeks of CRD + Corvitin)	0.72 ± 0.133*†	0.08 ± 0.012

* $p < 0.05$ compared to control. † $p < 0.05$ compared to cholesterol diet group. Control – n = 8; Corvitin – n = 10

thereby affecting the proteasome proteolysis involved in inducing atherogenic lesions of the blood vessel wall.

Effect of a 4- and 8-week Quertin-containing diet on proteasome activity in blood cells in CRD rabbits

Because Corvitin had an antiatherogenic effect when intravenously administrated, it would be difficult to use it for the prevention of atherosclerotic injury due to a slow development of disease. Therefore, we analyzed the ability of quercetin to influence the proteosomal system and atherosclerosis development, choosing its oral pharmacological form (Quertin) for further investigations.

CRD resulted in the following changes of proteasomal proteolysis: after a 4-week CRD, CTL activity decreased by 40% in rabbit monocytes. Simultaneous application of Quertin for 4 weeks decreased CTL activity up to 2.5-fold when compared to CRD. Addition of Quertin to the CRD after 8 weeks decreased the CTL activity in monocytes by 3-fold when compared with the group of rabbits that received only CRD for the same amount of time. There were no significant changes of PGPH activity in monocytes after 4 weeks, but after 8 weeks of CRD, it increased 15 times ($p < 0.05$) compared to control. Quertin prevented this increase and reduced PGPH activity by 70% compared to CRD alone ($p < 0.05$) (Tab. 3).

Furthermore, we evaluated changes of proteasomal activities in lymphocytes and found that CTL activity

Tab. 3. Changes of CTL and PGPH activity of the proteasome in monocytes, lymphocytes and PMN cells in a model of cholesterol atherosclerosis due to Quertin

	CTL activity (nM AMC/10^6 cells per min)	PGPH activity (nM AMC/10^6 cells per min)
Monocytes of control rabbits	21.01 ± 3.412	0.06 ± 0.008
Monocytes of rabbits after 4 weeks of CRD	15.21 ± 1.823	0.06 ± 0.010
Monocytes of rabbits (4 weeks of CRD + Quertin)	8.33 ± 0.421*#	0.10 ± 0.042
Monocytes of rabbits (8 weeks of CRD)	30.23 ± 9.125	0.94 ± 0.240*
Monocytes of rabbits (8 weeks of CRD + Quertin)	9.57 ± 2.243*	0.57 ± 0.142#
Lymphocytes of control rabbits	7.83 ± 1.402	0.12 ± 0.031
Lymphocytes of rabbits after 4 weeks of CRD	9.41 ± 2.812	0.10 ± 0.030
Lymphocytes of rabbits (4 weeks of CRD + Quertin)	4.24 ± 0.435	0.27 ± 0.142
Lymphocytes of rabbits after 8 weeks of CRD	7.41 ± 1.515	0.41 ± 0.103*
Lymphocytes of rabbits (8 weeks of CRD + Quertin)	6.12 ± 1.734	0.44 ± 0.112*
PMN of control rabbits	3.82 ± 0.625	0.27 ± 0.052
PMN of rabbits after 4 weeks of cholesterol diet	3.56 ± 0.552	0.37 ± 0.113
PMN of rabbits (4 weeks of CRD + Quertin)	0.15 ± 0.053*#	0.42 ± 0,072
PMN of rabbits after 8 weeks of CRD	3.04 ± 0.524	0.43 ± 0.051*
PMN of rabbits (8 weeks of CRD + Quertin)	0.06 ± 0.005*#	0.05 ± 0.005*#

* $p < 0.05$ compared with control. # $p < 0.05$ compared with the cholesterol diet group at the corresponding term. Control – n = 16; 4 weeks of CRD – n = 15; 4 weeks of CRD + Quertin – n = 15; 8 weeks of CRD – n = 15; 8 weeks of CRD + Quertin – n = 15

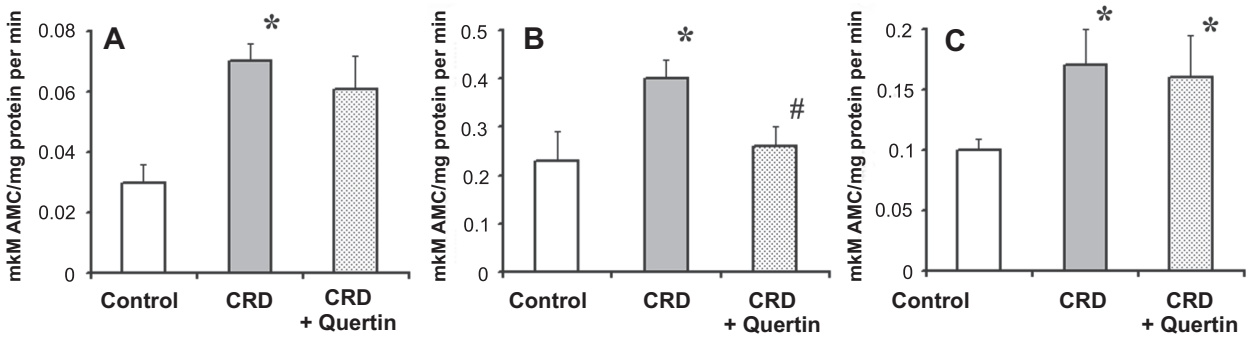

Fig. 3. Changes in proteasome activity in aortic tissues of control (n = 16), cholesterol-rich diet (CRD; n = 15), and CRD + Quertin (n = 15) groups. (**A**) Trypsin-like; (**B**) chymotrypsin-like; and (**C**) PGPH activity. * $p < 0.05$ compared with control, # $p < 0.05$ compared with CRD

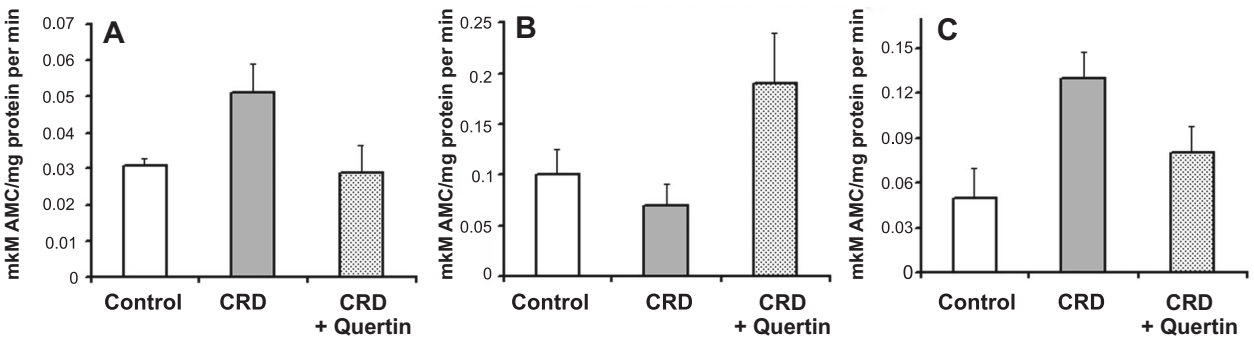

Fig. 4. Changes in proteasome activities in tissues of the venous wall. (**A**) trypsin-like; (**B**) chymotrypsin-like; and (**C**) PGPH activity

increased by 20% after 4 weeks of CRD and decreased by 80% in a group with simultaneous Quertin administration compared with control. The pattern of changes in both groups after 8 weeks of CRD and simultaneous Quertin administration was similar but not significant. PGPH activity increased equally in both groups, those that received CRD alone or received a combination of CRD with Quertin, compared with control. Therefore, there was no effect of drug administration on this particular proteasome activity.

Effect of an 8-week CRD with Quertin on proteasomal activity in aortic and vena cava posterior tissues of rabbits

TL activity in aortic tissues increased 2.3-fold after 8 weeks of CRD compared to control ($p < 0.05$) and was reduced by Quertin by 15% compared with the CRD group (Fig. 3). Cholesterol forage resulted in a 60% ($p < 0.05$) increase of CTL activity, and Quertin administration brought this value down to the control level ($p < 0.05$). PGPH activity also increased by 70% in the CRD group compared with control, and the effect of Quertin on this type of proteasome activity was not significant (Fig. 3).

The pattern of changes of proteasome activities in tissues of the venous wall was quite different. Whereas TL activity increased in the CRD group and decreased in the group with simultaneous administration of Quertin, like it did in aortic tissues, the changes in CTL activity had absolutely controversial tendencies, and it was reduced in both these groups. PGPH activity changed in the same way as TL (Fig. 4), and, importantly, the control levels of CTL and PGPH activities in the venous wall were significantly lower than corresponding levels in aortic tissues.

These data clearly indicate that, like Corvitin, the simultaneous use of Quertin and CRD prevents the increase of proteasome activity, but Quertin action seems to be less pronounced.

Morphological changes in aorta

Pathohistological changes in rabbit aorta were different among experimental groups. The endothelial layer in the control group had no signs of desquamation, edema and lipid infiltration (Oil Red staining). The elastic inner membrane, media and adventitia were intact. Histological investigation of aorta in the CRD group showed local disturbances in intima and media.

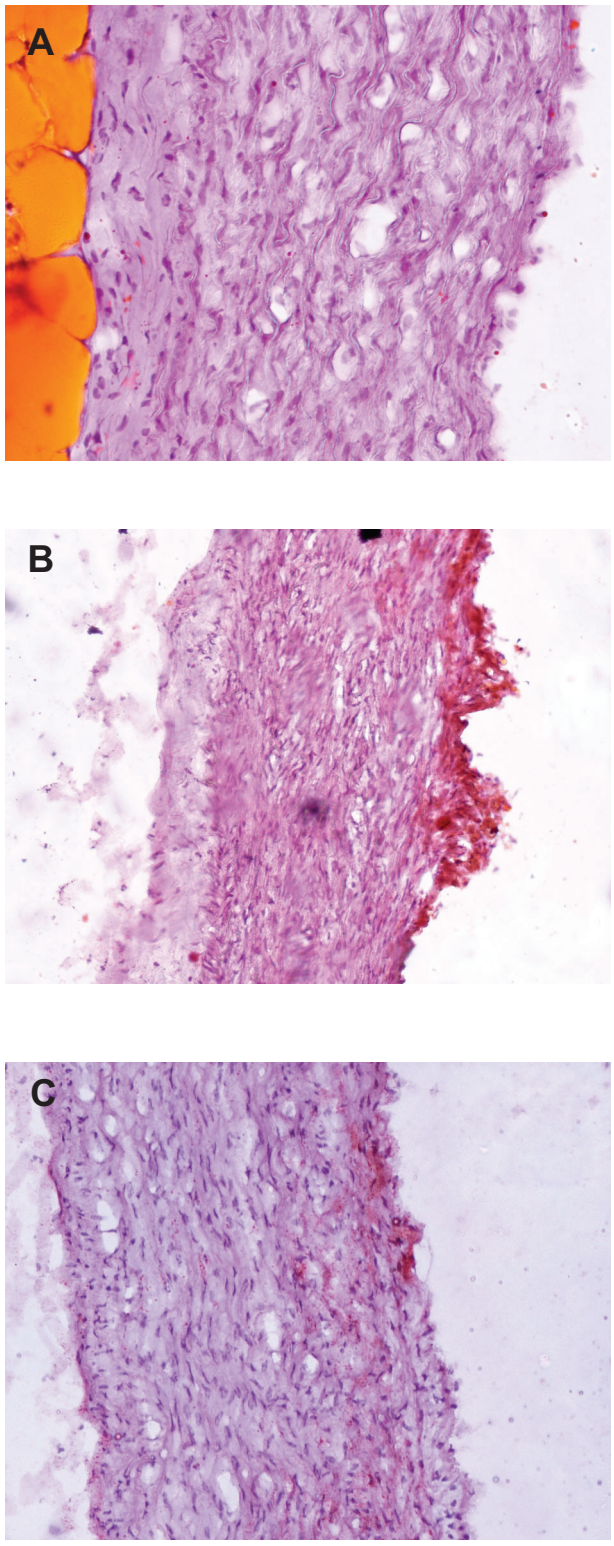

Fig 5. (**A**) Morphology of aortic tissues of control rabbits. (**B**) Local CRD disturbances in intima and media, endothelial desquamation, lipid accumulation in different layers of vessels, SMC migration to the intima, and excrescence of connective tissue were detected in the areas of the plaque formation in the CRD group. (**C**) These changes were abolished in the CRD + Corvitin group. Double-staining: Oil Red and hemotoxylin

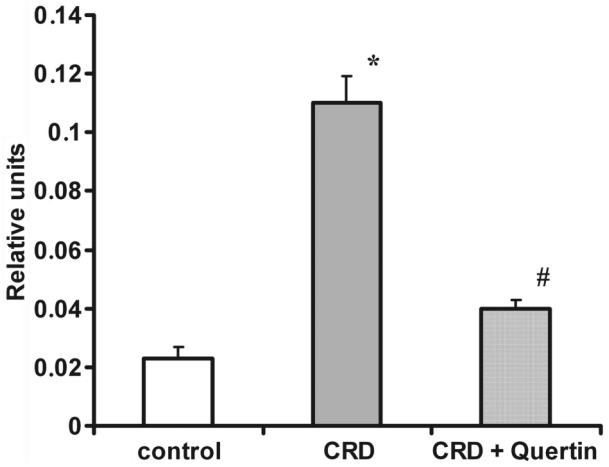

Fig. 6. Aortic intima/media ratio of control, CRD, and CRD + Quertin rabbits. * $p < 0.05$ compared with control, # $p < 0.05$ compared with CRD

Endothelial desquamation, lipid accumulation in different layers of the vessel, SMC migration to the intima, and excrescence of connective tissue were detected in the areas of plaque formation. Corvitin administration considerably abolished this effect (Fig. 5), especially at the level of lipid accumulation and the intima/media ratio, which was strongly decreased compared to the CRD group (Fig. 6).

Thus, Corvitin promotes the protection of the structure and functions of vessel walls and prevents lipid accumulation in intima and lipid plaque formation.

Discussion

Analysis of the collected data allows us to conclude that the pathological role of the increase of all three types of proteasome proteolytic activities or separate subunits in atherogenesis is due to significant changes in proteasomal activities observed by hypercholesterolemia modeling in tissues and in isolated blood cells. The use of bioflavonoids in intravenous and oral forms inhibited the activity of the proteasome complex and significantly decreased lipid accumulation in intima. TL activity was most prominently increased in aorta, by 2.4- and 2.3-fold after 4 and 8 weeks of CRD, respectively. These data are to some extent consistent with the work of Herrmann et al., who used the atherogenic diet in pigs (a high-cholesterol diet for 12 weeks) [7]. Bioflavonoids caused the most prominent decrease of the proteasomal β5 subunit of CTL, independently of the mode of drug administration. The majority of researchers consider this subunit to be the most important for multicatalytic complex formation and functioning. Our data correlate to a certain extent with results of American studies [3]. Chen et al. found that one of the bioflavonoids, apigenin, which has a very similar structure to quercetin, inhibits proteasomal activity in transformed cells (culture of Jurkat T cells) [3].

Because we found that proteasome activity was the lowest in venous walls in normal conditions, it is possible to assume that this regional feature of proteasome proteolysis is one of the factors that promotes the antiatherogenic defense in veins. Changes in proteasome activity the in wall of veins differed compared to aorta in animals on a high-cholesterol diet. There was no significant increase of any proteasome subunit activity. Oral application of Quertin decreased proteasomal TL and PGPH activities, but CTL activity had a tendency to increase. These data also indirectly prove the pathogenic action of increased proteasomal proteolysis in cholesterol-induced atherosclerosis.

We have shown in this work that the protective effects of bioflavonoids are not associated with an influence on lipid indices of blood: the cholesterol, triglyceride, LDL and VLDL content did not change with quercetin administration. Although these data are inconsistent with the results from Juźwiak et al. [14], they can be explained by the significantly (300 times) lower dose of quercetin administered to experimental animals. In contrast, our findings are in agreement with the results of Herrmann et al., who similarly showed that proteasome inhibitors did not affect the lipid spectrum of blood. Thus, this is another reason to consider that the antiatherogenic effect of flavonoids is mediated by their influence on proteasome activity. It is clear that flavonoids have many antiatherogenic properties in addition to their effect on proteasome activity. Quercetin is one of the most widespread natural polyphenols used by humans. Its role as antioxidant and an inhibitor of adenylate cyclase, LOX and most protein kinases has been proved by a number of studies [12, 16, 25, 27]. Together, these and other characteristics provide the mechanisms by which quercetin influences the induction of apoptosis, mediates vasorelaxation, inflammation, and proliferation and participates in the diversity of other atherosclerotic processes. Additionally, biofla-

vonoids are known to decrease the intensity of LDL oxidation and aggregation [22]. Treatment with flavonoids decreases the activity of endotelin-1 synthesis in experiments using an oxidative stress model [13, 26]. Quercetin decreases the expression of E-selectin and molecules of cell adhesion [18]. The important characteristic of these substances is their ability to prevent hypertrophy of smooth muscle cells of the vessel wall due to the inhibition of MAPK. This can be a possible mechanism of the antioxidant action of quercetin. Alternatively, this could be explained by the ability of quercetin to act as antioxidant. Moreover, some studies show that quercetin accumulates in regions of the aorta injured by atherosclerosis and prevents oxidation of lipids in these areas [15].

Proteasomal PGPH activity comes from the β1 subunit of the constitutional proteasome or the LMP-2 subunit of the immunoproteasome. The data of Gaczynska et al. demonstrate that imunoproteasome assembly (substitution of the β1 subunit by LMP-2) is characterized by a decrease in PGPH activity. This means that CRD may cause changes in proteasome activity by increasing the β1 subunit and/or decreasing LMP-2. Some disturbances in the immune system typical of atherogenesis may be caused by these changes in proteasome activity [9]. The proinflammatory response of an organism that takes place during atherogenesis also involves proteasomal proteolysis, including the activation of NF-κB, expression of cell adhesion molecules, etc. [9]. Experiments have shown that a single dose of the proteasomal inhibitor MG-132 reduces formation of neointima after endothelial denudation of carotid arteries by up to 75% and decreases infiltration by monocytes and proliferation of smooth muscle cells [21]. It was also shown that inhibition of proteasome activity decreases hypertrophy of vessel walls in hypertension, which can be explained by the decreased secretion of endotelin-1 [26]. Overall, these data support the results of the present work concerning the antiatherogenic effect of quercetin mediated by inhibition of proteasome activity.

Notably, CRD also impacted proteasome activity in blood cells, with the most prominent effect on the upregulation of PGPH activity in the 2nd month of the experiment. Administration of flavonoids profoundly reduced proteasomal activity, and importantly, this inhibitory action was highly dependent on the form of drug administration. The most prominent reduction was observed during intravenous injections of Corvitin and was apparent in all types of cells (monocytes, lymphocytes and PMN). In contrast, oral Quertin significantly influenced proteasome activity only in monocytes and PMN.

These data indicate that the increase of proteasome activity in aorta and blood cells plays a pathogenic role during the development of atherosclerosis. Here, we report for the first time that the antiatherogenic action of quercetin is due to its inhibitory effect on proteasome activity. Two different pharmacological forms of quercetin produced in Ukraine, water-soluble Corvitin and pelletized Quertin, are shown to be effective treatments of experimental atherosclerosis and might be considered as perspective human antiatherosclerotic drugs.

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Received: August 27, 2010; **in the revised form:** January 24, 2011; **accepted:** March 3, 2011.