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Bortezomib and quercetin as effective modulators of lipopolysaccharide-induced systemic inflammatory response and metabolic disorders

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inflammation, systemic inflammatory response, lipopolysaccharide, bortezomib, quercetin, transcription factors, oxidative stress, nitrosative stress, carbohydrate metabolism, lipid metabolism, rats.

Aim. This study aimed to evaluate the effects of combined administration of bortezomib and quercetin on serum ceruloplasmin levels, carbohydrate and lipid metabolism parameters, and secondary products of lipid peroxidation in a lipopolysaccharide (LPS)-induced rat model.

Materials and methods. Systemic inflammatory response (SIR) was modeled in male Wistar rats by intraperitoneal administration of *Salmonella typhi* LPS. Rats were divided into groups: intact controls, LPS-induced SIR, and SIR groups treated with bortezomib, quercetin, or their combination. Serum ceruloplasmin, glucose, lipid profiles, nitric oxide synthase (NOS) activity, and thiobarbituric acid-reactive substances (TBARS) levels were measured. Biochemical analyses were conducted using validated spectrophotometric and enzymatic methods.

Results. Combined administration of bortezomib and quercetin showed superior efficacy in mitigating SIR markers and metabolic disruptions compared to individual treatments. Serum ceruloplasmin levels, a marker of acute-phase reaction, were normalized, indicating robust anti-inflammatory effects. Hyperglycemia associated with SIR was significantly reduced, with glucose levels returning to baseline in the combined treatment group. Lipid profile analysis revealed a marked increase in high-density lipoprotein cholesterol and reductions in very low-density lipoprotein cholesterol and triglycerides, demonstrating improved lipid metabolism. Oxidative and nitrosative stress markers, including TBARS and inducible NOS activity, were significantly lower in the combined treatment group. Enhanced constitutive NOS activity and arginase levels further supported the restoration of nitric oxide metabolism.

Conclusions. The dual administration of bortezomib and quercetin is an example of a synergistic approach to managing SIR and its metabolic consequences. This combination effectively targets both inflammatory (NF-κB inhibition) and oxidative stress (Nrf2 activation) pathways, providing better therapeutic results compared to monotherapy. These findings suggest potential clinical application of the combined use of bortezomib and quercetin in conditions characterized by chronic inflammation and metabolic disturbances.

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Бортезоміб і кверцетин як ефективні коректори ліпополісахарид-індукованої системної запальної відповіді та метаболічних розладів

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Мета роботи – оцінити вплив комбінованого застосування бортезомібу та кверцетину на концентрацію гострофазового білка церулоплазміну в сироватці крові, показників у ній вуглеводного й ліпідного обмінів і вторинних продуктів пероксидного окиснення ліпідів на моделі щурів з індукованою ліпополісахаридом (ЛПС) запальною реакцією.

Матеріали і методи. Системну запальну відповідь (СЗВ) моделювали у самців щурів лінії Вістар шляхом внутрішньоочеревинного введення ЛПС *Salmonella typhi*. Щурів поділили на групи: інтактний контроль, ЛПС-індукована СЗВ, а також групи тварин, яким відтворили СЗВ і вводили бортезоміб, кверцетин або їх комбінацію. У сироватці крові визначали вміст церулоплазміну, глюкози, ліпідний профіль, активність NO-синтази (NOS) та рівень сполук, що реагують із тіобарбітуровою кислотою (ТБК-реактантів). Біохімічні дослідження здійснили за допомогою валідованих спектрофотометричних та ензиматичних методів.

Результати. Комбіноване застосування бортезомібу та кверцетину мало більшу ефективність щодо зменшення рівнів маркерів СЗВ і корегування метаболічних порушень порівняно з окремим введенням препаратів. Вміст церулоплазміну в сироватці, який є маркером реакції гострої фази, нормалізувався, що свідчить про сильний протизапальний ефект. Гіперглікемія, пов'язана із СЗВ, значно зменшилася, а рівень глюкози у групі комбінованого лікування повернувся до базового. Аналіз ліпідного профілю показав значне підвищення рівня холестерину ліпопротеїнів високої щільності, зниження рівнів ліпопротеїнів дуже низької щільності та тригліцеридів; це свідчить про покращення ліпідного обміну. Маркери оксидативного та нітрозативного стресу, включаючи ТБК-реактанти й активність індуцибельної NOS, суттєво нижчі у

групі комбінованого лікування. Підвищення активності конститутивних ізоформ NOS й аргінази додатково підтвердило відновлення метаболізму оксиду азоту.

Висновки. Комбіноване введення бортезомібу та кверцетину є прикладом синергічного підходу до управління СЗВ та її метаболічними наслідками. Ця комбінація ефективно діє на запальні (інгібування NF-κB) та оксидативні (активація Nrf2) шляхи, забезпечуючи кращі терапевтичні результати порівняно з монотерапією. Результати дослідження дали змогу зробити висновок, що комбіноване застосування бортезомібу та кверцетину має потенціал у клінічній практиці, коли виявлено хронічне запалення та діагностовано метаболічні розлади.

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Inflammation is known to be an integral component of many diseases, including oncological diseases, where the systemic inflammatory response (SIR) plays a key role in tumor progression and the development of associated metabolic disorders [1]. It has been established that the presence of SIR is linked to reduced survival rates in patients with cancer [2].

SIR-associated diseases are among the most dangerous conditions to humanity. These include atherosclerosis and coronary artery disease, type 2 diabetes mellitus, metabolic syndrome, stroke, depression and anxiety disorders, neurodegeneration and Alzheimer's disease, steatohepatitis, and others [3]. Comorbidity combining malignant tumors with other SIR-associated conditions represents a significant unfavorable prognostic factor [4], as it complicates disease progression, reduces therapy effectiveness, and worsens patient survival outcomes. Such comorbidity exacerbates inflammatory and metabolic imbalances in the body, contributing to tumor progression and the development of complications [1,5].

Lipopolysaccharide (LPS)-induced SIR serves as an experimental model for studying the mechanisms of inflammation and developing strategies for the pharmacological correction of metabolic imbalances [6]. Metabolic disturbances, particularly those involving L-arginine metabolism, activate oxidative and nitrosative stress, thereby amplifying systemic inflammatory reactions. It is well-established that the regulation of inflammatory and metabolic processes is closely linked to the functionality of the NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells), AP-1 (Activator Protein-1), and Nrf2 (Nuclear factor erythroid 2-related factor 2) signaling pathways [3]. These pathways represent promising targets for the development of therapeutic strategies in the treatment of inflammatory and oncological diseases.

Bortezomib, a proteasome inhibitor widely used in oncology for the treatment of multiple myeloma and mantle cell lymphoma, suppresses the chymotrypsin-like activity of the 26S proteasome in mammalian cells. The 26S proteasome is a large protein complex essential for the degradation of key intracellular proteins, playing a crucial role in regulating protein metabolism and maintaining cellular homeostasis. By inhibiting the 26S proteasome, bortezomib disrupts proteolysis, initiating a cascade of reactions that ultimately result in cancer cell apoptosis [7]. The 26S proteasome is crucial for the degradation of IκB, the inhibitor of NF-κB [8]. By preventing IκB degradation, bortezomib blocks the nuclear translocation and activation of NF-κB, thereby reducing the expression of pro-inflammatory cytokines and mediators regulated by this pathway. In the context of SIR, bortezomib may help mitigate excessive inflammation by downregulating NF-κB-driven inflammatory processes. Additionally, its recently identified ability to inhibit the activation of the NLRP3 (NOD-like receptor family pyrin domain-containing 3) inflammasome, a multiprotein complex critical to the innate immune system, has garnered attention [9].

Current studies have shown that bortezomib exhibits anti-inflammatory effects in conditions such as rheumatoid arthritis [10], myocardial and retinal ischemia-reperfusion injury [11], experimental autoimmune uveitis [8], psoriasis [12], experimental autoimmune neuritis [13], all of which involve pathogenesis closely linked to the activation of NF-κB and the NLRP3 inflammasome.

The application of bortezomib has been studied under LPS-induced inflammation conditions. In a model of LPS-stimulated equine monocytes *in vitro* and an *in vivo* endotoxemia model, bortezomib was shown to reduce TNF-α production. According to the authors, its inhibitory effect on LPS-induced TNF-α production is mediated through NF-κB suppression [14]. *In vitro* studies have shown that bortezomib dose-dependently reduces NO and chemokine production, inhibits IκB degradation, and suppresses NF-κB activation in LPS-stimulated RAW 264.7 mouse macrophage cells [15]. At the same time, a murine model demonstrated that NF-κB inhibition by bortezomib in the presence of elevated TNF-α levels under bacterial superantigen-induced toxic shock syndrome conditions may be detrimental, as NF-κB-dependent anti-apoptotic pathways protect hepatocytes from TNF-α-induced cell death [16].

Thus, the use of bortezomib as an effective inhibitor of NF-κB and/or NLRP3 inflammasome activation is limited by its adverse effects at certain concentrations, including specific side effects such as peripheral neuropathy, acute interstitial nephritis, and thrombotic microangiopathy. Another significant factor that limits the potential use of bortezomib is its high cost. However, achieving the beneficial effects of bortezomib in the treatment of processes associated with SIR and related metabolic disorders may be possible through its combined administration with non-toxic modulators of redox-sensitive transcription factors, such as bioflavonoids.

Quercetin, a natural flavonoid, demonstrates potent antioxidant and anti-inflammatory properties by activating Nrf2 and suppressing NF-κB-dependent pro-inflammatory mediator activity [3]. Combining these agents may create a synergistic effect, amplifying their anti-inflammatory and metabolic benefits.

Nonetheless, the efficacy of combining bortezomib and quercetin in the context of SIR modeling remains insufficiently studied.

Aim

This study aimed to evaluate the effects of combined administration of bortezomib and quercetin on serum ceruloplasmin levels, carbohydrate and lipid metabolism parameters, and secondary products of lipid peroxidation in a lipopolysaccharide-induced rat model.

Materials and methods

The study was conducted on 35 male Wistar rats weighing 180–220 g, divided into six groups of seven animals each: Group 1 – intact rats (Control I); Group 2 – animals with lipopolysaccharide (LPS)-induced systemic inflammatory response (SIR) (Control II); Group 3 – rats with SIR treated with bortezomib, an NF-κB inhibitor (via proteasome suppression); Group 4 – animals with SIR treated with quercetin, a flavonoid that acts as both an NF-κB inhibitor and an Nrf2 pathway activator; Group 5 – rats with SIR treated with a combination of bortezomib and quercetin.

The animals were housed under standard vivarium conditions (temperature: +22 ± 2 °C, humidity: 30–60 %) in accordance with the Standard Rules for the Organization, Equipment, and Maintenance of Experimental Biological Clinics (Vivariums). The rats had free access to food and water. All procedures involving animals were conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the current legislation of Ukraine. The study was approved by the Bioethics Committee of Poltava State Medical University (Protocol No. 229, dated 28.08.2024).

SIR was modeled by intraperitoneal administration of *Salmonella typhi* LPS (Sigma-Aldrich, Inc., USA) at a dose of 0.4 μg/kg body weight. LPS was administered three times during the first week and then once weekly for the next seven weeks [17].

Transcription factor modulators were administered intraperitoneally during the final week of the experiment: bortezomib (Sigma-Aldrich, Inc., USA) at a dose of 0.05 mg/kg [17]; water-soluble dosage form of quercetin (Corvitin, Borshchahivskiy Chemical-Pharmaceutical Plant, Ukraine) at a dose of 100 mg/kg (equivalent to 10 mg/kg of quercetin) [18].

Euthanasia was performed at the end of the study using an intraperitoneal injection of thiopental sodium (Kyivmedpreparat, Arterium Corporation, Ukraine) at a dose of 50 mg/kg body weight. Once deep anesthesia was achieved, thoracotomy was performed, and blood was collected via cardiac puncture. Blood samples were placed in special tubes containing lithium heparin at a concentration of 30 IU/mL blood (Sky Medica, Ukraine). The samples were centrifuged at room temperature (3000 g, 15 minutes). The serum was separated from the upper layer and used for subsequent biochemical analysis.

Serum ceruloplasmin, an acute-phase protein and a marker of SIR, was measured using a method based on the oxidation of *p*-phenylenediamine.

The concentrations of glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were measured using reagent kits from Felicit-Diagnostics (Dnipro, Ukraine).

Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels were calculated using Friedewald's formula: LDL-C = Total cholesterol – (HDL-C + triglycerides / 2.2); VLDL-C = triglycerides / 2.2.

To measure total NOS activity, serum samples were incubated in a solution containing 2.5 mL of 0.1 M Tris buffer, 0.3 mL of 320 mM L-arginine solution, and 0.1 mL of 1 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH). The incubation lasted 30 minutes, after which nitrite ion (NO_2^-) concentrations were determined spectrophotometrically using a Ulab-101 device at a wavelength of 540 nm. The method was based on the formation of colored diazo compounds through a reaction with 1 % sulfanilamide acid followed by the addition of 1-naphthylamine (Griess–Ilosvay reagent) [19].

Constitutive NOS (cNOS) activity was determined by inhibiting the enzyme with a 1 % solution of aminoguanidine hydrochloride (98 %, Sigma-Aldrich, Inc., USA) [6]. Inducible NOS (iNOS) activity was calculated as the difference between total NOS activity and cNOS activity.

Total arginase activity was measured based on the reaction of L-ornithine with ninhydrin (Chinard reagent). The amount of colored product formed was assessed spectrophotometrically using a Ulab-101 device at a wavelength of 515 nm [19].

Protein concentrations were measured using the biuret method. Lipid peroxidation (LPO) levels in blood samples were assessed by measuring the formation of a colored trimethine complex in the reaction of thiobarbituric acid (TBA) with TBA-reactive substances (TBARS) before and after 1.5-hour incubation. The antioxidant potential was evaluated by the increase in TBA-reactive compounds during incubation in an iron-ascorbate buffer solution [20].

Statistical calculations were performed using Microsoft Office Excel with the Real Statistics add-on module. The normality of data distribution was assessed by applying the Shapiro–Wilk test. For normally distributed data, comparisons were made by Student's t-test for independent samples; for non-normally distributed data, the Mann–Whitney U test was applied.

Results

The concentration of the acute-phase response protein ceruloplasmin, considered a marker of systemic inflammatory response (SIR) development, in the blood serum of intact animals was 279.8 ± 8.3 mg/L *(Fig. 1)*. Administration of *S. typhi* LPS significantly increased the serum ceruloplasmin content to 420.6 ± 13.4 mg/L, exceeding the result of Group 1 by 50.3 % ($p < 0.001$).

Administration of bortezomib under LPS-induced SIR reduced the serum ceruloplasmin level to 322.6 ± 8.8 mg/L, which was 23.3 % lower than in Control II ($p < 0.001$), but still 15.3 % higher ($p < 0.01$) compared to the result of Group 1. Quercetin administration decreased the ceruloplasmin concentration to 327.5 ± 8.0 mg/L, representing a 22.1 % decrease compared to Control II ($p < 0.001$) and a 17.0 % increase ($p < 0.01$) compared to intact animals. The combined treatment with bortezomib and quercetin further lowered serum ceruloplasmin levels to 266.5 ± 7.2 mg/L. This value did not differ significantly from

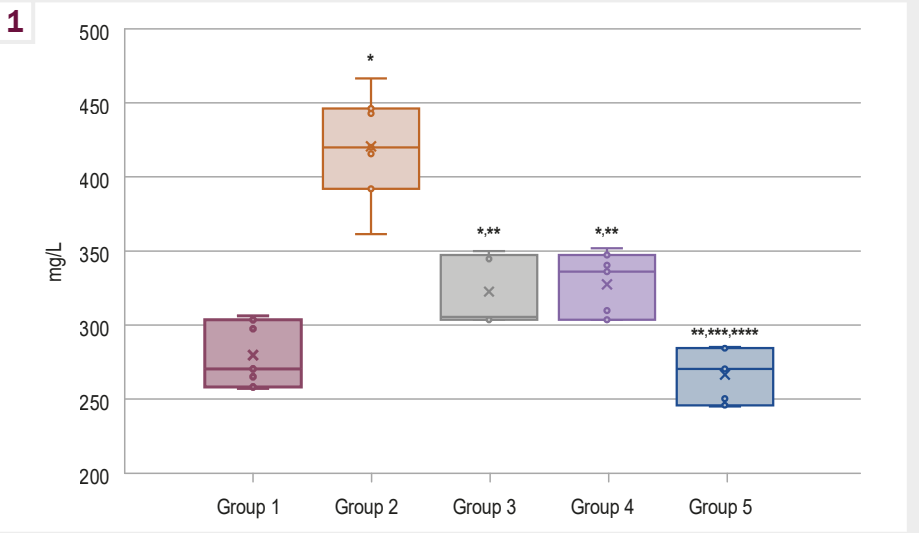

Fig. 1. Serum ceruloplasmin concentration in the following groups: intact animals (1); after modeling of LPS-induced SIR (2); following the administration of bortezomib during modeled SIR (3); following administration of quercetin during modeled SIR (4); and after combined administration of bortezomib and quercetin during modeled SIR (5). ***:** $p < 0.05$ compared to Group 1; ****:** $p < 0.05$ compared to Group 2; *****:** $p < 0.05$ compared to Group 3; ******:** $p < 0.05$ compared to Group 4.

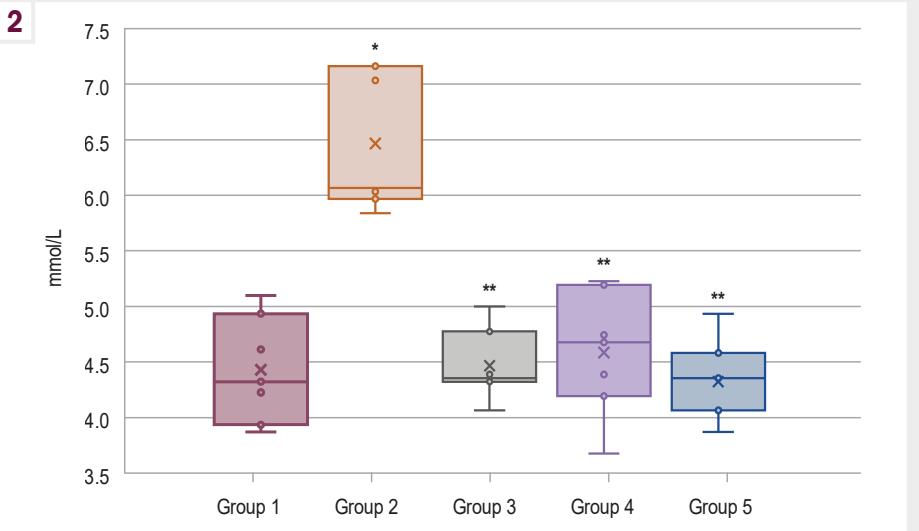

Fig. 2. Serum glucose concentration in the following groups: intact animals (1); after modeling of LPS-induced SIR (2); following administration of bortezomib during modeled SIR (3); following administration of quercetin during modeled SIR (4); and after combined administration of bortezomib and quercetin during modeled SIR (5). ***:** $p < 0.05$ compared to Group 1; ****:** $p < 0.05$ compared to Group 2; *****:** $p < 0.05$ compared to Group 3; ******:** $p < 0.05$ compared to Group 4.

Control I but was 36.6 % lower than Group 2 ($p < 0.001$), 17.4 % lower than Group 3 ($p < 0.001$), and 18.6 % lower than Group 4 ($p < 0.001$).

Administration of *S. typhi* LPS led to a moderate increase in serum glucose concentration in rats *(Fig. 2)* to 6.47 ± 0.23 mmol/L, which was 46.0 % higher than the value in Group 1 (4.43 ± 0.18 mmol/L, $p < 0.001$).

Treatment with bortezomib and quercetin under the experimental conditions reduced serum glucose levels to 4.47 ± 0.12 mmol/L and 4.59 ± 0.21 mmol/L, respectively. Both values were comparable to those in Control I and showed reductions of 30.9 % and 29.1 %, respectively, compared to Group 2 ($p < 0.001$ for both). Combined administration of bortezomib and quercetin further reduced serum glucose concentration to 4.32 ± 0.13 mmol/L, representing a 33.2 % decrease compared to Group 2 ($p < 0.001$). This value did not significantly differ from those observed in Groups 3 and 4.

The analysis of the blood lipid profile under the experimental conditions *(Table 1)* revealed a notable reduction in HDL-C level by 26.4 % ($p < 0.001$) following the administration of *S. typhi* LPS compared to Control I. Conversely, VLDL-C and triglycerides concentrations increased by 89.4 % and 88.5 %, respectively ($p < 0.001$ for both). The levels of total cholesterol and LDL-C did not undergo significant changes.

Table 1. Effect of bortezomib and quercetin on blood lipid profile parameters in rats following lipopolysaccharide-induced systemic inflammatory response modeling (M ± SE)

Group	Cholesterol, mmol/L				Triglycerides, mmol/L
	Total	High-density lipoproteins	Low-density lipoproteins	Very low-density lipoproteins	
Intact animals (Control I)	2.66 ± 0.32	1.06 ± 0.04	1.14 ± 0.32	0.47 ± 0.03	1.04 ± 0.06
After SIR modeling (Control II)	2.92 ± 0.39	0.78 ± 0.05*	1.25 ± 0.36	0.89 ± 0.03*	1.96 ± 0.06*
Administration of bortezomib during SIR	2.76 ± 0.27	0.94 ± 0.09	1.20 ± 0.23	0.62 ± 0.01*,**	1.37 ± 0.03*,**
Administration of quercetin during SIR	2.82 ± 0.27	1.06 ± 0.06**	1.15 ± 0.28	0.61 ± 0.01*,**	1.35 ± 0.02*,**
Combined administration of bortezomib and quercetin during SIR	2.86 ± 0.31	1.40 ± 0.19**,***	0.97 ± 0.29	0.49 ± 0.01**,***,****	1.07 ± 0.03**,***,****

:** p < 0.05 compared to the values of Group 1; *:** p < 0.05 compared to the values of Group 2; *****:** p < 0.05 compared to the values of Group 3; ******:** p < 0.05 compared to the values of Group 4.

Table 2. Effect of bortezomib and quercetin on NO-synthase and arginase activity in rat serum following lipopolysaccharide-induced systemic inflammatory response modeling (M ± SE)

Groups	NO-synthase activity, μmol NO_2^-/min·g protein			Arginase activity, μmol/min·g protein
	Total	Constitutive	Inducible	
Intact animals (Control I)	0.72 ± 0.01	0.13 ± 0.01	0.59 ± 0.01	0.76 ± 0.04
After SIR modeling (Control II)	1.54 ± 0.02*	0.04 ± 0.01*	1.50 ± 0.02*	0.44 ± 0.03*
Administration of bortezomib during SIR	0.94 ± 0.02*,**	0.08 ± 0.01*,**	0.86 ± 0.02*,**	0.64 ± 0.03*,**
Administration of quercetin during SIR	1.08 ± 0.04*,**	0.08 ± 0.01*,**	1.00 ± 0.04*,**	0.59 ± 0.03*,**
Combined administration of bortezomib and quercetin during SIR	0.78 ± 0.01*,**,***,****	0.11 ± 0.02**	0.67 ± 0.03*,**,***,****	0.73 ± 0.02*,***,****

:** p < 0.05 compared to the values of Group 1; *:** p < 0.05 compared to the values of Group 2; *****:** p < 0.05 compared to the values of Group 3; ******:** p < 0.05 compared to the values of Group 4.

Administration of bortezomib during SIR modeling reduced VLDL-C and triglycerides levels by 30.3 % and 30.1 %, respectively (p < 0.001 for both). However, these values remained 31.9 % and 31.7 % higher (p < 0.001 for both) than those in Group 1. The concentration of HDL-C under these conditions did not change significantly.

Quercetin treatment increased serum HDL-C levels by 35.9 % (p < 0.01). At the same time, VLDL-C and triglycerides concentrations decreased by 31.5 % and 31.1 %, respectively (p < 0.001 for both) compared to Group 2. However, these levels remained 29.8 % higher (p < 0.001 for both) than those observed in Control I.

Combined administration of bortezomib and quercetin resulted in a 79.5 % increase in HDL-C levels (p < 0.01), which was 48.9 % higher (p < 0.05) than in Group 3, but not significantly different from Group 4. Meanwhile, serum VLDL-C levels decreased by 44.9 % (p < 0.001) compared to Group 2 and were 21.0 % and 19.7 % lower than those in Groups 3 and 4, respectively (p < 0.001 for all). Under these conditions, triglycerides concentrations decreased by 45.4 % compared to Group 2 and by 21.9 % and 20.7 % compared to Groups 3 and 4, respectively (p < 0.001 for all).

During LPS-induced SIR, total NOS activity in serum *(Table 2)* increased by 113 %, while iNOS activity rose by 154 % (p < 0.001 for both) compared to Control I. Conversely, cNOS activity decreased by 69.2 %, and arginase activity dropped by 42.1 % (p < 0.001 for both) relative to the corresponding values in Group 1.

Administration of bortezomib under SIR conditions reduced total and inducible NOS activity by 39.0 % and 42.7 %, respectively, compared to Control II. However, these values remained 30.6 % and 45.8 % higher than the respective results in Group 1 (p < 0.001 for all). Quercetin treatment decreased total and inducible NOS activity by 29.9 % and 33.3 %, respectively, compared to Control II, yet these levels were still 50.0 % and 69.5 % higher than those in Group 1 (p < 0.001 for all).

Combined administration of bortezomib and quercetin resulted in a twofold increase (P < 0.001 for both) in cNOS activity compared to Control II. However, this parameter remained 38.5 % lower (P < 0.01 for both) than in Group 1. The combination also increased arginase activity by 45.5 % (P < 0.001) and 34.1 % (P < 0.01), respectively, compared to Group 2. Despite this improvement, the results were still 15.8 % (P < 0.05) and 22.4 % (P < 0.01) lower than those observed in Control I.

Table 3. Effect of bortezomib and quercetin on the levels of secondary lipid peroxidation products in rat blood following lipopolysaccharide-induced systemic inflammatory response modeling (M ± SE)

Groups	Concentration of thiobarbituric acid-reactive substances, μmol/L		
	Before incubation	After incubation	Increase during incubation
Intact animals (Control I)	13.50 ± 1.18	31.18 ± 2.06	17.69 ± 2.20
After SIR modeling (Control II)	28.74 ± 0.88 *	64.56 ± 3.28*	35.82 ± 2.78*
Administration of bortezomib during SIR	18.65 ± 0.84*,**	40.73 ± 1.19*,**	22.08 ± 0.77**
Administration of quercetin during SIR	16.66 ± 0.74*,**	37.02 ± 1.86**	20.36 ± 1.95**
Combined administration of bortezomib and quercetin during SIR	11.26 ± 0.83**,***,****	31.35 ± 2.79**,***	20.09 ± 3.00**

:** p < 0.05 compared to the values of Group 1; *:** p < 0.05 compared to the values of Group 2; *****:** p < 0.05 compared to the values of Group 3; ******:** p < 0.05 compared to the values of Group 4.

Modeling LPS-induced SIR led to an increase in the concentration of TBARS, secondary lipid peroxidation products, both before and after blood incubation in a pro-oxidant iron-ascorbate buffer solution *(Table 3)* by 112 % and 107 %, respectively (p < 0.001 for both) compared to Control I. Additionally, the incubation-induced increase in TBARS rose by 102 % (P < 0.001), indicating a depletion of the blood's antioxidant potential.

Bortezomib treatment under SIR conditions decreased TBARS levels by 35.1 % and 36.9 % before and after blood incubation in the pro-oxidant buffer solution, respectively, (p < 0.01 for both) compared to Control II. Despite this reduction, the levels remained 38.1 % and 30.6 % higher (p < 0.01 for both) than those observed in Group 1. Quercetin reduced TBARS levels by 42.0 % and 42.7 % before and after blood incubation in a pro-oxidant buffer solution, respectively (p < 0.01 for both), compared to Control II. After incubation, the TBARS levels showed no significant difference from those in Group 1.

Combined administration of bortezomib and quercetin reduced the TBARS concentration before blood incubation by 60.8 % compared to Group 2, 39.6 % compared to Group 3, and 32.4 % compared to Group 4 (p < 0.001 for all).

The incubation-induced increase in TBARS decreased by 38.4 % and 43.2 % compared to Control 2 after separate administration of bortezomib and quercetin, respectively (p < 0.001 for both). Combined administration further reduced this increase to 43.9 % (p < 0.01). However, no significant differences were observed between the effects of separate and combined treatments.

Discussion

The combined administration of bortezomib and quercetin demonstrated a superior ability to modulate SIR markers and metabolic imbalances induced by *S. typhi* LPS in rats compared to monotherapy. This finding highlights the synergistic potential of these agents and their respective mechanisms of action in mitigating the deleterious effects of SIR.

Bortezomib and quercetin independently reduced ceruloplasmin levels, a key acute-phase response protein, under SIR conditions. However, their combined administration achieved a normalization of ceruloplasmin levels, reflecting a more robust attenuation of the inflammatory cascade.

An increase in ceruloplasmin content is considered an informative marker of SIR, along with elevated levels of specific pro- and anti-inflammatory cytokines in blood serum – interleukins 6 and 10, tumor necrosis factor-α, and C-reactive protein. These changes have been noted by researchers using *S. typhi* LPS in the specified protocol, confirming the model adequacy [21].

The inhibition of the NF-κB pathway by bortezomib and the dual modulation of NF-κB and Nrf2 by quercetin likely underpin the reduction of SIR markers. NF-κB activation is a critical driver of acute-phase protein synthesis and pro-inflammatory cytokine production during SIR [22]. The combined treatment may enhance anti-inflammatory effects through a more comprehensive suppression of NF-κB and simultaneous activation of Nrf2, which promotes antioxidant and cytoprotective gene expression [23,24].

Compared to studies reporting monotherapeutic benefits of NF-κB inhibitors or flavonoids in reducing inflammation, the observed effects of combined treatment emphasize the importance of targeting inflammatory and oxidative stress pathways. For instance, previous research demonstrated that bortezomib alone attenuates NF-κB-driven inflammation [12]. Similarly, the ability of quercetin to suppress inflammatory cytokine production has been widely documented [18]. Our results align with these findings but reveal an amplified effect when these agents are used together.

LPS-induced SIR significantly elevated serum glucose levels, reflecting a state of metabolic dysregulation. Both bortezomib and quercetin effectively reduced hyperglycemia, yet their combined administration further normalized glucose levels. This suggests that simultaneous modulation of inflammatory and oxidative stress pathways exerts a more profound impact on glucose homeostasis. The observed improvements may result from the restoration of insulin signaling pathways disrupted by NF-κB-mediated inflammation and oxidative damage [25].

Comparable reductions in glucose levels were reported in studies utilizing NF-κB inhibitors or antioxidants in rodent models of metabolic disorders [20]. However, the combined approach in this study not only decreased hyperglycemia but also improved the lipid profile. HDL-C levels increased significantly with the combined treatment, surpassing the effects of monotherapies, while

reductions in VLDL-C and triglycerides were more pronounced. These findings support the hypothesis that addressing multiple pathogenic mechanisms is critical for managing the metabolic derangements associated with SIR.

LPS-induced SIR triggered marked oxidative stress, as evidenced by elevated TBARS levels. Both agents individually reduced lipid peroxidation, yet their combination produced the most significant reduction, approaching baseline levels. This effect is consistent with the complementary actions of bortezomib and quercetin. Suppression of NF-κB by bortezomib reduces pro-oxidant gene expression [7], while quercetin activates Nrf2, enhancing the expression of antioxidant enzymes [26].

Nitrosative stress, characterized by increased iNOS activity and decreased cNOS activity, was also attenuated more effectively by the combined treatment. Restoration of cNOS activity and arginase levels suggests improved L-arginine metabolism, which is often impaired during SIR. Previous studies have shown that modulating iNOS and cNOS activities can mitigate nitrosative stress and related tissue damage [18]. Our findings extend this knowledge by demonstrating that a dual-targeted approach is more effictive in achieving this goal.

The findings of this study highlight the importance of targeting multiple signaling pathways to achieve comprehensive modulation of SIR and its metabolic consequences. By simultaneously inhibiting NF-κB and activating Nrf2, the combined administration of bortezomib and quercetin effectively targets both inflammatory and oxidative stress pathways. This dual modulation may explain the superior therapeutic results observed.

The findings also have implications for clinical practice, particularly in conditions characterized by chronic inflammation and metabolic dysfunction, such as cancer, diabetes, and cardiovascular diseases. Although the high cost and side effects of bortezomib limit its widespread use, combining it with quercetin, a relatively safe and available compound, may reduce the required dose of bortezomib, thereby decreasing its adverse effects.

Conclusions

1. The combination of bortezomib and quercetin under LPS-induced SIR significantly reduced the serum ceruloplasmin concentration to levels comparable to intact animals. This reduction exceeded the effects observed with either agent alone, highlighting the synergistic anti-inflammatory potential of these compounds.

2. Combined treatment with bortezomib and quercetin normalized serum glucose levels, reducing them to values similar to those in intact animals. This outcome underscores the enhanced efficacy of dual therapy in decreasing hyperglycemia induced by LPS-induced SIR.

3. The combination of bortezomib and quercetin increased HDL-C levels and decreased VLDL-C and triglyceride concentrations more effectively than individual treatments. These findings indicate a pronounced improvement in the lipid profile, which is critical in the context of SIR-associated metabolic disturbances.

4. Combined administration of bortezomib and quercetin under LPS-induced SIR decreased the iNOS activity and enhanced cNOS activity, restoring a more balanced nitric oxide metabolism. This treatment also markedly reduced secondary lipid peroxidation product levels, highlighting its role in mitigating oxidative and nitrosative stress.

Prospects for further research should focus on elucidating the precise molecular mechanisms underlying the synergistic effects of bortezomib and quercetin, optimizing their dosing regimens, and evaluating their efficacy in chronic inflammatory and metabolic disorders through comprehensive preclinical and clinical studies. Broadening this approach to include additional experimental models and combination therapies may further enhance its therapeutic applications.

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References

1. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct Target Ther. 2021;6(1):263. doi: 10.1038/s41392-021-00658-5
2. Yuk HD, Ku JH. Role of Systemic Inflammatory Response Markers in Urothelial Carcinoma. Front Oncol. 2020;10:1473. doi: 10.3389/fonc.2020.01473
3. Kostenko V, Akimov O, Gutnik O, Kostenko H, Kostenko V, Romantseva T, et al. Modulation of redox-sensitive transcription factors with polyphenols as pathogenetically grounded approach in therapy of systemic inflammatory response. Heliyon. 2023;9(5):e15551. doi: 10.1016/j.heliyon.2023.e15551
4. Dolan RD, McMillan DC. The prevalence of cancer associated systemic inflammation: Implications of prognostic studies using the Glasgow Prognostic Score. Crit Rev Oncol Hematol. 2020;150:102962. doi: 10.1016/j.critrevonc.2020.102962
5. Mleko M, Pitynski K, Pluta E, Czerw A, Sygit K, Karakiewicz B, et al. Role of Systemic Inflammatory Reaction in Female Genital Organ Malignancies – State of the Art. Cancer Manag Res. 2021;13:5491-508. doi: 10.2147/CMAR.S312828

6. Yelins'ka AM, Akimov OY, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. Ukr Biochim J. 2019;91(1):80-5. doi: 10.15407/ubj91.01.080
7. Sogbein O, Paul P, Umar M, Chaari A, Batuman V, Upadhyay R. Bortezomib in cancer therapy: Mechanisms, side effects, and future proteasome inhibitors. Life Sci. 2024;358:123125. doi: 10.1016/j.lfs.2024.123125
8. Mao Y. Structure, dynamics and function of the 26S proteasome. In: Harris JR, Marles-Wright J, editors. Macromolecular Protein Complexes III: Structure and Function. Subcell Biochem. Cham: Springer; 2021. Vol. 96. doi: 10.1007/978-3-030-58971-4_1
9. Zhu X, Yu J, Hua M, Xu N, Wang L, Chen L, et al. Function of NLRP3 inflammasome activation in multiple myeloma. Hematology. 2024;29(1):2399367. doi: 10.1080/16078454.2024.2399367
10. Lassoued S, Moyano C, Beldjerd M, Pauly P, Lassoued D, Billey T. Bortezomib improved the joint manifestations of rheumatoid arthritis in three patients. Joint Bone Spine. 2019;86(3):381-2. doi: 10.1016/j.jbspin.2019.01.019
11. Liu C, Zhou J, Wang B, Zheng Y, Liu S, Yang W, et al. Bortezomib alleviates myocardial ischemia reperfusion injury via enhancing of Nrf2/HO-1 signaling pathway. Biochem Biophys Res Commun. 2021;556:207-14. doi: 10.1016/j.bbrc.2021.03.154
12. Chen X, Chen Y, Ou Y, Min W, Liang S, Hua L, et al. Bortezomib inhibits NLRP3 inflammasome activation and NF-κB pathway to reduce psoriatic inflammation. Biochem Pharmacol. 2022;206:115326. doi: 10.1016/j.bcp.2022.115326
13. Klimas R, Sgodzai M, Motte J, Mohamad N, Renk P, Blusch A, et al. Dose-dependent immunomodulatory effects of bortezomib in experimental autoimmune neuritis. Brain Commun. 2021;3(4):fcab238. doi: 10.1093/braincomms/fcab238
14. Sato H, Matsuda K, Amagai Y, Tanaka A, Matsuda H. Suppressive effect of bortezomib on LPS-induced inflammatory responses in horses. J Equine Vet Sci. 2018;61:114-20. doi: 10.1016/j.jevs.2017.05.003
15. Yang CH, Liu YC. Inhibitory effects of proteasome inhibitor bortezomib on endotoxin-induced uveitis in Lewis rats. Invest Ophthalmol Vis Sci. 2010;51(13):827.
16. Tilahun AY, Theuer JE, Patel R, David CS, Rajagopalan G. Detrimental effect of the proteasome inhibitor, bortezomib in bacterial superantigen- and lipopolysaccharide-induced systemic inflammation. Mol Ther. 2010;18(6):1143-54. doi: 10.1038/mt.2010.53
17. Chen FT, Yang CM, Yang CH. The protective effects of the proteasome inhibitor bortezomib (velcade) on ischemia-reperfusion injury in the rat retina. PLoS One. 2013;8(5):e64262. doi: 10.1371/journal.pone.0064262
18. Kozaeva R, Klymenko MO, Katrushov OV, Kostenko VO. Bioflavonoids as agents for correcting nitro-oxidative stress and salivary gland functions in rats exposed to alcohol during modeled lipopolysaccharide-induced systemic inflammatory response. Wiad Lek. 2022;75(3):685-90. doi: 10.36740/WLek202203121
19. Akimov OY, Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. Ukr Biochem J. 2016;88(6):70-5. doi: 10.15407/ubj88.06.070
20. Frenkel Y, Cherno V, Kostenko H, Chopra H, Gautam RK, Kostenko V. Dietary Supplementation with Resveratrol Attenuates Serum Melatonin Level, Pro-Inflammatory Response and Metabolic Disorder in Rats Fed High-Fructose High-Lipid Diet under Round-the-Clock Lighting. Pathophysiology. 2023;30(1):37-47. doi: 10.3390/pathophysiology30010005
21. Kozaeva RS, Klymenko MO, Kostenko VO. [Lipopolysaccharide-induced systemic inflammatory response enhances the development of oxidative-nitrosative stress in salivary glands of rats under alcohol damage]. Fiziologichnyi zhurnal. 2021;67(6):60-7. Ukrainian. doi: 10.15407/fz67.06.060
22. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther. 2024;9(1):53. doi: 10.1038/s41392-024-01757-9
23. Frenkel YD, Cherno VS, Kostenko VO. Nrf2 induction alleviates metabolic disorder and systemic inflammatory response in rats under a round-the-clock lighting and high-carbohydrate-lipid diet. Rom J Diabetes Nutr Metab Dis. 2022;29(2):194-201. doi: 10.46389/rjd-2022-1092
24. Gao W, Guo L, Yang Y, Wang Y, Xia S, Gong H, et al. Dissecting the Crosstalk Between Nrf2 and NF-κB Response Pathways in Drug-Induced Toxicity. Front Cell Dev Biol. 2022;9:809952. doi: 10.3389/fcell.2021.809952
25. Gao T, Chen S, Han Y, Zhang D, Tan Y, He Y, et al. Ameliorating Inflammation in Insulin-resistant Rat Adipose Tissue with Abdominal Massage Regulates SIRT1/NF-κB Signaling. Cell Biochem Biophys. 2022;80(3):579-89. doi: 10.1007/s12013-022-01085-1
26. Yousefi Zardak M, Keshavarz F, Mahyaei A, Gholami M, Moosavi FS, Abbasloo E, et al. Quercetin as a therapeutic agent activates the Nrf2/Keap1 pathway to alleviate lung ischemia-reperfusion injury. Sci Rep. 2024;14(1):23074. doi: 10.1038/s41598-024-73075-7