

ORIGINAL ARTICLE

QUERCETIN LIMITS THE PROGRESSION OF OXIDATIVE AND NITROSATIVE STRESS IN THE RATS' TISSUES AFTER EXPERIMENTAL TRAUMATIC BRAIN INJURY

10.36740/WLek202010104

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ABSTRACT

The aim: To investigate the effect of water-soluble form of quercetin on the indices reflecting the progression of oxidative-nitrosative stress in the cerebral tissues and the periodontium of rats after experimental TBI.

Materials and methods: The studies were conducted on 30 white rats of the Wistar line weighing 180-220 g, divided into 3 groups: the 1st group included pseudo-injured animals (subjected to ether anaesthesia, fixation without TBI modeling), the 2nd group included the animals exposed to modeled moderate TBI, the 3rd group involved the rats, which were given injections with water-soluble form of quercetin (corvitin, "Borshchahivskiy CPP", Ukraine) intraperitoneally in a daily dose of 10 mg/kg recalculated for quercetin for 7 days following the TBI modeling. The formation of superoxide radical anion (O_2^-), activity of NO-synthase – total (NOS), its constitutive and inducible isoforms (cNOS, iNOS) – and concentration of peroxynitrite were evaluated spectrophotometrically. The level of lipid peroxidation (LPO) in the tissues was evaluated by the formation of a stained trimethine complex during the reaction of tiobarbituric acid (TBA). The activity of the antioxidant system was assessed by increasing in the concentration of TBA active products during 1.5 hour incubation in iron-ascorbate buffer solution, as well as by the activity of antioxidant enzymes – superoxide dismutase (SOD) and catalase.

Results: The use of quercetin under the experimental conditions significantly reduced the O_2^- generation by NADPH- and NADH-dependent electron transport chains by 30.2 and 35.0% (in the cerebral hemispheres) and by 23.5 and 32.5% (in the soft periodontal tissues), respectively, compared to the findings in the 2nd group. The production of this radical by leukocyte NADPH oxidase in these organs was inferior to the value of the 2nd group by 39.3 and 29.9%. We revealed that the use of quercetin in the experimental conditions probably reduced the activity of NOS, including iNOS, by 38.2 and 45.3% (in the cerebral hemispheres) and by 53.5 and 66.9% (in the soft periodontal tissues), respectively, compared with the findings in the 2nd group. Under these conditions, the cNOS activity went up by 50.0% and doubled, the peroxynitrite content was lower by 19.5 and 32.1% than that in the 2nd group. The administration of quercetin in the experimental conditions significantly reduced the concentration of TBA-active products in the homogenate of cerebral hemispheres and soft periodontal tissues. The development of decompensated LPO is also confirmed by a decrease in the activity of SOD and catalase.

Conclusions: on the 7th day after modeling moderate TBI in rats the signs of oxidative-nitrosative stress are found not only in locus morbi (in the tissue of the cerebral hemisphere), but also in distant organs (periodontal tissues). Applying of water-soluble form of quercetin significantly reduces signs of oxidative-nitrosative stress in the tissue of the cerebral hemisphere of rats, as well as in periodontal tissues on the 7th day after moderate TBI modeling.

KEY WORDS: water-soluble form of quercetin, traumatic brain injury, oxidative-nitrosative stress, brain, periodontium

Wiad Lek. 2020;73(10):2127-2132

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of death in people younger than 45 years of age [1]. Furthermore, World Health Organization has reported that TBI is ranked as the 3rd leading cause of death and disability in 2020 [2]. TBI results in an astounding 6 billion USD in direct costs and over 40 billion USD in indirect costs annually in the USA [3].

The most researchers consider hypoxia and local cerebral ischemia as the leading pathogenetic mechanisms of TBI [1, 4]. In these conditions, the production of reactive oxygen and nitrogen species (ROS / RNS) considerably increases that in turn triggers the activation of free radical oxidation in the cerebral tissues and further emergence of a complex of structural and functional disorders of nerve cell membranes.

Depending on the concentration and other causes, ROS / RNS can perform either a physiological role in the regulation of cerebral functions (inter- and intra-neural signaling, synaptic plasticity, cerebral hemodynamics, oscillatory activity of neurons, etc.), or have a negative effect on the CNS functioning by causing oxidative-nitrosative stress [4].

The latter results in the activation of the kappa B (NF- κ B) transcription factor [5], NLRP3 inflammasome activation, the expression of controlled genes encoding pro-inflammatory and pro-oxidant molecules (cytokines, inducible NO-synthase, matrix metalloprotein, etc.), and as a consequence, leads to the development of systemic inflammatory response [6, 7] and damage to other target organs, including periodontium [8].

Quercetin, a dietary flavonoid, possesses anti-inflammatory, anti-blood coagulation, anti-ischemic and anti-cancer

activities, and neuroprotective effects in the context of brain injury [9]. Nowadays, the ability of quercetin to influence the activity of enzymes involved in the degradation of phospholipids (phospholipase, lipoxygenase, cyclooxygenase), and block oxidative stress has been proved [10]. There is evidence of quercetin ability to suppress ubiquitin-dependent proteolysis of NF- κ B with an inhibitory protein I κ B, which disturbs degradation of the latter under the proteasome action [11]. This creates the prerequisites for eliminating the possibility of NF- κ B-dependent genes expression [12]. Moreover, quercetin may protect against NF- κ B-induced oxidative-nitrosative stress in skeletal muscle through Nrf2-mediated heme oxygenase-1 induction accompanied by inactivation of NF- κ B [13]. Additionally, quercetin causes a marked inhibition of extracellular signal-regulated kinase 1/2 phosphorylation and activates Akt serine/threonine protein kinase phosphorylation, which may result in attenuation of neuronal apoptosis [9]. Quercetin administration can potentially attenuate experimental TBI by increasing the activities of mitochondrial biogenesis via the mediation of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) pathway [14].

However, the effects of quercetin on the development of oxidative-nitrosative stress in the brain and other organs in mammals in the TBI conditions are still remaining unclear. Clarifying these issues will contribute to existing modalities of preventing neurological deficits and treating TBI-related complications.

THE AIM

The aim of this study was to investigate the effect of water-soluble form of quercetin on the indices reflecting the progression of oxidative-nitrosative stress in the cerebral tissues and the periodontium of rats after experimental TBI.

MATERIALS AND METHODS

The studies were conducted on 30 white rats of the Wistar line weighing 180-220 g, divided into 3 groups: the 1st group included pseudo-injured animals (subjected to ether anaesthesia, fixation without TBI modeling), the 2nd group included the animals exposed to modeled TBI, the 3rd group involved the rats, which were given injections with water-soluble form of quercetin (corvutin, "Borshchahivskiy CPP", Ukraine) intraperitoneally in a daily dose of 10 mg/kg recalculated for quercetin [10] for 7 days following the TBI modeling.

The research was conducted in compliance with the standards of the Convention of the Council of Europe on Bioethics 'European convention for the protection of vertebrate animals used for experimental and other scientific purposes' (Strasbourg, 18.III.1986).

We reproduced moderate TBI model [15]. For this purpose, we anesthetized the rats with ether and then fixed them paying special attention to fixing head on the rubber plate under a vertical metal tube for dropping weight (m=66.7g); the drop height was 65 cm, the impact area

was 0.5 cm². This enabled us to produce an impact with energy of 0.425 J.

We removed the animals from the experiment under ether anaesthesia by decapitation on the 7th days following TBI modeling. We carried out all biochemical studies in 10% homogenate of cerebral hemispheres and soft tissues of the periodontium (gingiva and periodontal ligament).

The formation of superoxide radical anion (O_2^-) was evaluated by a test with nitroblue tetrazolium with using spectrophotometry of the tissue homogenate with the following inductors: nicotinamide adenine dinucleotide reduced (NADH) was used for the evaluation of O_2^- production by the mitochondrial electron transport chain (ETC), nicotinamide adenine dinucleotide phosphate reduced (NADPH) was used to evaluate O_2^- production by endoplasmic reticulum and NO-synthase (NOS), and *Salmonella typhi* lipopolysaccharide (pyrogenalum, "Medgamal", Russia) was used to assess O_2^- production by phagocytic NADPH oxidase [16].

The activity of NOS was determined by the difference in the concentration of nitrite ions before and after the incubation of homogenate into the medium containing L-arginine (NO-synthase substrate) and NADPH [17]. To evaluate the activity of constitutive isoforms (cNOS), we added 1% solution of aminoguanidine hydrochloride (98%, "Sigma Aldrich") [18]. The activity of inducible NOS (iNOS) was evaluated by subtracting the cNOS activity from the overall activity of NOS.

The cNOS coupling index was calculated as the ratio between the cNOS activity and the O_2^- generation rate by the NADPH-dependent ETCs. This index indicates the presence of substrates (L-arginine, O_2) and tetrahydrobiopterin for NO production, but not for O_2^- generation under oxidative metabolism of L-arginine. It is considered that any increase in O_2^- production (by mitochondria, xanthine oxidase, or NADPH-oxidase) leads to cNOS uncoupling. This cNOS becomes not only a powerful O_2^- generator by itself, but at the same time it activates other sources of O_2^- [19].

Peroxyntirite concentration was measured by using its reaction with potassium iodide under pH 7.0 in 0.2 M phosphate buffer with the same pH [17].

The level of lipid peroxidation (LPO) in the tissues was evaluated by the formation of a stained trimethine complex during the reaction of tiobarbituric acid (TBA) [20]. The activity of the antioxidant system was assessed by increasing in the concentration of TBA active products during 1.5 hour incubation in iron-ascorbate buffer solution, as well as by the activity of antioxidant enzymes – superoxide dismutase (SOD) and catalase [20].

The findings obtained were statistically processed. To verify the normality distribution, the calculation of the Shapiro-Wilk criterion was applied. If findings corresponded to the normal distribution, then the Student's t-test was used to compare independent samples. When the results ranges were not subject to normal distribution, statistical processing was performed using a nonparametric method, the Mann-Whitney test. Statistical calculations were performed using the "StatisticSoft 6.0" program.

Table I. Effect of quercetin on the production of superoxide radical anion in homogenate of cerebral hemispheres and periodontal soft tissues under traumatic brain injury, nmol/s·g homogenate (M+m, n=30)

Groups of the animals	Sources of the superoxide radical anion production		
	NADPH-dependent electron-transport chains	NADH-dependent (mitochondrial) electron-transport chain	NADPH-oxidase of white blood cells
The homogenate of the cerebral hemispheres			
Pseudo-injured animals	11.31±0.40	13.57±0.47	1.25±0.04
TBI modeling	21.08±0.40 *	27.19±1.54 *	2.67±0.18 **
TBI modeling + quercetin	14.72±0.22 *,**	17.67±0.27 *,**	1.62±0.02 *,**
The homogenate of the soft periodontal tissues			
Pseudo-injured animals	12.97±1.02	16.52±1.09	1.82±0.19
TBI modeling	19.75±0.95 *	32.04±1.58 *	2.98±0.14 *
TBI modeling + quercetin	15.11±0.93 **	21.63±1.30 *,**	2.09±0.14 **

Note (in table 1-3): * – p<0.05 compared with values in the control group I (pseudo-injured rats); ** – p<0.05 compared with values in the control group II (TBI modeling).

Table II. Effect of quercetin on the indices of nitrosative stress in homogenate of cerebral hemispheres and periodontal soft tissues under traumatic brain injury (M+m, n=30)

Groups of the animals	NOS activity, μmol (NO ₂ ⁻) / min·g of protein			cNOS coupling index	Peroxynitrite concentration, μmol/g
	Total	cNOS	iNOS		
The homogenate of the cerebral hemispheres					
Pseudo-injured animals	7.26±0.77	1.60±0.13	5.65±0.75	0.141±0.010	1.55±0.07
TBI modeling	11.49±1.27 *	0.86±0.06 *	10.62±1.26 *	0.041±0.003 *	2.41±0.11 *
TBI modeling + quercetin	7.10±0.67 **	1.29±0.11 *,**	5.81±0.66 **	0.087±0.007 *,**	1.94±0.05 *,**
The homogenate of the soft periodontal tissues					
Pseudo-injured animals	5.41±0.66	1.11±0.12	4.30±0.66	0.089±0.016	0.97±0.13
TBI modeling	7.72±0.96	0.60±0.06 *	7.12±0.94 **	0.031±0.004 *	1.56±0.08 *
TBI modeling + quercetin	3.59±0.61 **	1.23±0.35 **	2.36±0.33 **	0.087±0.029	1.06±0.11 **

RESULTS AND DISCUSSION

In 7 days after TBI modeling we observed increased O₂⁻ generation by NADPH- and NADH-dependent ETCs (Table I) in the homogenate of the cerebral hemispheres in 1.9 times and twofold compared with the findings in the 1st group. O₂⁻ generation by leukocyte NADPH-oxidase increased in 2.1 times.

The use of quercetin under the experimental conditions significantly reduced the O₂⁻ generation by NADPH- and NADH-dependent ETCs in the homogenate of the cerebral hemispheres by 30.2 and 35.0%, respectively, compared to the findings in the 2nd group, i.e. to values, which were not statistically different from the values in the 1st group. The production of this radical by leukocyte NADPH oxidase was inferior to the value of the 2nd group by 39.3%.

In 7 days after TBI modeling we also observed increase in O₂⁻ generation by NADPH- and NADH-dependent ETCs in the homogenate of the soft periodontal tissues by 52.3 and 93.9%, respectively, compared with the findings in the 1st group. O₂⁻ generation by leukocyte NADPH oxidase elevated by 63.7%.

The introduction of quercetin under the experimental conditions significantly reduced O₂⁻ generation by NADPH- and NADH-dependent ETCs in the homogenate of the soft periodontal tissues by 23.5 and 32.5%, respectively, compared with the findings in the 2nd group. The production of this radical by leukocyte NADPH oxidase was lower by 29.9% compared with the 2nd group.

At the same time, in the homogenate of the cerebral hemispheres we observed significant increase in the NOS activity (Table II), including its inducible isoform, by 58.3 and 88.0%, respectively, compared with the findings in the 1st group. In these conditions, cNOS activity considerably decreased by 46.2%.

cNOS coupling index went down by 70.9%, indicating cNOS uncoupling, when this isoenzyme shows signs of a powerful O₂⁻ generator and can activate other sources of ROS generation [19]. The concentration of peroxynitrite rose by 55.5%.

We revealed that the use of quercetin in the experimental conditions probably reduced the activity of NOS, including iNOS, in the homogenate of the cerebral hemispheres by

Table III. Effect of quercetin on lipid peroxidation and antioxidant protection in homogenate of cerebral hemispheres and soft tissues of periodontium under traumatic brain injury (M+m, n=30)

Groups of the animals	Concentration of agents reacting with thiobarbituric acid, $\mu\text{mol/kg}$ homogenate, $\mu\text{mol/kg}$ homogenate			Antioxidant enzymes activity	
	Before incubation	After incubation	Increment for incubation time	Superoxide dismutase activity, c.u.	Catalase activity, $\mu\text{kat/g}$ homogenate
The homogenate of the cerebral hemispheres					
Pseudo-injured animals	36.61 \pm 1.2	45.3 \pm 1.0	8.7 \pm 0.5	0.34 \pm 0.01	0.40 \pm 0.01
TBI modeling	52.6 \pm 1.0 *	67.7 \pm 1.2 *	15.1 \pm 0.5*	0.14 \pm 0.01 *	0.18 \pm 0.01 *
TBI modeling + quercetin	43.7 \pm 1.2 *,**	52.3 \pm 1.1 *,**	8.7 \pm 0.4**	0.25 \pm 0.02 *,**	0.30 \pm 0.02 *,**
The homogenate of the soft tissues of periodontium					
Pseudo-injured animals	35.4 \pm 2.1	42.3 \pm 1.9	6.8 \pm 0.4	0.31 \pm 0.03	0.35 \pm 0.01
TBI modeling	52.8 \pm 1.2 *	68.0 \pm 1.6 *	15.2 \pm 0.6 *	0.10 \pm 0.03 *	0.17 \pm 0.01 *
TBI modeling + quercetin	40.0 \pm 0.9 **	48.4 \pm 1.1 *,**	8.3 \pm 0.8 **	0.20 \pm 0.02 *,**	0.33 \pm 0.04 **

38.2 and 45.3%, respectively, compared with the findings in the 2nd group. Under these conditions, the cNOS activity went up by 50.0%, and its coupling index nearly doubled compared to the findings in the 2nd group. The content of peroxynitrite was lower by 19.5% than that in the 2nd group.

In the homogenate of the soft periodontal tissues in 7 days after the TBI modeling, total NOS activity did not probably change, but iNOS activity increased by 65.6% compared with the findings in the 1st group. The activity of cNOS under these conditions decreased considerably by 45.9%, cNOS coupling index – by 65.2%. The concentration of peroxynitrite increased by 60.8%.

The administration of quercetin under the experimental conditions significantly reduced the activity of NOS, including iNOS, in the homogenate of the soft periodontal tissues by 53.5 and 66.9%, respectively, compared with the findings in the 2nd group. Under these conditions, the cNOS activity doubled, and its coupling index increased in 2.8 times compared with the findings in the 2nd group. Peroxynitrite content was inferior by 32.1% compared to the 2nd group.

The increase in ROS / RNS production was accompanied by changes in LPO and antioxidant protection indices in the tissues of rats (Table III). In 7 days after the TBI modeling in the homogenate of the cerebral hemispheres, the concentration of TBA-active products significantly increased before and after 1.5-hour incubation in the pro-oxidant iron-ascorbate buffer solution – by 43.8 and 49.5%, respectively, compared with the findings in the 1st groups. The increase in the concentration of TBA-active compounds during 1.5 hour incubation in iron-ascorbate buffer solution was higher by 73.5% than the values in the control that indicates the depletion of antioxidant potential in cerebral tissue and the development of uncompensated lipid peroxidation. This is also evidenced by lowered activity of SOD and catalase – by 58.8 and 55.0%.

The administration of quercetin in the experimental conditions significantly reduced the concentration of

TBA-active products in the homogenate of cerebral hemispheres before and after 1.5-hour incubation in the pro-oxidant iron-ascorbate buffer solution by 17.0 and 22.7% respectively, compared with the findings in the 2nd group. The increase in the concentration of TBA-active compounds during 1.5 hour incubation in iron-ascorbate buffer solution slowed down by 42.6%, the activity of SOD and catalase increased by 78.6 and 66.7% respectively, compared with the findings in the 2nd group.

At the same time, the experimental TBI was accompanied by an increase in the concentration of TBA-active products before and after 1.5-hour incubation of the soft periodontal tissues homogenate in the pro-oxidant iron-ascorbate buffer solution by 49.2 and 60.8%, respectively, compared with the findings in the 1st group. The increase in the concentration of TBA-active compounds during 1.5 hour incubation in iron-ascorbate buffer solution was 2.2 times higher than that in the control. The development of decompensated LPO is also confirmed by a decrease in the activity of SOD and catalase – by 67.7 and 51.4%.

The administration of quercetin under the experimental conditions also resulted in the decrease in TBA-active products concentration in the soft periodontal tissues homogenate before and after 1.5-hour incubation in the pro-oxidant iron-ascorbate buffer solution – by 24.2 and 28.8% respectively compared with the findings in the 2nd group. The increase in the concentration of TBA-active agents during 1.5 hour incubation in iron-ascorbate buffer solution went down by 45.4%, and the activity of SOD and catalase increased twofold and in 1.9 times respectively compared with the findings in the 2nd group.

In our previous works we reported on the dependence of the development of oxidative-nitrosative stress in the cerebral hemispheres and periodontal tissues on the activity of the NF- κ B-dependent signaling pathway [21]. It has been shown that TBI is accompanied by prolonged (through weeks or months) activation of NF- κ B in neurons, astrocytes, and microglial cells that causes inflammation [22].

The administration of the NF- κ B nuclear translocation inhibitor (JSH-23) reduces NOS activity, O₂ production and the level of LPO in cerebral tissue, as well as enhances antioxidant protection and energy potential [21].

The ROS / RNS formed are the means of redox-sensitive transcription factors (NF- κ B, Nrf2, etc.) regulating, whose changes in the activity affect not only oxidative metabolism in the cerebrum, but also in other organs through the development of a systemic inflammatory response (SIR). The latter is known as an important mechanism of damage to periodontal tissues as it induces oxidative-nitrosative stress [8].

The ability of quercetin to inhibit NF- κ B-associated free radical processes in tissues [10] and to enhance Nrf2-mediated processes associated with the expression of antioxidant response element-dependent genes [23], according to the results of our study, limits the development of oxidative-nitrosative stress in cerebral tissues after TBI as well as in distant organs, in particular, periodontium.

CONCLUSIONS

1. On the 7th day after modeling moderate TBI in rats the signs of oxidative-nitrosative stress are found not only in locus morbi (in the tissue of the cerebral hemisphere), but also in distant organs (periodontal tissues).
2. Applying of water-soluble form of quercetin significantly diminishes signs of oxidative-nitrosative stress in the tissue of the cerebral hemisphere of rats, as well as in periodontal tissues on the 7th day after moderate TBI modeling: it reduces the generation of superoxide anion radical and the activity of inducible NO-synthase, improves coupling of its constitutive isoform, limits the concentration of peroxy nitrite, increases the antioxidant potential.

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 11.05.2020

Accepted: 31.08.2020

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis,
D – Writing the article, **E** – Critical review, **F** – Final approval of the article