DOI 10.26724 / 2079-8334-2017-3-61-97-100
UDC 614. 312 – 76 – 18:4

Demkovych, Yu. Bondarenko, P. Hasiuk
SHEI "I. Horbachevsky Ternopil State Medical University", Ternopil

HUMORAL IMMUNE REACTIVITY DISORDERS IN EXPERIMENTAL PARODONTITIS AND THEIR CORRECTION BY FLAVONOL

e-mail: p.gasyuk@gmail.com

In the article investigated disorders of humoral immune defense (immunoglobulins A, M, G classes and circulating immune complexes (CIC)) on the 7th, 14th and 30th days of experimental bacterial-immune periodontitis, and the possibility of correcting them with the corvitin (quercetin). Its use for 7 days intramuscularly at a dose of 100 mg / kg positively affected to the course of the inflammatory process with this modeled pathology. At the same time, the content of the CIC in the blood serum was determined as an important link in the pathogenesis of immune damage during experimental inflammation in periodontal tissues. The dynamics of the CIC indices was similar to the dynamics of immunoglobulins on the 14th day of the experiment, that is, their concentration was significantly higher, as compared with the indices of intact animals. The use of corvitin caused decrease concentration of circulating immune complexes in the blood serum as compared to animals that did not treat. Thus, it has been proved that flavonoid corvitin, influencing to immune processes, promote to restrict the inflammatory reaction in the periodontal complex in this pathology.

Key words: Periodontitis, immunoglobulins, circulating immune complexes, immune status, corvitin.

Despite the work of many authors which were engaged upon improving existing and developing new methods for treating periodontitis [8, 9, 11, 12], and methods for their prevention [9, 17], the problem of inflammatory processes in the periodontal complex remains unresolved and urgent in now day. The authors of the research in the treatment and prevention did not consider damaging agents of periodontitis (activity immune response, nature flora, the general state of the patient), which are important factors in the emergence of generalized periodontitis [5, 7]. The treatment of periodontitis is one of the most urgent tasks and requires not ordinary approaches to their decisions. In this regard, the effectiveness of plant origin substances, which are related to polyphenolic compounds, which are capable to affect the immune and inflammatory processes in the organism, has not been sufficiently studied.

The corvitin (quercetin) is classified as flavonols with antioxidant, anti-ischemic, membrane-stabilizing and immunomodulating properties [3, 14]. It has a large reduction potential and exhibits anti-inflammatory, anabolic, anti-apoptotic features [10, 13]. Antioxidant activity of the medicine is associated with its ability to suppress lipid peroxidation, reduce the concentration of free radicals and toxic peroxidation products, stimulate catalase and superoxide dismutase activity of the organism [18]. Anti-inflammatory and antiallergic effects also associated with the ability of corvitin to suppress calcium-ATPase and the synthesis of leukotrienes. The flavonoid inhibits the activity of hyaluronidase. It is able to increase the content of immune cells (phagocytosis, T-lymphocytes, B-lymphocytes), resulting in reduced manifestations of secondary immunosuppression [16].

The purpose of this study was to determine the effect of flavonol corvitin on the indices of the humoral link of immune reactivity in experimental bacterial-immune periodontitis.

Materials and methods. The investigation was performed with use of white clinically healthy rats, 150-200 g weight in conditions of vivarium in accordance to the sanitary standards and GLP requirements. The animals were supported on standard diet, balanced by nutritional elements. The investigations was performed according to the general rules and regulations of the European Convention for the Protection of vertebrate animals that use for experimental and other scientific purposes (Strasbourg, 1986), the General ethical animal experimentation (Kyiv, 2001). The experimental animals were randomly selected and divided into 3 groups: I – intact animals, control (n=10); II – animals with experimental periodontitis on the 14th day study (n=8); III – animals with experimental periodontitis on the 14th day study (n=8), which was introduced corvitin (n=8). Experimental bacterial-immune periodontitis in experimental animals was caused by injection into the tissue of the periodontal complex of the microorganism's mixture diluted with egg protein [4]. To enhance the immune response, an injection of complete Freund's adjuvant was simultaneously injected into the rats paw. In the third group of rats were used corvitin by intramuscular injection (100 mg / kg) for 7 days (from 7th to 14th day).

On the 14th day experimental animals were sacrificed by blood-letting under thiopental anesthesia. For further research the blood serum was selected. The level of Ig A, Ig M, Ig G and the concentration of circulating immune complexes (CIC) was determined in the blood serum. The principle of determining immunoglobulins in the blood serum is based on their ability to precipitate blood serum at

different pH and ionic strength [1]. Protein-buffer complexes, which are formed, changed the optical density of the medium. Photometer on the photoelectric colorimeter with a blue light filter against water. The calculation of the immunoglobulins number was carried out according to the calibration table and expressed in g / l. The maintenance of circulating immune complexes in the blood serum was determined by precipitation with their solution of polyethylene glycol-6000 [6]. The samples were also incubated with photometric methods on a SF-46 spectrophotometer at 450 nm. The concentration of CIC was expressed in conditioned. units. The results were statistically analyzed with using parametric and nonparametric indexes [15] in the Excel software (Microsoft, USA) and STATISTICA 10.0 (Statsoft, USA). The reliability of the differences in values between independent quantitative values was determined with a normal distribution according to the Mann-Whitney U criterion [2].

Results and discussion. The development of the inflammatory process in the periodontal complex was accompanied an increase in the blood serum activity of the humoral link immune system of the organism, in particular, the immunoglobulins A, M, and G classes (table 1). Thus, in the rats of the second experimental group (on the 14th day after modeling of experimental periodontitis), we detected an increase of Ig A content in the blood serum by 1.13 times ($p<0.01$) relative to the control animal group. A similar trend was observed to relatively of immunoglobulins M and G classes content, that is, an increase by 1.41 times ($p<0.01$) and by 1.51 times ($p<0.01$), respectively, as compared with the indices of intact animals.

Table 1

Effects of the corvitin to indices of immunoglobulin A, M, G classes in blood serum rats with experimental periodontitis (M±m)

Indices	Groups of the experimental animals		
	Intact group (n=10)	Experimental periodontitis, 14th day (n=8)	Experimental periodontitis, 14th day corrected corvitin (n=8)
Ig A, g / l	1.301±0.002	1.463±0.010 p1<0.01	1.327±0.004 p1<0.01, p2<0.01
Ig M, g / l	1.196±0.020	1.688±0.006 p1<0.01	1,323±0,013 p1<0.01, p2<0.01
Ig G, g / l	1.315±0.001	1.990±0.002 p1<0.01	1.569±0.017 p1<0.01, p2<0.01

Notes: 1. p1 – significant of differences relatively to intact animals; 2. p2 – significant of differences relatively to animals with experimental periodontitis on the 14th day of the research.

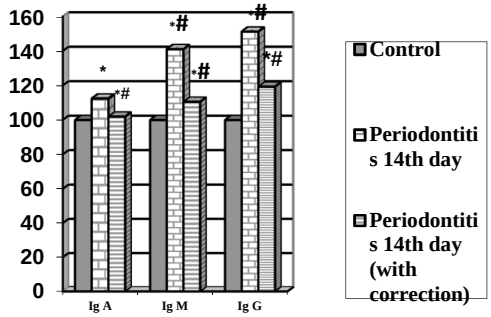

Fig. 1 – Changes of the immunoglobulins A, M, G indices of the rat's blood serum in the experimental periodontitis and correction corvitin (% of control). Notes: * – significant of differences relatively to the intact animals ($p<0.01$); # – significant differences relatively to the animals with periodontitis on the 14th day of the experiment ($p<0.01$).

The introduction of the flavonol corvitin intramuscularly at a dose 100 mg / kg for 7 days resulted the decrease content of Ig A – by 1.10 times ($p<0.01$), Ig M – by 1.28 times ($p<0.01$) and Ig G – by 1.27 times ($p<0.01$) in the blood serum in comparison with such indices of the animals group with experimental periodontitis on the 14th day which did not treatment this substance (table 1, fig. 1).It should be noted, in comparison of the humoral immune indices on the 14th day of experimental periodontitis without correction with correction, we found that they remained slightly higher than the parameters of the control group, in particular, Ig A – by 1.02 times ($p<0.01$), Ig M – by 1.11 times ($p<0.01$) and Ig G – by 1.19 times ($p<0.01$).

Table 2

Effects of corvitin to indices of circulating immune complexes in blood serum rats with experimental periodontitis (M±m)

Stages of experiment		Number of animals	CIC, conditioned. units.
Intact animals, control		10	56.80±1.73
Rats with experimental periodontitis (14th day)	Without correction	8	86.25±1.96 p1<0.01
	After correction with corvitin	8	78.38±2.53 p1<0.01, p2<0.05

Notes: 1. p1 – significant of differences relatively to intact animals; 2. p2 – significant of differences relatively to animals with experimental periodontitis on the 14th day of the research.

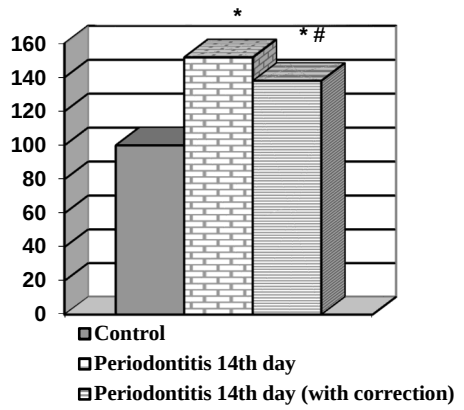

Fig. 2 – Changes of circulating immune complexes indices in the rat's blood serum with the experimental periodontitis and the correction with corvitin (% of control). Notes: * – significant of differences relatively to the intact animals ($p<0.01$); # –significant differences relatively to the animals with periodontitis on the 14th day of experiment ($p<0.05$).

It is known, that one of the important function of immunoglobulins is the neutralization of the antigens, including autoantigens, with the formation of the CIC and subsequent elimination them from the organism in order to supporting immunobiological homeostasis. Therefore, studies of humoral factors of local protection were also performed for assessment of circulating immune complexes content in the blood serum (table 2). It is found that their content in the blood serum on the 14th day of the experiment was higher, as compared with the intact group of animals (by 1.82 times, $p<0.01$). It should be noted that an increase of circulating immune complexes concentration leads to the activation of components complement and tissue damage.

Analyzing the changes of circulating immune complexes content in the blood serum of experimental animals with periodontitis, which treatment corvitin for the inflammatory development, it should be noted that the therapeutic efficacy of this flavonol is confirmed decreased content of circulating immune complexes in the blood serum (by 1.10 times, $p<0.05$) in comparison with animals, that were examined on the 14th day and did not treatment (table 2, fig. 2).At the same time, the data were higher than those in the rats of the control group (by 1.38 times, $p<0.01$). That is, this flavonol is able to suspend and stabilize the further development of the inflammatory process, destructive phenomenas and thus weaken the immune response associated with this pathology.

Conclusions

1. Disorders of the humoral link of immunological reactivity for experimental bacterial-immune periodontitis in animals is an important pathogenetic chain in the mechanisms that formate features of development, course and completion of the inflammatory process.
2. Flavonol corvitin reduces the titre of immunoglobulins A, M, G classes and circulating immune complexes in the blood serum of animals with experimental bacterial-immune periodontitis that contributes to the stabilization and remission of the inflammatory process.

References

1. Andreychyn M. A. Clinical Immunology and Allergology / M. A. Andreychyn, V. V. Chop'yak, I. Y. Hospodarskyy // - Ukrmedknyha, Ternopil. - 2004 – 372 p.
2. Berger. R. L. Statistical Inference 2nd ed / R. L. Berger., G. Casella. // Duxbury Press; - 2001. 374 p.
3. D'Andrea G. : Quercetin: A flavonol with multifaceted therapeutic applications? / G. D'Andrea // - Fitoterapia. - 2015, Vol.106, P. 256-271.
4. Demkovych A. Pathogenetic bases periodontitis modeling in rats / A. Demkovych, Yu. Bondarenko // - J. Achievements of Clin. and Exper. Med. - 2015; Vol. 1 (22) P. 54-57.
5. Eming S. A. Interrelation of immunity and tissue repair or regeneration / S. A. Eming, M. Hammerschmidt, T. Krieg [et al.] // - Semin. Cell. Dev. Biol. – 2009, Vol.20 (5), P. 517-527.
6. Gayevska M. Yu. Circulating immune complexes in the conditions of health and disease / M. Yu. Gayevska // - Journal of Research. – 2000, Vol. 4, P. 37-40.
7. Harutyunyan A. Comparative evaluation of indicators of local, humoral immunity and nonspecific resistance in patients with different pathology of the cervix. Georgian Med. News. – 2011, Vol.190, P. 7-15.
8. Kiseleva E. A. Directed immunocorrection of chronic periodontitis / E. A. Kiseleva, E. A. Te, O. Yu. Nikolaeva // - Clinical stomatology. – 2011, Vol.4: P.80-83.
9. Khorsavi Samani M. Introducing cut-points for salivary nitric oxide to distinguish periodontitis from the normal periodontium / M. Khorsavi Samani, A. Poorsattar Bejeh, M. Kashiri [et al.] // - Minerva Stomatol. – 2012, Vol. 61 (10), P. 443-448.
10. Kravets B. B. The role of corvitin in the correction of violations of lipid peroxidation processes and the activity of the antioxidant system in guinea pigs blood in experimental bacterial keratitis against the background of bronchial asthma and pneumonia / B. B. Kravets, M. S. Regeda // - Medical and Clinical chemistry. – 2015, Vol.17 (2), P. 69-71.
11. Madrid C. What impact do systemically administrated bisphosphonates have on oral implant therapy? A systematic review / C. Madrid, M. Sanz // - Clin. Oral. Implants. Res. – 2009, Vol.4, P. 87-95.
12. Machavariani A. Use of osteoplastic material to guide bone tissue regeneration defect / A. Machavariani, K. Mazmishvili, T. Grdzelidze // - Georgian Med. News. – 2011, Vol. (201), P.70-73.
13. Mikhailova Yu. A. The influence of cardioprotection on the state of hemodyncmics at treatment of cardiorenal syndrome in patients with chronic heart failure and chronic kidney disease / Yu. A. Mikhailova, P. H. Kravchun, O. V. Petyunina [et al.] // - International Medical Journal. – 2013, Vol.19 (1), 44-49.
14. Miles S. L. McFarland M. Molecular and physiological actions of quercetin: need for clinical trials to assess its benefits in

human disease / Miles S. L. McFarland M., R. M. Niles // - Nutrition Reviews. – 2014, Vol. 72 (11), P. 720-734.
15. Orlov A. Cases Mathematics, Probability and Statistics / A. Orlov // - Basic facts. MZ-Press, - 2004, Moscow. – 455 p.
16. Posokhova K. A. Influence of corvitin-containing compounds on myocardium status in diabetes mellitus type 2. / K. A. Posokhova, I. P. Stechyshyn, V. V. Pidhirnyy // - J. Achievements of Clin. and Exper. Med. – 2014, Vol.2, P. 17-21.
17. Sergeeva I. E. Research of functional features of cells of the immune system in patients with generalized periodontitis / I. E. Sergeeva // - Zaporozhye Med. Journal. – 2010, Vol.12 (3), P. 48-50.
18. Williams R. J. Flavonoids: antioxidants or signalling molecules? (review) / R. J. Williams, J.P. Spencer, C. Rice-Evans // - Free Radical Biology & Medicine. 2004; Vol.36 (7): 838-849.
3. Yip J. K. Association between oral bisphosphonate use and dental implant failure among middle-aged women / J. K. Yip, L. N. Borrell, S. C. Cho [et al.] // - J. Clin. Periodontol. – 2012, Vol.39 (4), P. 408-414.

Реферати

ПОРУШЕННЯ ГУМОРАЛЬНОЇ ЛАНКИ ІМУННОЇ РЕАКТИВНОСТІ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ПАРОДОНТИТІ ТА КОРЕКЦІЯ ЇХ ФЛАВОНОЛОМ

Демкович А. Є., Бондаренко Ю. І., Гасюк П. А.

У статті вивчалися порушення гуморальної ланки імунного захисту організму (імуноглобуліни класів А, М, G, концентрацію в сироватці крові циркулюючих імунних комплексів (ЦІК)) на 7-у та 14-ту доби розвитку експериментального бактеріально-імунного пародонтиту, а також можливість корекції їх під впливом корвітину (кверцетину). Застосування його впродовж 7-ми днів внутрішньом'язово в дозі 100 мг/кг позитивно вплинуло на перебіг запального процесу при даній модельованій патології. Паралельно проводилось визначення вмісту ЦІК в сироватці крові як важливої ланки патогенезу імунного ураження за умови експериментального запалення в тканинах пародонта. Застосування корвітину привело до зниження рівня ЦІК в сироватці крові, порівняно з тваринами, яким не вводили даний препарат. Таким чином, доведено, що флавоноїд корвітин, впливаючи на імунні процеси, здатний обмежувати запальну реакцію в пародонтальному комплексі за даної патології.

Ключові слова: пародонтит, імуноглобуліни, циркулюючі імунні комплекси, імунний статус, корвітин.

Стаття надійшла 28.08.2017 р.

НАРУШЕНИЕ ГУМОРАЛЬНОГО ЗВЕНА ИММУННОЙ РЕАКТИВНОСТИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ПАРОДОНТИТЕ И КОРРЕКЦИЯ ИХ ФЛАВОНОЛОМ

Демкович А. Е., Бондаренко Ю. И., Гасюк П. А.

В статье изучались нарушения гуморального звена иммунной защиты организма (иммуноглобулины классов А, М, G, концентрацию в сыворотке крови циркулирующих иммунных комплексов (ЦИК)) на 7 и 14 день развития экспериментального бактериально-иммунного пародонтита, а также возможность коррекции их под влиянием корвитина (кверцетина). Применение его в течение 7-ми дней внутримышечно в дозе 100 мг / кг положительно повлияло на ход воспалительного процесса при данной моделируемой патологии. Параллельно проводилось определение содержания ЦИК в сыворотке крови как важного звена патогенеза иммунного поражения при экспериментальном воспалении в тканях пародонта. Применение корвитина привело к снижению уровня ЦИК в сыворотке крови по сравнению с животными, которым не вводили этот препарат. Таким образом, доказано, что флавоноид корвитин, влияя на иммунные процессы, способен ограничивать воспалительную реакцию в пародонтальном комплексе при данной патологии.

Ключевые слова: пародонтит, иммуноглобулины, циркулирующие иммунные комплексы, иммунный статус, корвитин.

Рецензент Запорожець Т.М.

DOI 10.26724 / 2079-8334-2017-3-61-100-107
УДК 616.833-003.93:577.112

І. М. Довгань, Н. О. Мельник, І. Ф. Лабунець, Н. А. Утко, С. І. Савосько
Національний медичний університет ім. О. О. Богомольця, Лабораторія експериментального моделювання, відділ клітинних і тканинних технологій, ДУ "Інститут генетичної та регенеративної медицини НАМН України", м. Київ

АКТИВНІСТЬ АНТИОКСИДАНТНИХ ФЕРМЕНТІВ У СІДНИЧНОМУ НЕРВІ ЩУРІВ ЗА УМОВ МОДЕЛЮВАННЯ ГЕМОРАГІЧНОГО ІНСУЛЬТУ

e-mail: savosko_s@ukr.net

Досліджено біохімічні зміни у сідничому нерві лабораторних щурів за умов геморагічного інсульту та введення лікарських засобів (пірацетам, кверцетин, ліпін). Встановлено демієлінізацію у сідничому нерві щурів з інсультом та збільшення активності супероксиддисмутази. Введення пірацетаму, ліпіну та кверцетину достовірно відновлювало функціонування системи ферментів обміну глутатіону (глутатіонпероксидаза, глутатіонредуктаза).

Ключові слова: геморагічний інсульт, мозок, сідничий нерв, демієлінізація, супероксиддисмутаза, каталаза, глутатіонпероксидаза, глутатіонредуктаза.

Робота є фрагментом НДР «Органи нервової, імунної та сечостатевої систем в умовах експериментального пошкодження», № державної реєстрації 0112U001413.

Ішемічне ураження мозку та інсульт є основними причинами смерті і тяжких неврологічних розладів. Гіпоксія і гіпоглікемія, які при цьому розвиваються, є причинами загибелі нейронів у анатомічних структурах головного мозку. Як показано у наших попередніх дослідженнях [8], інсульт не обмежується лише деструктивними змінами у корі мозку, а нейродегенеративні зміни