**The article is published in Ukrainian in the journal.
The English text is given in the author's version.**

*UDC:* [615.276:547.814.5:611.127]:57.084.1

*S.K. Shebeko, O.S. Ruskin*

*National University of Pharmacy, Ukraine*

***Тhe experimental study of the myocardium functional state under the influence of Diclocor in conditions of furazolidone-isadrine lesion.***

|  |
| --- |
| **Key words:** myocardium, furazolidone-isadrine lesion, biochemical indicators, electrophysiological parameters, antialterative properties, drug Diclocor. |

The frequency of cardiovascular complications among the patients with autoimmune lesions (pseudorheumatism, etc.) is caused by single immunopathological mechanisms of inflammatory and degenerative lesion, that is a complex systemic pathological process caused at the first stage by destructive influence of pro-inflammatory cytokines [1,2]. In connection with the existing prevalence of cardiovascular lesions among patients with rheumatoid arthritis (RA) [3,8] on the one hand, and with the impossibility of wide use of existing anti-inflammatory remedies, due to a number of limitations [9], it is advisable to pay appropriate attention to the search for new anti-inflammatory remedies with cardioprotective properties [1,8,13].

Original drug Diclocor (capsule), manufactured by PJSC SIC "Borshchahivskiy CPP", is a combined anti-inflammatory drug based on the composition of classical NSAID, non-selective inhibitor of cyclooxygenase of sodium diclofenac (38,20% of total volume of active ingredients) and known for the cytoprotective properties of lipoxygenase inhibitor of flavonoid quercetin (61,80% of total volume of active ingredients) [4,6]. Considering the fact that at initial stage of pathological lesion of cardiovascular system of patients with RA the alterative processes causing metabolic and functional disturbances prevail, experimental studying of influence of preparation Diclocor on the functional state indicators of rat’s myocardium at the conditions of alterative lesion [7, 8].

 The aim of this experimental study was the assessment of the influence of the drug Diclocor on the functional state indicators of rat’s myocardium at the conditions of alterative lesion.

 **Materials and methods.** Experimental investigation of the influence of the drug Diclocor on the functional state indicators of rat’s myocardium compared with the reference objects was conducted on furazolidon-isadrin myocarditis (FIM) [8]. Modeling was performed on 50 white rats (nonlinear) of both sexes weighing from180 to 200 g divided into five groups: "I" (intact animals), "CL" – untreated animals with FIM (control lesions), "DC" – animals with FIM under the influence of the preparation Diclocor in dose of 17.8 mg/kg (ED50 for anti-inflammatory activity [5]); "DN" – animals with FIM under the influence of reference drug of diclofenac sodium in a dose of 6.8 mg/kg (corresponds to Diclocor on the content of diclofenac sodium); "Q" – animals with FIM under the influence of the reference drug with the active ingredient quercetin in a dose of 11.0 mg / kg (corresponds to Diclocor on the content of quercetin). Within 5 days (starting from the first day of the study) each animal from "DC", "DS" and "Q" groups orally received appropriate drugs in the form of aqueous suspensions, stabilized TWEEN-80, once a day and animal from "I" and "CL" received equivalent volume of 0.9% aqueous solution of sodium chloride. On the Day 5 of the experiment the electrocardiographic (ECG) examination indicators were registered. To register the ECG indicators after the preliminary injection of 1% solution of 5-ethyl-5 sodium isoamyl barbiturate, sleeping rats were fixed in a prone position with the help of a machine and connected with the cardiograph by subcutaneous injection of needle electrodes. The ECG indicators were recorded in standard branches with the speed of the tape recorder of 50 mm/s and sensitivity of the channels of 10 mm/mV using the electrocardiograph ЕК1Т-03М2. Also on the 5th day of the experiment the activity of some marker enzymes of alterative lesion of cardiomyocytes: aspartate aminotransferases (AST) and lactate dehydrogenases (LDH) was estimated, the determination of content in the blood of rats TBA-reactants (TBAr) and diene conjugates (DC).

 Experimental study was conducted according to “European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes" requirements. [10] Statistical analysis of the results was performed by methods of variation using Student’s t-test and nonparametric analysis (Mann-Whitney U Test) using computer programs STATISTICA 7.0, StatPlus 2009 and MS Exel 2007 [12]; it was represented by a comparative table with the results of different groups.

 **Results and discussion.** The analysis of the results of ECG-examinations of rats of “KP” group confirms the development of ischemic myocarditis. In rats of this group an obvious increase of wave S and a formation of a deep negative wave of QS type concerning group of intact control were fixed, that is characteristic of the expressed ischemic lesion of myocardium. The sinus arrhythmia was displayed by the increasing of the heart rate (HR) indicator from 428 to 555 b/min and by the corresponding reduction of PQ intervals (atrial conduction) and QT (ventricular activation at the moment of systole). The reduction of the waves P, T and R amplitude and the shift of the ST segment concerning the isoline also testified in favor of myocardium ischemia. The changes of the QRS complex characteristic of focal injuries of myocardium occurred in 4 cases from 10 in rats of "CL" group. The nature of changes of the wave T corresponded to the expected level of diffusive disturbances of metabolism at the conditions of a furazolidone-isadrine lesion (Tab. 1). At the same time, the results of biochemical research of the blood serum of rats of "CL" group confirmed alternative disturbance of myocardium and activation of processes of the peroxide oxidation of lipids (POL) because of the expected increase in the AST and LDH levels 1,7 and 2,1 times respectively, and the growth of indicators of TBAr and DC 1,8 times relatively to intact animals (Tab. 2).

 After an application of the drug Diclocor, a reduction of the manifestations of alterative lesion and recovery of functional state of the myocardium of rats with FIM was observed. The accurate line of obvious distinctions existed concerning rats of KP group. So, the results of ECG-test reflected the tendency to the normalization of the duration of intervals of RR, PQ, QT, the QRS complex, and the level of voltage of the waves P, T, R (Tab. 1). The decrease of the level of deviation of the ST segment concerning the isoline (Tab. 1) and the stabilization of HR at the level of 472 beats/min also showed the decrease in negative impact of furazolidone-isadrine lesion on a functional state of rat’s myocardium under the influence of preparation Diclocor. The results of biochemical research of blood serum of rats of “DC” group reflected an obvious decrease in level of enzyme release and normalization of processes of POL (the level of TBAr and DC has decreased 1,3 times) relatively to the group of rats with control pathology (Tab. 2).

 After an application of reference object of diclofenac sodium an insignificant normalizing influence on the indicators of functional state of rat’s myocardium with FIM was observed. So, the results of ECG-examinations of this group of rats reflected insignificant stabilization of the studied parameters (Tab. 1). The changes of the QRS complex characteristic of focal injuries of myocardium were observed in 2 cases from 10 rats of “DN” group. The results of biochemical research of blood serum of rats of “DN” group also testified in favor of insignificant tendency to the normalization of the corresponding indicators. It is necessary to remark, that the stabilization of the content of AST and Dc in the serum of rats of “DN” group had probabilistic character relatively to the group of rats with control pathology.

A further analysis of the obtained data has revealed a cytoprotective vector of the reference object Quercetin at the conditions of FIM (Tab. 1, 2). After an application of Quercetin, the decrease of the manifestations of tachycardia and expressed ischemia of myocardium was observed relatively to the group of rats with control pathology: the normalization of the duration of intervals of RR, PQ, QT, the QRS complex; the decrease of the level of deviation of the ST segment concerning the isoline (Tab. 1). The results of biochemical research of blood serum of rats of “Q” group reflected an obvious decrease in level of enzyme release and the normalization of processes of POL relatively to the group of rats with control pathology (Tab. 2).

 Assessment results of the influence of the preparation studied and reference objects on the functional state indicators of myocardium of rats with FIM show the advantages of Diclocor. In the realization of its cytoprotective activity at the conditions of FIM, Quercetin has a significant role, namely, anti-alterative, antioxidant and membrane-stabilizing activity of given substance. The presence of Quercetin causes the ability of the studied preparation to normalize electro-physiologic functional state indicators of myocardium of animals with FIM. The content of diclofenac sodium in the composition of Diclocor promotes essential increase of expressiveness and speed of implementation of anti-inflammatory potential of the studied preparation, at the same time its cardiotoxic action is leveled due to the content of flavonoids as a part of pharmaceutical composition.

**Conclusions.**

From the ability to functional recovery of the affected myocardium and the level of the normalization of blood biochemical indicators of rats the studied objects should be placed as follows: Diclocor → Quercetin → Sodium Diclofenac. Cytoprotective activity of the preparation Diclocor at the conditions of FIM is caused by the potentiation of expressed anti-inflammatory properties of diclofenac sodium by anti-alterative, antioxidant and membrane-stabilizing properties of quercetin.

Received results allow to consider the possibility of further experimental study of cytoprotective potential of original preparation Diclocor on different models of inflammatory and degenerative lesions of myocardium.

*Table 1*

***Influence of the drug Diclocor and reference objects on the functional state indicators of rat’s myocardium with FIM (n=50)***

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Group* | *Duration of the interval RR, c* | *Duration of the interval PQ, с* | *Duration of the interval QT, с* | *Duration of the interval QRS, с* | *Voltage of wave P, mV* | *Voltage of wave T, mV* | *Voltage of wave R, mV* | *Shift ST from isoline, mm* |
| *Intact rats “I”* | *0,140**±0,006* | *0,046**±0,002* | *0,077**±0,003* | *0,024**±0,001* | *0,087**±0,003* | *0,158**±0,006* | *0,430**±0,017* | *0,20**±0,08* |
| *Control pathology, “CL”* | *0,108**±0,004\** | *0,036**±0,001\** | *0,056**±0,002\** | *0,018**±0,001\** | *0,061**±0,002\** | *0,084**±0,003\** | *0,320**±0,013\** | *1,90**±0,12\** |
| *Quercetin, “Q”* | *0,124**±0,005\*\** | *0,039**±0,002\*\** | *0,062**±0,002\** | *0,022**±0,001\*\*/●* | *0,076**±0,003\*/\*\** | *0,101**±0,004\*/\*\** | *0,384**±0,015\*\*/●* | *0,85**±0,08\*\*/●* |
| *Diclofenac, “DN”*  | *0,112**±0,004\** | *0,040**±0,002\** | *0,064**±0,003\*/\*\** | *0,019**±0,001\** | *0,069**±0,003\** | *0,097**±0,004\*/\*\** | *0,339**±0,014\** | *1,40**±0,12\*\** |
| *Diclocor, “DC”*  | *0,127**±0,005\*\*/●* | *0,042**±0,002\*\** | *0,066**±0,003\*/\*\** | *0,023**±0,001\*\*/●* | *0,084**±0,003\*\*/●* | *0,109**±0,004\*/\*\*/●* | *0,390**±0,016\*\*/●* | *0,65**±0,17\*\*/●* |

*Notes: \* - P≤ 0,05 relatively to intact animals; \*\* - P≤ 0,05 relatively to the control lesion group; ● - p≤ 0,05 relatively to the group of rats under the influence of reference sample of Diclofenac sodium; n=10 – quantity of animals in group.*

*Table 2*

***Some blood biochemical indicators of the rats with FIM under the influence of the drug Diclocor and reference objects (n = 50)***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *Group* | *AST, mmol / hr \* l* | *Blood LDH, mmol / hr \* l* | *TBA-reactants, mumol/l* | *DC, mumol/l* |
| *Intact rats “I”* | *0,75±0,02* | *5,53±0,27* | *2,84±0,08* | *56,55±1,46* |
| *Control pathology, “CL”* | *1,24±0,03\** | *11,37±0,56\** | *5,13±0,14\** | *103,40±2,67\** |
| *Quercetin, “Q”* | *0,93±0,02\*/\*\*/●* | *8,19±0,40\*/\*\*/●* | *4,14±0,11\*/\*\*/●* | *75,40±1,95\*/\*\*/●* |
| *Diclofenac, “DN”*  | *1,12±0,03\*/\*\** | *10,07±0,50\** | *4,96±0,13\** | *95,70±2,48\*/\*\** |
| *Diclocor, “DC”*  | *0,88±0,02\*/\*\*/●* | *7,32±0,36\*/\*\*/●* | *3,94±0,11\*/\*\*/●* | *77,60±2,01\*/\*\*/●* |

*Notes: \* - P≤ 0,05 relatively to intact animals; \*\* - P≤ 0,05 relatively to the control lesion group; ● - p≤ 0,05 relatively to the group of rats under the influence of reference sample of Diclofenac sodium; n=10 – quantity of animals in group*

***References***

1. Бобров В.О. Адаптаційні ішемічні та реперфузійні синдроми у хворих ішемічною хворобою серця: механізми, діагностика, обґрунтування терапії / В.О. Бобров, С.К. Кулішов. − Полтава: Дивосвіт, 2004. - 240 с.
2. Meek I.L., Picavet H.S., Vonkeman H.E. et al. Increased cardiovascular risk factors in different rheumatic diseases with the general population // Oxford (United Kingdom). — Р. Rheumatology. – 2012 – 52 (1). – Р. 210-216.
3. World Health Statistics 2012. − WHO, 2012. − 176 p. − <http://www.who.int/healthinfo/> EN\_WHS2012\_Full.pdf.
4. T. Gusdinar, R. Herowati, R.E. Kartasasmita and I.K. Adnyana, Anti-inflammatory and Antioxidant Activity of Quercetin-3, 3’, 4’-Triacetate. Journal of Pharmacology and Toxicology.2011, 6: 182-18.
5. Експериментальне дослідження протизапальних властивостей препарату «Диклокор» / О.С. Попов, С.К. Шебеко, О.І. Шевченко, А.С. Шаламай // Клінічна фармація. – 2015. – Т. 19, № 4. – С. 35-39.
6. Zupanets I.A. The effect of the medication «Diclocor», containing diclofenac and quercetin, on clinico-biochemical parameters in rats with osteoarthritis / I.A. Zupanets, S.K. Shebeko, O.S. Popov // International Journal of Pharmacy and Pharmaceutical Sciences. – 2015. – Vol. 7 (9). – P. 101-104.
7. Diclocor is superior to diclofenac sodium and quercetin in normalizing biochemical parameters in rats with collagen-induced osteoarthritis / I.A. Zupanets, S.K. Shebeko, O.S. Popov, A.S. Shalamay // Inflammopharmacology. – 2016. – Р. 1-5. – DOI: 10.1007/s10787-015-0258-8.
8. Кардиоваскулярная патология при ревматоидном артрите. Монография/А.А. Дзизинский, Н.А.Храмцова//Иркутск: РИО ГИУВа – 2010. – 150 с.
9. Зайченко Г.В. НПВП: что нового в начале XXI века? // Медична газета «Здоров’я України». – Квітень 2014 р. – № 7 (332). – С. 30-31.
10. European convention for the protection of vertebrate animals used for experimental and other scientific purpose: Council of Europe 18.03.1986. − Strasbourg, 1986. − 52 p.
11. Доклинические исследования лекарственных средств: Методические рекомендации / Под ред. А.В. Стефанова. – К: Авиценна. – 2002. – 528 с.
12. Реброва О.Ю. Статистический анализ медицинских данных. Применение пакета прикладных программ STATISTICA. – 3-е изд. – М-ва: МедиаСфера. – 2006. – 312 с.
13. C. P. Chung, J. T. Giles, M. Petri et al., “Prevalence of traditional modifiable cardiovascular risk factors in patients with rheumatoid arthritis: Comparison with control subjects from the multi-ethnic study of atherosclerosis,” Seminars in Arthritis and Rheumatism. 2012, vol. 41, no. 4, pp. 535–544.

*UDC: [615.276:547.814.5:611.127]:57.084.1*

*S.K. Shebeko, O.S. Ruskin*

*National University of Pharmacy, Ukraine*

***Тhe experimental study of the myocardium functional state under the influence of Diclocor in conditions of furazolidone-isadrine lesion***

*It has been presented the results of the studying on the influence of Diclocor on the functional state indicators of rat’s myocardium in conditions of furazolidone-isadrine lesion. After a medical application of Diclocor the normalization of both, general functional indicators − electrophysiological parameters of myocardium, and some blood biochemical indicators of experimental rats − the AST, LDH level, TBA-reactants, DC was observed. The obtained data testify about expressed anti-inflammatory action of the investigated medication in comparison with reference objects, which mechanism is caused by the membrane stabilizing and antioxidant types of activity as for the affected cardiomyocytes. Received results allow considering the perspectives of further experimental study of cytoprotective potential of original medication Diclocor on different models of inflammatory and dystrophic in condition of myocardium lesion.*

***Key words:*** *myocardium, furazolidone-isadrine lesion, biochemical indicators, electrophysiological parameters, antialterative properties, drug Diclocor.*