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Original Article

THE EFFECT OF THE MEDICATION "DICLOCOR", CONTAINING DICLOFENAC AND QUERCETIN, ON CLINICO-BIOCHEMICAL PARAMETERS IN RATS WITH OSTEOARTHRITIS

ZUPANETS I. A., SHEBEKO S. K., POPOV O. S*.

National University of Pharmacy, 61057, 27 Pushkinskaya str., Kharkiv, Ukraine
Email: oleksii.s.popov@gmail.com

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ABSTRACT

Objective: The aim of this study was to evaluate chondroprotective effect of a combined medication Diclocor in comparison with its active monocomponents (diclofenac sodium and quercetin).

Methods: The study was conducted on the model of steroid dystrophy of connective tissue in rats. Steroid dystrophy was induced by dexamethasone injections. For evaluation of chondroprotective effect, the following biochemical markers were analyzed: serum concentrations of chondroitin sulfate, sialic acids, glycoproteins, N-acetylglucosamines, total glycosaminoglycans and their fractions, as well as clinical features of the animals' condition.

Results: The analyzed parameters under the action of Diclocor were significantly closer to the intact control than those after treatment with diclofenac sodium. There were no significant differences between Diclocor and quercetin groups.

Conclusion: The results have led to a conclusion that Diclocor exhibits pronounced chondroprotective effect which is of the same level as quercetin and significantly exceeds diclofenac sodium.

Keywords: Diclocor, Diclofenac sodium, Quercetin, Osteoarthritis, Experimental.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used groups of drugs in clinical practice, however, despite the undoubted clinical effectiveness, their use is associated with high risk of side effects which are generally found in 25% of cases and in 5% constitute a serious threat to life [1-3].

One of the most well-known problems of NSAIDs use is anti-inflammatory therapy in patients with osteoarthritis (OA), namely, the negative effect of some NSAIDs on the metabolism of articular cartilage [4, 5].

One solution to the above-mentioned problem is to explore new combinations of NSAIDs with substances that could lessen their chondrotoxic effect due to the presence of antioxidant, antihypoxic, and cytoprotective properties [6]. In this regard, the medication Diclocor, newly developed by PJSC SIC "Borshchagivskiy HFZ" (Ukraine), arouses scientific interest [7]. The purpose of the present study was to investigate chondroprotective activity of Diclocor in the setting of steroid dystrophy of connective tissue in rats in comparison with its monocomponents (diclofenac and quercetin).

MATERIALS AND METHODS

Ethical permissions

All animal studies were performed in accordance with Directive 2010/63/EU on the protection of animals used for scientific purposes. The plan of this study was approved by the Ethical Committee of National Pharmaceutical University, Kharkiv, Ukraine.

Test drug

Diclocor is a combination drug in capsules for oral use (270 mg of the capsule mass) containing 25 mg of diclofenac sodium and 40 mg of quercetin. Diclocor is being developed by PJSC SIC "Borshchagivskiy HFZ", Ukraine.

Reference drugs

1. Quercetin granules containing 40 mg of the quercetin substance per granule, manufactured by PJSC SIC "Borshchahivskiy CPP": Ukraine.

2. Voltaren coated tablets containing 50 mg of diclofenac sodium: manufactured by Novartis Pharma, Switzerland.

Test animals and grouping

60 white rats of both sexes with body weight of 250-300 g were divided into 5 experimental groups with 10 animals in each group, except for control pathology group consisting of 20 animals.

Group 1–intact control

Group 2–control pathology (untreated animals)

Group 3–rats treated with Diclocor at a dose of 17.8 mg/kg

Group 4–rats treated with Quercetin at a dose of 11 mg/kg

Group 5–rats treated with Voltaren at a dose of 6.8 mg/kg

Experimental design

The study was conducted in the settings of steroid dystrophy of connective tissue in our modification [8] by triple intramuscular injection of dexamethasone phosphate ("KRKA", Slovenia) at a dose of 7 mg/kg with intervals of 1 week between injections. 10 rats from the control pathology group were withdrawn from the experiment for assessment of pathology development on day 28. Starting from day 28 of the experiment, the animals were receiving corresponding drugs which were administered intragastrically as extemporal suspensions. On day 56, the rats were withdrawn from the experiment, decapitated under ether anesthesia for blood samples to be taken for biochemical tests. Clinical observations were made throughout the study, and body mass measurements were taken on day 0, 14, 28, and 56.

Biochemical tests analyzed

blood serum concentrations of sialic acids (SA), glycoproteins (GP), chondroitin sulfate (CS), glycosaminoglycans (GAG)-total and fractions-and N-acetylglucosamine (NAG). NAG was also measured in the articular cartilages.

Statistical analysis of the results

The received results were analyzed by means of variation statistics using Student's t-test and nonparametric methods (Mann–Whitney

U-test). Utilized computer software included STATISTICA 7.0, StatPlus 2009, and MS Excel 2007 [9, 10].

RESULTS AND DISCUSSION

Clinical observations

The results of the study suggest that clinical signs of musculoskeletal system damage occurred in all animals after administration of 3 doses of dexamethasone. First of all, this was demonstrated by the fact that animals were lethargic and inactive. Swelling of knee joints, decrease in the range of motion in them, mobility difficulties, and reduction of exercise tolerance was observed. In addition, loss of appetite and abnormal state of wool cover were present. Clinical picture of OA development, that was later observed, was confirmed by results of biochemical studies on day 28 of the experiment.

Clinical observations of rats with OA receiving Diclocor have shown that significant changes in the functional state of animals were noted starting from the second week of administration. There was an increase in motor activity, exercise tolerance, visual normalization of joint state, and an increase in appetite. When reference drugs Quercetin and Voltaren were administered, functional state of animals differed from the control pathology group to a slightly lesser extent.

Biochemical tests

It should be noted that among the parameters presented, the most important one in relation to the development of OA is the level of CS as it is the main GAG of articular cartilage matrix. Such parameters as GP and SA are less specific and largely reflect acute phase pathological processes in the connective tissue. The results of determination of blood serum concentration of the main connective tissue metabolites are presented in table 1.

Table 1: Blood serum concentration of the main connective tissue metabolites

Group	Chondroitin sulfate, g\l	Glycoproteins, g\l	Sialic acids, mmol\l
Initial data			
Intact control	0.311±0.019○	2.73±0.16	3.55±0.17
Day 28			
Control pathology	0.406±0.024○	3.88±0.28○	5.14±0.32○
Day 56			
Control pathology	0.425±0.026○	4.15±0.38○	4.83±0.19○
Diclocor	0.351±0.015●	3.37±0.30	3.91±0.26●
Quercetin	0.338±0.030●	3.59±0.32○	4.27±0.38
Voltaren	0.407±0.036○	3.82±0.34○	3.76±0,33●

○–p≤ 0.05 in relation to intact animals; ●-p≤ 0.05 in relation to the control pathology group; ■-p≤ 0.05 in relation to animals receiving reference drug Voltaren.

It is noteworthy that after administration of dexamethasone in rats of the control group on day 28 of the experiment, there was a significant increase in concentration of all metabolites in comparison with the intact control group. This fact confirms development of the pathological process of connective tissue in all test animals. Later this tendency continued, and 56 days after study initiation, the level of CS and GP increased more significantly (1.4-fold and 1.5-fold respectively) in relation to intact. SA concentration has slightly declined and was 1.25 times as much as in the intact group.

Under the action of Diclocor, a moderate decrease in concentration of CS and SA was observed with significant differences from the control pathology group. Thus, the CS, GP, and SA levels decreased by 21, 23, and 23% respectively. This pattern is due to the fact that the test drug has an oral pharmaceutical form and, as a result, causes systemic effect through which it can actively influence the metabolic processes of the connective tissue in the entire body of animals.

In the Quercetin group, CS was at the level of Diclocor (decrease by 25% in comparison with control pathology). GP and SA changes were slightly less prominent with SA concentration decreasing without significant differences from untreated animals. Under the action of Voltaren, no significant decrease in CS and GP in comparison with untreated animals was noted. This indicates a lack of positive effect of this drug on the metabolism of articular tissue. However, positive trend in SA concentration was identified, caused by anti-inflammatory properties of the drug, but without significant differences from rats receiving Diclocor.

The most important biochemical indicator characterizing intensity of pathological changes in joint tissues in the setting of OA is the total amount and fractions of GAG in blood serum. It is known that, under normal conditions, the main GAG fraction is hyaluronate and chondroitin-6-sulfate. As the disease progresses, the ratio of fractions of GAG undergoes specific changes, the result of which being GAG total amount increasing at the expense of chondroitin-4-sulfate, since this particular GAG is contained in the greatest amounts in cartilage matrix and leaves it in the result of destruction [5]. Further, when the pathological process is enhanced, an increase in the concentration of highly sulfated GAG fraction, represented mainly by keratan sulfate, is observed. Thus, analysis of the ratio of different GAG fractions as well as the blood serum concentration of its total amount make it possible to estimate the process of development of OA and efficiency of treatment carried out.

The results of investigation of fraction composition and total concentration of GAG in the blood serum of rats with OA under the action of Diclocor and comparator drugs are provided in table 2.

Analysis of data provided suggests that, under normal conditions in intact animals, the fraction of hyaluronates and chondroitin-6-sulfates amounts to 60% of the total amount of GAG, the fraction of chondroitin-4-sulfates–33.5%, and highly sulfated GAG fraction–6.5%. When the pathology developed (day 28 of the experiment), beside a significant increase in the amount of GAG, the change in the percentage ratio of fractions was also observed with chondroitin-4-sulfates amounting to 40% of the total GAG.

Further over the period of pathology development (day 56 of the experiment), this trend remained. A 1.4-fold increase in the total amount of GAG in comparison with initial data was observed mainly due to chondroitin-4-sulfates since this fraction amounted to more than 43% of the GAG total amount.

It should be noted that the level of highly sulfated GAG in the course of OA development had a slight tendency to increase and did not reach significant differences from the intact group even on day 56 of the experiment. Thus, this parameter was subjected to fewer changes in comparison with other fractions owing to the much smaller concentration of highly sulfated GAG in the structures of cartilage matrix compared to CS. This indicates moderate severity of experimental joint disease. In the Diclocor group, a certain pharmacological activity was observed. This was demonstrated by a significant decrease in the total amount of GAG in relation to control pathology not only due to the reduction of chondroitin-4-sulfates, but also owing to reduction of the fraction of chondroitin-6-sulfates whose concentration reached the level of intact animals. It should be noted that, in this case, the ratio of fractions was almost without changes, and the above changes were significant in comparison with the control pathology group only in terms of the dynamic of changes

of chondroitin-4-sulfates. In case of Quercetin, the study showed a similar dynamic of these parameters without significant differences from animals receiving Diclocor. Voltaren, on the contrary, had almost no positive effect on the concentration of GAG and their fractions as the majority of the parameters were at the level of the untreated group of rats.

Table 2: Fraction composition and total concentration of GAG in blood serum

Group	GAG concentration, g/l			Total GAG concentration, g/l
	Hyaluronates and chondroitin-6-sulfates	Chondroitin-4-sulfates	Highly sulfated GAG	
Initial data				
Intact control	0.195±0.013	0.109±0.008	0.021±0.002	0.325±0.023
Day 28				
Control pathology	0.236±0.022○	0.176±0.016○	0.023±0.002	0.435±0.040○
Day 56				
Control pathology	0.233±0.014	0.195±0.012○	0.025±0.002	0.453±0.028○
Diclocor	0.199±0.011	0.155±0.009○/●	0.025±0.001	0.379±0.021●
Quercetin	0.204±0.012	0.131±0.007●/■	0.020±0.002	0.356±0.021●/■
Voltaren	0.238±0.013○	0.183±0.010○	0.024±0.002	0.445±0.026○

○–p≤ 0.05 in relation to intact animals; ●-p≤ 0.05 in relation to the control pathology group; ■-p≤ 0.05 in relation to animals receiving reference drug Voltaren.

Analysis of the study results suggests that the best effect on the total amount and fractions of GAG in the blood serum were recorded under the action of Quercetin, since the ratio of GAG fractions was the closest to the normal level and two chondroitin-containing GAG fractions were reduced in comparison with untreated animals. At the same time, Diclocor exhibited activity at the level of Quercetin without significant differences which suggest a high chondroprotective potential of this drug.

NAG determination in blood and cartilage

The parameters of endogenous NAG may be considered as non-specific, but, at the same time, informative markers of destructive processes of connective tissue and efficiency of the drug therapy.

The results obtained are shown in the table 3 and indicate that, in the control group, a significant increase in blood serum concentration of both overall (1.2-fold) and bound (1.3-fold) fractions of NAG was observed in comparison with intact rats on day 28 of the experiment. At the same time, although the level of free NAG had a downward tendency, this reduction was not significant in comparison with the intact group. In addition, a significant reduction (1.2-fold) of NAG concentration in the homogenate of the cartilaginous tissue was noted in rats from this group.

Table 3: Endogenous N-acetylglucosamine metabolism

Group	Endogenous NAG concentration			
	Blood serum, mmol/l			Articular cartilage, mg/g
	Total	Bound	Free	
Initial data				
Intact control	7.02±0.49	5.17±0.33	1.84±0.18	0.225±0.014
Day 28				
Control pathology	8.46±0.44○	6.85±0.43○	1.62±0.18	0.183±0.012○
Day 56				
Control pathology	7.96±0.57	6.81±0.49○	1.15±0.08○	0.154±0.013○
Diclocor	7.24±0.46	5.49±0.36●	1.75±0.11●/■	0.207±0.018●/■
Quercetin	7.61±0.68	5.89±0.52	1.72±0.15●	0.212±0.019●/■
Voltaren	8.10±0.72	6.73±0.60○	1.37±0.12○	0.145±0.013○

○–p≤ 0.05 in relation to intact animals; ●-p≤ 0.05 in relation to the control pathology group; ■-p≤ 0.05 in relation to animals receiving reference drug Voltaren.

Data presented indicate development of destructive processes in the matrix of articular cartilage and passage of destroyed remnants of biopolymers (proteoglycans, GAG, etc.), including NAG, into the bloodstream. In this regard, blood concentration of this hexosamine is increased owing namely to the bound fraction. This demonstrates the intensity of the destructive processes of the articular cartilage.

By contrast, the level of free NAG reflects the intensity of regenerative processes in the connective tissue, and a decrease in its concentration may be due to its capture by chondrocytes and synoviocytes followed by inclusion of newly formed GAGs into biosynthesis processes. Thus, the lower the concentration of this fraction is, the fewer capabilities for regeneration the damaged cartilaginous tissue has.

In the course of OA development, the above-mentioned pattern has been enhanced. And on day 56 of the study, NAG concentration in articular cartilage tissues was reduced by 1.5 times in the control pathology group. At the same time, the concentration of overall NAG in the blood serum of the control group has slightly decreased and had no significant differences from the intact group.

However, the level of bound NAG still remained increased by 1.3 times. The concentration of free amino sugar fraction decreased even more and reached 1.15 mmol/l which is 1.16 times lower than in the intact control group and 1.4 times lower than the values on day 28 of the experiment. Thus, the regenerative potential of the cartilaginous tissue was significantly decreased and reached its minimum level.

In case of Diclocor use, a positive dynamic in NAG concentration was detected with respect to all fractions since the values obtained have a tendency to normalization with significant differences from the control pathology group (except for the free fraction). At the same time, the level of free NAG increased by 52% in comparison with untreated animals, which in its turn indicates an enhancement of the regenerative potential of the cartilaginous tissue in rats of this group. The picture presented is supported by the results of analysis of NAG concentration in the cartilaginous tissue, where the level of hexosamine reached 0.207 mg/g (without significant differences from the intact group) and was significantly (by 1.4 times) higher than the parameter values of the control pathology group.

A similar but slightly less pronounced pattern was observed with reference drug Quercetin. Concentration of overall and bound NAG fraction in the blood also decreased, but without significant differences from untreated animals. However, the concentration of the bound fraction increased significantly and reached values of animals receiving Diclocor.

Thus, in terms of the effect on NAG concentration in articular cartilage, Quercetin is slightly inferior to Diclocor which indicates a better effect of Diclocor on reducing the intensity of destructive processes in joint tissues and enhancement of reparative capabilities of the articular cartilage.

At the same time, no positive changes in endogenous NAG metabolism were noted under the action of Voltaren. All parameters studied were at the level of control pathology. A slight upward trend at the expense of the bound fraction was noted in overall blood serum NAG along with the respective decrease in NAG in the cartilaginous tissue, but without significant differences, indicating enhancement of destructive processes in the cartilage matrix and reflecting the negative characteristics of Voltaren.

It should be noted that the positive trend in parameters of endogenous NAG metabolism, observed with administration of the test medication Diclocor, apparently, is associated with the presence of quercetin in its composition with its antioxidant, antiapoptotic, and membrane-protective effects which enable chondroprotection.

CONCLUSION

Diclocor exhibits a positive effect on the course of experimental OA in rats and, consequently, has chondroprotective properties being not inferior to Quercetin and significantly exceeding Voltaren. This makes Diclocor a promising perspective for anti-inflammatory treatment of joint diseases while, undoubtedly, further studies are needed.

CONFLICT OF INTERESTS

All authors have none to declare

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