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The influence of glucosamine and Ketoprofen combination in the form of cream-gel on connective tissue metabolism indicators of rats with experimental osteoarthritis

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Abstract
The article presents the results of experimental study of chondroprotective activity of glucosamine hydrochloride and ketoprofen combination in topical dosage form. The study was carried out on a model of systemic steroid osteoarthritis in rats. To evaluate the chondroprotective activity the following biochemical parameters like the content of sialic acids, glycoproteins, chondroitin sulphates, total and fractions glycosaminoglycans in blood serum and content of endogenous N-acetylglucosamine were used. Fastum gel 5%, glucosamine cream gel 5% were used as a comparison agents that were applied in a similar way with equivalent therapeutic dose. As a result of biochemical studies research combination was showing a positive effect on the experimental osteoarthritis in rats that proves its chondroprotective properties, and some data exceeding the level of activity of comparison drugs.

Keywords: chondroprotective activity, glucosamine hydrochloride, ketoprofen, topical dosage form, osteoarthritis, biochemical parameters.

1. Introduction
Osteoarthritis (OA) is the most common musculoskeletal system disease. According to WHO data, about 14% of people aged over 60 years suffer from symptomatic knee osteoarthritis. Recently, statistical data about healthy aging of the European population were published. The most common cause of disability in older people was dementia, followed by factors such as hearing loss and osteoarthritis [1]. Due to population aging, including Ukrainian population, problems of diagnostics, prevention and treatment of OA are becoming more and more essential. Decreasing peoples' life quality is OA consequence, so the drugs' form have to be invented for continued and painless using [2].
Chondroprotective agents are still used in OA treatment [3]. Drugs from this group can change not only main OA clinical aspects, but also provide a pathogenetic influence on disease progress by preventing articular cartilage damage and stimulating its regeneration [4, 5, 6]. A lot of attention also should be paid to anti-inflammatory and analgesics drugs, because pain is the main symptom, causing patients suffering [5]. The purpose of the present research became the experimental evaluation of using biochemical parameters to prove the chondroprotective activity of topical dosage forms containing glucosamine hydrochloride and ketoprofen combination.

2. Materials and Methods
The study of chondroprotective activity of the combination of glucosamine hydrochloride and ketoprofen as a topical dosage form was carried out on a model of systemic steroid osteoarthritis (SSOA) in rats. The object of the present research was glucosamine hydrochloride and ketoprofen combination in the form of a cream-gel (G\K cream-gel). Fastum gel 5%, glucosamine cream gel 5% were used as a comparison agents that were applied in a similar way with equivalent therapeutic dose of 50 mg. The 60 white male rats aged 4-5 months, weighing 250-300 g. had been used in the study.
According to the standard health and safety regulations the experimental animals were kept on the appropriate food diet in the vivarium at the Central Research Laboratory of the National University of Pharmacy certified by the State Enterprise "Centre for Drug Evaluation and Research at the Ministry of Public Health of Ukraine" as a base for research in experimental pharmacology [7].

The studies were conducted in accordance with EC Directive 86/609 EEC from November, 24, 1986 in compliance with laws, acts and regulations of the EU countries as to protection of animals used for experimentation and other scientific purpose [8, 9].
The rats were divided into 5 experimental groups:

group 1 – intact control (n=10),

group 2 – pathology control (n=20),

group 3 – rats with osteoarthritis treated by studied combination (G\K cream-gel) in conventionally-therapeutic dose of 50 mg (n=10),

group 4 – rats with osteoarthritis, treated by glucosamine cream-gel with an equivalent dose of 50 mg (n=10),

group 5 – rats with osteoarthritis, treated by Fastum gel 5% with an equivalent dose of 50 mg (n=10).

Experimental osteoarthritis was reproduced by three times intramuscular injections in the thigh muscle of dexamethasone in a single dose of 7 mg / kg at intervals of 1 week in accordance with the methodological recommendations [10, 11, 12]. Starting from 28 research day and during 4 weeks period, all animals daily received appropriate agents that were applied externally by cutaneous applications to the knees area on both legs in equivalent dose of 50 mg on each.
During 56 research day, biological material for biochemical studies was taken by decapitation of animals under anesthesia. Next biochemical parameters were received: content of sialic acids (SA), glycoproteins (GP), chondroitin sulphates (CS), total and fractions glycosaminoglycans (GAG) in blood serum and content of endogenous N-acetylglucosamine (N-acGA) in blood serum (total, free and connected fractions) and articular cartilage tissues [12]. Investigated data were taken into account as initial data and for this purpose intact animals were used, and by 28 day result (control pathology group) and 56 day results of the present research.
Statistical analysis of the results were carried out by methods of variation statistics using Student's t-test and nonparametric methods of analysis (Mann-Whitney U Test) using a computer program STATISTISA 7.0 and presented in a comparative table with the different groups results [13, 14].

3. Results and Discussion
Results of biochemical parameters research on rats with SSOA under the influence of the experimental treatment were presented in tables 1, 2, 3. It is noteworthy that control pathology group after dexamethasone administration showed a significant increase level of content of all metabolites compared with the intact control group during 28 days of research, that is confirming the pathological process of connective tissue in all studied animals.
Later growth trend was taking place and after 56 days of present research CS and GP levels in control pathology group increased to 1.44 and 1.6 accordingly than in the intact group. The content of SA slightly decreased and stopped with the result 4.97 mmol/l, which is in 1.3 times higher than in the intact group.
Under the influence of G\K cream-gel showed a moderate decrease of content of all markers of connective tissue in the serum of animals. So, CS levels decreased by 24%, GP – 27%, and the rate of SA – 22%. Results of the present research shown that the studied agent was used in external dosage form, and as a result had the local action effect. During reference medications, treatment of animals with SSOA dynamics of connective tissue metabolism in blood serum of animals compared to the control pathology group showed the results with no effects, that proves the lack of effect on the metabolism of cartilage in the selected schema application.
Intact control group data analysis showed that intact animals fractions of hyaluronates and chondroitin-6-sulfates were 61.0% of total GAG, the fraction of chondroitin-4-sulfates – 32.7% and the fraction highly sulfated GAG – 6,5%. In the period of disease development (28th day of researches) a significant increase of GAG amount compared to intact animals was observed as well as percentage faction value changing – the share of chondroitin-4-sulfate had been already 43% of the total GAG. And further with the development of disease (56th day of researches) this tendency was continued. The increasing of the GAG total amount in comparison with the original data observed was in 1.4 times, mainly due to chondroitin-4-sulfates, since this fraction accounted over 43% of the total GAG. When G\K cream-gel was applied to the joints of animals the pharmacological activity were observed by decreasing the total amount of GAG to 19.5% compared with the control disorders. It happened by reducing of chondroitin-4-sulfates and the fraction of chondroitin-6-sulfates as well with content decreasing from 22.5%. Should be noted that the proportion of fractions practically did not change. In case of comparison drug Fastum gel using all parameters of content and the total amount of GAG fractions were almost at the same level as control pathology. The presence of minimal changes had no significant character.
During the study indicators of metabolism of endogenous N-acGA in rats with experimental OA under the influence of investigated combination and reference drugs were also examined. These indicators can be considered as non-specific, but at the same time informative markers destructive processes of connective tissue and efficacy of drug therapy [15]. The results indicate that animals in the pathology control group as of 28 days of the experiment there was a significant increase in serum content as total (1.2 times) and connected (1.4 times) fractions N-acGA in compared with intact rats. The level of free-form N-acGA though tended to decrease, but not significantly compared to the intact group. There was a significant decrease (1.2 times) of content N-acGA in homogenate of cartilage in rats of this group. The data indicated the development of destructive processes in the matrix of articular cartilage and destroyed the remnants of the output of biopolymers [15]. By contrast, the level of free N-acGA reflected the intensity of regenerative processes in the connective tissue cells of the body. In connection with this, the content of hexosamine in blood exactly connected fractions by reflecting intensity of degradation of articular cartilage reducing its content may be explained seizure chondrocytes and synoviotcytes followed by incorporation into newly GAG biosynthesis [15].
During the experimental osteoarthritis development the data were deteriorating. In 56th day of study N-acGA concentration in articulate cartilage decreased in 1,8 times in control pathology group. At the same time the contents of general N-acGA in blood serum of control pathology group decreased and did not have a certainty differences from intact control group.

However, the level of connected N-acGA was remaining increased in 1.1 times. Content of free fraction decreased more and achieved the level of 1.10 mmol/l, which is in 1.8 times less than in the intact control group and in 1.3 times than on the 28th day data of the experiment. Thus, the regenerative potential of cartilage tissue was significantly decreased and reached its minimum level.

During the animal treatment with study drug the positive dynamics of N-acGA in serum of rats in all fractions were observed, since these parameters tended to be normalize with no significant differences as to the control pathology group, and intact control group (except free faction). The level of free N-acGA increased in 37.2% compared with untreated animals, which, in turn, shows the increasing in regenerative potential of cartilage tissue in rats of this group. Present data confirmed by content analysis N-acGA in cartilage, where it reached 0.2 mg/g (no significant difference from the intact group) and significantly (in 1.4 times) higher than the pathology control group data.

All studied data on comparison drugs were almost at the same level with control pathology group, while there was a slight decrease in the overall dynamics of the N-acGA serum by connected fraction and increasing the content of N-acGA in cartilage, but without significant differences. By the level of influence on the content of N-acGA in articular cartilage comparison drugs were far below from the data demonstrated by research combination drug. It should be noted that the positive dynamics data of N-acGA endogenous exchange that was observed during the application of the studied drug, connected with the presence of free glucosamine hydrochloride in it, that hence the transdermal exogenous hexosamines permeation to the tissues of affected joints.

Table 1: Contents of the main metabolites of connective tissue in the serum of rats with experimental osteoarthritis

The experimental conditions	Chondroitin sulfates, g/l	Glycoproteins, g/l	Sialic acids, mmol/l
Initial data			
Intact control group (n=10)	0,303±0,018	2,66±0,15	3,79±0,18
28th day			
Pathology control group (n=20)	0,412±0,024*	3,93±0,28*	5,17±0,32*
56th day			
Pathology control group (n=20)	0,436±0,026*	4,25±0,39*	4,97±0,20*
G\K cream-gel group (n=10)	0,333±0,012**/•	3,12±0,17**/••	3,87±0,25**
Glucosamine cream-gel group (n=10)	0,365±0,014 */**/•	3,78±0,24*	4,34±0,33
Fastum gel group (n=10)	0,420±0,016*	3,67±0,24*	3,97±0,21**

Notes:
1) * - $p \leq 0,05$ relatively intact control;
2) ** - $p \leq 0,05$ relative to the control pathology group;
3) • - $p \leq 0,05$ relative to animals treated Fastum gel;
4) •• - $p \leq 0,05$ relative to animals treated with glucosamine cream-gel.

Table 2: The fractional structure and total GAG content in the blood serum of rats with experimental osteoarthritis

The experimental conditions	The content of the GAGs fractions, g/l			Total GAG content, g/l
	hyaluronates and chondroitin-6-sulfate	chondroitin-4-sulfate	highly sulfated GAGs	
Initial data				
Intact control group (n=10)	0,197±0,013	0,106±0,008	0,021±0,002	0,324±0,023
28th day				
Pathology control group (n=20)	0,225±0,021	0,186±0,017*	0,023±0,002	0,434±0,040*
56th day				
Pathology control group (n=20)	0,236±0,014	0,200±0,012*	0,025±0,002	0,461±0,028*
G\K cream-gel group (n=10)	0,195±0,011	0,155±0,007*/**/•	0,021±0,001	0,371±0,021**
Glucosamine cream-gel group (n=10)	0,222±0,013	0,170±0,010*	0,023±0,002	0,415±0,024*
Fastum gel group (n=10)	0,227±0,013	0,195±0,010*	0,025±0,002	0,448±0,026*

Notes:
1) * - $p \leq 0,05$ relatively intact control;
2) ** - $p \leq 0,05$ relative to the control pathology group;
3) • - $p \leq 0,05$ relative to animals treated with Fastum gel.

Table 3: Indicators of metabolism of endogenous N-acetylglucosamine in rats with experimental osteoarthritis

The experimental conditions	The content of endogenous N-acetylglucosamine			
	blood serum, mmol/l			articular cartilage, mg/g
	total fraction	connected fraction	free fraction	
Initial data				
Intact control group (n=10)	7,13±0,50	5,20±0,33	1,93±0,19	0,220±0,014
28th day				
Pathology control group (n=20)	8,54±0,45	6,95±0,44*	1,59±0,18	0,180±0,011*
56th day				
Pathology control group (n=20)	7,89±0,57	6,79±0,49*	1,10±0,08*	0,147±0,013*
G\K cream-gel group (n=10)	7,15±0,45	5,64±0,37	1,51±0,10**/•	0,217±0,019**/•
Glucosamine cream-gel group (n=10)	7,35±0,48	5,95±0,39	1,40±0,09*/**	0,212±0,014**/•
Fastum gel group (n=10)	7,74±0,46	6,60±0,39*	1,14±0,10*	0,152±0,010*

Notes:
1) * - p≤ 0,05 relatively intact control;
2) ** - p≤ 0,05 relative to the control pathology group;
3) • - p≤ 0,05 relative to animals treated with Fastum gel.

4. Conclusion

1. Research combination G\K cream-gel showed the highest activity in effecting on metabolites in connective tissue in rats' serum with SSOA in compare with comparison drugs.
2. The best data of influence on the total amount of GAG and fractions in serum of rats were recorded under the influence of study combination because under this condition the percentage of fractions of GAG was mostly closer to normal levels, also the both chondroitin containing factions of GAG decreasing were observed in compare with control pathology group.
3. As a result of biochemical studies research G\K cream-gel was showing a positive effect on the experimental osteoarthritis in rats that proves its chondroprotective properties, and some data exceeding the level of activity of comparison drugs.

5. References

1. Data and statistics. Geneva. World Health Organization 2011. [Weblink: http://www.euro.who.int/ru/what-we-do/health-topics/Life-stages/healthy-ageing/data-and-statistics/10-facts-on-healthy-ageing-in-europe]
2. Mason L, Moore RA, Derry S, Edwards JE, McQuay HJ. Systematic review of topical medicines for the treatment of chronic pain. BMJ 2004; 328:991-994.
3. Hinton R, Moody RL, Davis AW, Thomas SF. Osteoarthritis: Diagnosis and Therapeutic Considerations. Am. Fam. Physician. 2002; 65:841-848.
4. Алексеева ЛИ, Кашеварова НГ. Применение локальных средств в лечении остеоартроза. РМЖ. 2008; 24:1622-1628.
5. Зупанець ІА, Попов СБ, Отрішко ІА. Експериментальне вивчення протизапальної активності композиції глюкозаміну гідрохлориду та диклофенаку натрію на моделі карагенінового набряку. Клінічна фармація. 2002; 6(2):48-50.
6. Чичасова НВ. Глюкозамин и хондроитин в лечении остеоартроза. Здоров'яУкраїни 2006; 19: 48-49.
7. Кожем'якін ЮМ, Хромов ОС, Філоненко МА, Сайфетдінова ГА. Науково-практичні рекомендації з утримання лабораторних тварин та роботи з ними. Державний фармакологічний центр МОЗ України, Київ, 2002, 155.
8. Стефанов ОВ, Бухтіарова ТА, Коваленко ВМ та ін. Настанова СТ-Н МОЗУ 42-6.0:2008. Лікарські засоби. Належна лабораторна практика (видання офіційне). «Моріон», Київ, 2009, 37-68.
9. European convention for the protection of vertebrate animals used for experimental and other scientific purpose: Council of Europe 18.03.1986. Strasbourg, 1986, 52.
10. Стефанов АВ. Доклинические исследования лекарственных средств: Методические рекомендации. «Авиценна», Киев, 2002, 528.
11. Усенко ВФ, Зупанець ІА, Тарасенко ОО, Шебеко СК. Дослідження впливу кверцетину на апоптоз хондроцитів в умовах розвитку експериментального остеоартрозу. Український ревматологічний журнал 2011; 3(45):62-66.
12. Зупанец ИА, Корж НА, Дедух НВ и др. Методические рекомендации по экспериментальному исследованию и клиническому изучению противоартрозных (хондромодулирующих) лекарственных средств. К., 1999, 56.
13. Лапач СН, Чубенко АВ, Бабич ПН Статистические методы в медико-биологических исследованиях с использованием Excel. К.: Морион 2000, 320.
14. Реброва О.Ю. Статистический анализ медицинских данных. Применение пакета прикладных программ STATISTICA. 3-е изд. «МедиаСфера», Москва, 2006, 312.
15. Зупанець ІА, Попов СБ., Шебеко СК та ін. Динаміка вмісту ендогенного N-ацетилглюкозаміну при запально-деструктивних процесах різної етіології та під впливом експериментальної терапії. Клінічна фармація 2004; 8(4):34-37.