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N-acetylglucosamine increases the efficacy of quercetin in the treatment of experimental acute kidney injury

[N-acetilglucosamina aumenta la eficacia de quercetina en el tratamiento de insuficiencia renal aguda experimental]

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Abstract

Context: Acute kidney injury (AKI) is a common complication of renal pathology and an adverse drug effect. Mortality caused by AKI reaches 80% in some groups of patients. Therefore, the search of the effective agents for AKI treatment is an important task of the pharmaceutical science.

Aims: To evaluate the efficacy of various combinations of N-acetylglucosamine (NAG) and quercetin in rats with AKI.

Methods: The study was conducted on the model of acute glycerol nephrosis in rats. Combinations of NAG and quercetin in ratios of 1:2, 1:1, 2:1 and 3:1 were studied at a dose of 50 mg/kg with daily intramuscular injection for a week. The efficacy of the combinations was assessed by the indicators of animal survival, excretory renal function, nitrogen metabolism and nephroprotective activity (NA).

Results: The combination of NAG and quercetin in the 1:1 ratio was the most effective. There was a significant increase of excretory renal function and normalization of nitrogen metabolism under the influence of this combination. This determined the NA level up to 69.7%, which was the highest value in the presented study. This combination was statistically superior to all others, as well as to quercetin, by the efficacy level. In addition, the combination exceeded the quercetin in NA index by 2.7 times.

Conclusions: Thus, the NAG/quercetin combination in 1:1 ratio in injectable dosage form is the most promising for kidney diseases treatment.

Keywords: N-acetylglucosamine; acute kidney injury; nephroprotective effect; quercetin.

Resumen

Contexto: La insuficiencia renal aguda (IRA) es una complicación frecuente de la patología renal y un efecto adverso de los medicamentos. La mortalidad por IRA alcanza el 80% en algunos grupos de pacientes. Por lo tanto, la búsqueda de agentes efectivos para el tratamiento de IRA es una tarea importante de las ciencias farmacéuticas.

Objetivos: Evaluar la eficacia de varias combinaciones de N-acetilglucosamina (NAG) y quercetina en ratas con IRA.

Métodos: El estudio se realizó sobre el modelo de nefrosis aguda inducida por glicerol en ratas. Las combinaciones de NAG y quercetina en proporciones de 1:2, 1:1, 2:1 y 3:1 se estudiaron a una dosis de 50 mg/kg con administración intramuscular diaria durante una semana. La eficacia de las combinaciones se evaluó mediante los indicadores de supervivencia animal, función renal excretora, metabolismo del nitrógeno y actividad nefroprotectora (AN).

Resultados: La combinación de NAG y quercetina en la proporción de 1:1 fue la más efectiva. Hubo un aumento significativo de función renal excretora y normalización de metabolismo del nitrógeno bajo la influencia de esta combinación. Esto determinó el nivel de AN hasta el 69.7%, que fue el valor más alto en el estudio presentado. Esta combinación fue estadísticamente superior a todas las demás, así como a la quercetina, por el nivel de eficacia. Además, la AN de la combinación superó la quercetina en 2,7 veces.

Conclusiones: Por lo tanto, la combinación de NAG/quercetina en la proporción de 1:1 en forma de dosis inyectable es la más prometedora para el tratamiento de enfermedades renales.

Palabras Clave: N-acetilglucosamina; efecto nefroprotector; insuficiencia renal aguda; quercetina.

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INTRODUCTION

Effective treatment of renal diseases is an important issue in the medical and pharmaceutical practice at the present time. Acute kidney injury (AKI) is a common complication not only for kidney diseases and may also be a result of drug therapy, palliative or surgical treatment methods (Gilbert et al., 2018). The prevalence of AKI is 5–10% of all hospitalized patients according to the world statistics (Lerma et al., 2019). AKI is found out more often in critically state patients, reaching the prevalence rates up to 67% (Feehally et al., 2019). Over the last 25 years, there has been an almost 20-times increase in the incidence of AKI, so it can be consumed that this pathology relentlessly spreads (Lerma et al., 2019). The AKI-related death is the most severe complication of this disease and occurs on an average 15% of the total population of the patients with AKI, but it is increase up to 80% among patients in critical state (Feehally et al., 2019). Therefore, the optimization of the treatment of patients with AKI and the search for effective nephroprotective agents is an important task of the pharmaceutical science.

The implementation of the combined drugs based on the membrane protectors and antioxidants of natural origin with different mechanisms of nephroprotective action is a promising approach in the solution of this problem. In view of this, a promising scientific objective is to study the pharmaceutical combinations based on the flavonoid quercetin and the amino sugar N-acetylglucosamine (NAG).

Quercetin is a well-known flavonoid of plant origin with a wide range of pharmacological effects. The antioxidant, antihypoxic, membrane stabilizing, and anti-inflammatory effects are the most significant among its properties (Anand et al., 2016; Li et al., 2016). As a result, quercetin has angioprotective action and reduces the permeability of glomerular capillaries. This complex of effects is useful in the treatment of renal diseases.

NAG is a biologically active form of amino sugar glucosamine (Chen et al., 2010), which is a natural metabolite of humans and virtually safe for the body (Lieberman and Peet, 2018; Baynes and Dominiczar, 2019). Glucosamine performs its physiological effects through the NAG and in this form, it is embedded in glycosaminoglycans and glycoproteins of biological membranes, including the glomerular basement membrane (Morita et al., 2008; Dalirfardouei et al., 2016). Therefore, potentially NAG has a more expressed nephroprotective effect because of the direct mechanism of action.

Based on the peculiarities of the pharmacological properties of quercetin and NAG, the combined drug on their basis is promising for the renal diseases treatment, since both components mutually complement each other's pharmacodynamics with the effects necessary for the therapy of kidney diseases. In the previous experimental studies, the high efficacy of oral combinations of quercetin with some glucosamine derivatives was proved in different models of kidney injury in rats (Shebeko et al., 2018). In this regard, the scientific interest was to study the combined drug in solution for injections, which may be more effective and useful not only in the latent course of kidney diseases, but in acute injuries and exacerbations of chronic nephropathies as well.

Taking into account all the above mentioned, the purpose of present work was to compare the efficacy of various combinations of NAG and quercetin in the injectable dosage forms in rats with AKI.

MATERIAL AND METHODS

Animals

Experimental study was performed using 68 random-bred male albino rats weighing 170-190 g, which were kept on a standard diet with free access to food and water, constant humidity 50%, and temperature 20–25°C, in a well-ventilated room in standard polypropylene cages (Guide, 2011; Sharp and Villano, 2013). All studies were conducted in accordance with EU Council Directive 2010/63/EU dated September 22, 2010 on compliance with the laws, regulations and admin-

istrative provisions of the EU Member States on the protection of animals used for scientific purposes. The experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy, Kharkiv, Ukraine (Approval No. 1 of 20 January 2016).

Test objects

As objects of research, the combinations of NAG and quercetin in injectable dosage forms in ratios from 1:2 to 3:1 were used. These combinations were selected based on the previous studies of the efficacy of oral combinations of quercetin with glucosamine derivatives in rats with kidney injury (Shebeko et al., 2018). To prepare the combinations, NAG was used as a 6% solution for injections, which has been developed and manufactured as a pilot series at PJSC SIC "Borschahivskiy CPP" (Ukraine). Quercetin was used as the medication "Corvitin®" (COR) (PJSC SIC "Borschahivskiy CPP", Ukraine), which is a lyophilized powder for injections. To prepare the combinations immediately prior to use, COR was diluted by solution of NAG to achieve a certain ratio and the 0.9% sodium chloride solution for injections was added to obtain the 20 mg/mL concentration of the sum of active substances. The compositions of test combinations are shown in Table 1.

Reference object

COR was used as a reference drug. It was diluted by the 0.9% sodium chloride solution for injections up to 10 mg/mL concentration immediately prior to use.

Experimental design

All animals were randomly divided into 5 experimental groups as follows.

Group 1 – intact control (healthy animals receiving intramuscularly (i.m.) 0.9% sodium chloride solution at 2.5 mL/kg, n=8).

Group 2 – control pathology (untreated animals receiving (i.m.) 0.9% sodium chloride solution at 2.5 mL/kg, n=10).

Group 3 – animals with AKI treated with COR intraperitoneally (i.p.) at 34 mg/kg (n=10).

Group 4 – animals with AKI treated with NAG/COR 1:2 (i.m.) at 50 mg/kg (n=10).

Group 5 – animals with AKI treated with NAG/COR 1:1 (i.m.) at 50 mg/kg (n=10).

Group 6 – animals with AKI treated with NAG/COR 2:1 (i.m.) at 50 mg/kg (n=10).

Group 7 – animals with AKI treated with NAG/COR 3:1 (i.m.) at 50 mg/kg (n=10).

The AKI was induced by the injection of 50% glycerol solution (Sigma-Aldrich, USA) in the hind limb muscle at a dose of 10 mL/kg. The day before, the animals were deprived of access to drinking water (Bao et al., 2018). After that, the animals were i.m. injected with the test combinations at a dose of 50 mg/kg (2.5 mL/kg), which corresponds to the estimated before effective dose of glucosamine on the model of autoimmune glomerulonephritis (Shebeko and Zupanets, 2006). COR was administered i.p. at a dose of 34 mg/kg, which corresponds to ED_{50} for nephroprotective effect (Shebeko et al., 2018). All test drugs were administered as solutions for injections daily for a week. Animals of control groups received i.m. the equivalent dose of 0.9% sodium chloride solution. A week after the modeling of pathology, the functional state of the kidneys was evaluated. Then, the animals were sacrificed under general anesthesia with ketamine/xylazine (75/10 mg/kg, i.p.) (Flecknell, 2015) to obtain the blood for biochemical assays.

Biological samples preparation and storage

Blood samples were collected from the inferior vena cava and were centrifuged at 1500 g at +4°C for 10 min using refrigerated centrifuge "Eppendorf 5702R" (Eppendorf, Germany). Urine samples were collected using individual metabolic cages and were centrifuged at 500 g for 10 min. The supernatants were separated and used for the biochemical assays. All biological samples were frozen and stored at -80°C until use.

Table 1. Compositions of the test combinations of NAG and COR in the form of solutions for injections.

Test objects	Ratio		Concentration (mg/mL)		Dose (mg/kg)	
	NAG	COR	NAG	COR	NAG	COR
NAG/COR 1:2	1	2	6.7	13.3	16.7	33.3
NAG/COR 1:1	1	1	10.0	10.0	25.0	25.0
NAG/COR 2:1	2	1	13.3	6.7	33.3	16.7
NAG/COR 3:1	3	1	15.0	5.0	37.5	12.5

NAG: N-acetylglucosamine; COR: Corvitin®.

Evaluation of the functional state of the kidneys

At the end of the study, the spontaneous daily diuresis and the volume of consumed water were determined by individual metabolic cages and the relative diuresis was calculated. The urine level of protein and its daily excretion was determined. The glomerular filtration rate (GFR) was evaluated by the endogenous creatinine clearance, the tubular reabsorption and urea clearance were also calculated, using the standard formulas [1-3] (Kamyshnikov, 2013; Gilbert et a1., 2018; Feehally et al, 2019).

$$\text{Creatinine clearance} = U_{cr} \times V / P_{cr} \quad [1]$$

$$\text{Tubular reabsorption} = (1 - P_{cr} / U_{cr}) \times 100\% \quad [2]$$

$$\text{Urea clearance} = U_{ur} \times V / P_{ur} \quad [3]$$

Where U_{cr} is the urine creatinine concentration, V is the daily diuresis, P_{cr} is the plasma creatinine concentration, U_{ur} is the urine urea concentration and P_{ur} is the plasma urea concentration.

Evaluation of biochemical parameters

To evaluate the parameters of nitrogen metabolism, the biochemical assays were performed using "Creatinine FS" (cat. No. 117119910021) and "Urea FS" (cat. No. 131019910021) kits ("DiaSys Diagnostic Systems GmbH", Germany) and the "Express Plus" automatic biochemical analyzer ("Bayer Diagnostics", Germany). The creatinine was determined in the blood and urine by the reaction of Jaffe and the urea was determined using the urease – glutamate dehydrogenase method (Kamyshnikov, 2013). The urinary excretion of creatinine and urea was calculated as well. The concentration of urine protein was determined using "Total protein UC FS" kit (cat. No. 102109910021) by a photometric test based on the reaction with pyrogallol red (Kamyshnikov, 2013).

Calculation of nephroprotective activity

The nephroprotective activity (NA) of the test combinations was evaluated by the total index, which was defined as the arithmetic mean of indicators, representing the influence on three different parameters, which fully reflect the course of nephropathy: GFR, blood urea and urea clearance. These activities were calculated as equation [4].

$$\text{Activity} = (X_c - X_t) / (X_c - X_i) \times 100\% \quad [4]$$

Where X_c is the indicator in the control pathology group, X_i is the indicator in the intact control group and X_t is the indicator in the group treated with the test drug.

Statistical analysis

All the results were processed by descriptive statistics and presented as the mean ± standard error of the mean (SEM) excluding the survival rate. Statistical differences between groups were analyzed using one-way ANOVA followed by Dunnett`s post-hoc test and using Fisher's exact test for survival analysis (Quirk et al., 2015; Islam and Al-Shiha, 2018). Utilized computer software included IBM SPSS Statistics v. 22 (IBM Corp.) and MS Excel 2016 (Microsoft Corp.). The level of statistical significance was considered as $p<0.05$.

RESULTS

A week later after the modeling of glycerol nephrosis, a high mortality rate was observed in the control pathology group, with only 50% animal survived (Fig. 1). Rats were in poor functional state, with reduced motor activity, edema and ascites.

At the same time, there was a significant decrease ($p<0.05$) in relative diuresis, GFR and tubular reabsorption compared to the intact animals. In addition, untreated animals represented the pronounced proteinuria, which reached about 12 g/L (Table 2).

The deterioration of the renal function led to the development of azotemia. The creatinine and urea blood levels were 3.8 and 3.7 times higher ($p<0.05$) than in intact rats, respectively. At the same time, the urea clearance was significantly ($p<0.05$) decreased by 4.5 times (Table 3).

The similar changes were observed in the nitrogen compounds elimination: the creatinine and urea excretion were significantly decreased ($p<0.05$) by 14.4% and 21.9%, respectively (Fig. 2). The described state suggests an impairment of the renal excretory function and the development of AKI.

The COR provides positive effect on the course of AKI. Under its influence the animal survival rate was increased to 70%, which, however, was insignificant ($p>0.05$ *versus* intact control group) (Fig. 1). There was a significant increase ($p<0.05$) in the excretory renal function compared to untreated animals. In this case, the relative diuresis was increased by 25.7%, GFR – by 75.2%, and tubular reabsorption – by 0.76%. Also, there was a significant decrease ($p<0.05$) in proteinuria by 93.5% (Table 2). In addition, COR exerted the hypoazotemic action, significantly reducing ($p<0.05$) the creatinine and urea blood levels by 44.8% and 25.0%, respectively, and increasing the urea clearance by 97.9% (Table 3). The creatinine and urea excretion were 1.2 and 1.6 times higher, respectively (Fig. 2). These data allowed to calculate the NA, which was 25.8% (Fig. 3).

When combining NAG and COR in the ratio of 1:2, there was an insignificant increase in the efficacy of the last one. There was no significant difference between animals received this combination and COR-treated group. But the combination significantly increased ($p<0.05$) renal excretory function, decreased proteinuria, blood creatinine and urea level compared to untreated animals (Table 2, 3). The NA index was 30.5% (Fig. 3).

An increase in quantity of NAG in combination with COR up to 1:1 ratio caused expressed increase in efficacy in contrast with previous group. Under the influence of the combination the mortality disappeared, and the survival rate was equal to the intact group – 100% (Fig. 1). The excretory renal function was significantly enhanced ($p<0.05$) compared to control pathology group: the relative diuresis was increased by 35.8%, tubular reabsorption by 1.8%, and GFR index was 3.2 times higher. The proteinuria level was 4.1 times lower ($p<0.05$) than that in untreated animals (Table 2).

The NAG/COR combination (1:1) provided positive influence on the nitrogen metabolism. The combination significantly increased ($p<0.05$ *versus* control pathology group) the urinary excretion of creatinine and urea by 43.2% and 79.5%, respectively (Fig. 2). As a result, the blood creatinine and urea levels were 2.2 and 2.0 times lower, respectively. There was a significant increase ($p<0.05$) in urea clearance by 3.7 times (Table 3). This group showed the NA of 69.7% (Fig. 3).

The increase of NAG level relative to COR in the test combinations in ratios of 2:1 and 3:1 showed a reverse trend that was manifested in the lowering efficacy of combinations. Thus, the animal survival rate under the influence of both combinations was 80%, which was insignificant ($p>0.05$) compared to untreated animals (Fig. 1). There was a significant increase ($p<0.05$ *versus* control pathology group) in the excretory renal function. The relative diuresis was increased by 20.9% when the NAG/COR (2:1) has been applied and by 17.0% under the influence of NAG/COR (3:1). The GFR was 2.2–2.3 times higher, and the tubular reabsorption index was increased by 1.3%. In addition, both combinations contributed to a

significant decrease ($p<0.05$) in proteinuria (Table 2).

Both these combinations significantly reduced ($p<0.05$ *versus* untreated animals) the nitrogen compounds blood level: creatinine by 70.1–75.5% and urea by 55.7–58.3% (Table 3). Moreover, the creatinine and urea excretion indices were significantly increased ($p<0.05$) compared to control pathology group (Fig. 2). The NA index was 45.1% for the combination in 2:1 ratio and 42.5% for the combination in 3:1 ratio (Fig. 3).

Table 2. Influence of NAG/COR combinations on excretory renal function in rats with AKI.

Groups	Relative diuresis (%)	GFR (mL/day)	Tubular reabsorption (%)	Proteinuria (g/L)
Intact control (n=8)	52.0 ± 0.4	420.3 ± 15.2	98.44 ± 0.05	0.21 ± 0.01
Control pathology (n=5)	35.8 ± 0.6[a]	95.9 ± 3.4[a]	96.39 ± 0.12[a]	11.96 ± 0.27[a]
COR (n=7)	45.0 ± 0.2[abd]	168.0 ± 4.3[abd]	97.12 ± 0.10[ab]	6.18 ± 0.58[abd]
NAG/COR 1:2 (n=7)	45.6 ± 0.4[abd]	174.8 ± 4.1[abd]	97.24 ± 0.07[abd]	5.94 ± 0.55[abd]
NAG/COR 1:1 (n=10)	48.6 ± 0.5[abc]	304.0 ± 8.2[abc]	98.12 ± 0.07[abc]	2.90 ± 0.17[abc]
NAG/COR 2:1 (n=8)	43.3 ± 0.6[abd]	218.0 ± 7.0[abcd]	97.65 ± 0.08[abcd]	5.38 ± 0.43[abd]
NAG/COR 3:1 (n=8)	41.9 ± 0.4[abcd]	206.7 ± 7.1[abcd]	97.61 ± 0.11[abcd]	5.18 ± 0.48[abd]

Data are expressed as mean ± SEM (n is the number of animals at the end of experiment). [a]$p<0.05$ compared to intact control group, [b]$p<0.05$ compared to control pathology group, [c]$p<0.05$ compared to COR-treated group, [d]$p<0.05$ compared to group treated with NAG/COR 1:1 (ANOVA, Dunnett's post-hoc test). GFR: Glomerular filtration rate, COR: Corvitin®, NAG: N-acetylglucosamine.

Table 3. Indicators of nitrogen metabolism in rats with AKI under the influence of NAG/COR combinations.

Groups	Blood creatinine (μmol/L)	Blood urea (mmol/L)	Urea clearance (mL/day)
Intact control (n=8)	56.30 ± 1.80	5.1 ± 0.3	175.1 ± 5.4
Control pathology (n=5)	215.16 ± 8.21[a]	19.0 ± 0.8[a]	38.4 ± 1.7[a]
COR (n=7)	148.57 ± 6.23[abd]	15.2 ± 0.6[abd]	76.0 ± 2.4[abd]
NAG/COR 1:2 (n=7)	143.01 ± 4.99[abd]	14.2 ± 0.3[abd]	83.1 ± 2.2[abcd]
NAG/COR 1:1 (n=10)	97.49 ± 3.02[abc]	9.3 ± 0.4[abc]	141.5 ± 4.5[abc]
NAG/COR 2:1 (n=8)	122.57 ± 2.97[abcd]	12.0 ± 0.5[abcd]	102.6 ± 3.2[abcd]
NAG/COR 3:1 (n=8)	125.89 ± 3.09[abcd]	12.2 ± 0.4[abcd]	98.9 ± 3.3[abcd]

Data are expressed as mean ± SEM (n is the number of animals at the end of experiment). [a]$p<0.05$ compared to intact control group, [b]$p<0.05$ compared to control pathology group, [c]$p<0.05$ compared to COR-treated group, [d]$p<0.05$ compared to group treated with NAG/COR 1:1 (ANOVA, Dunnett's post-hoc test). COR: Corvitin®, NAG: N-acetylglucosamine.

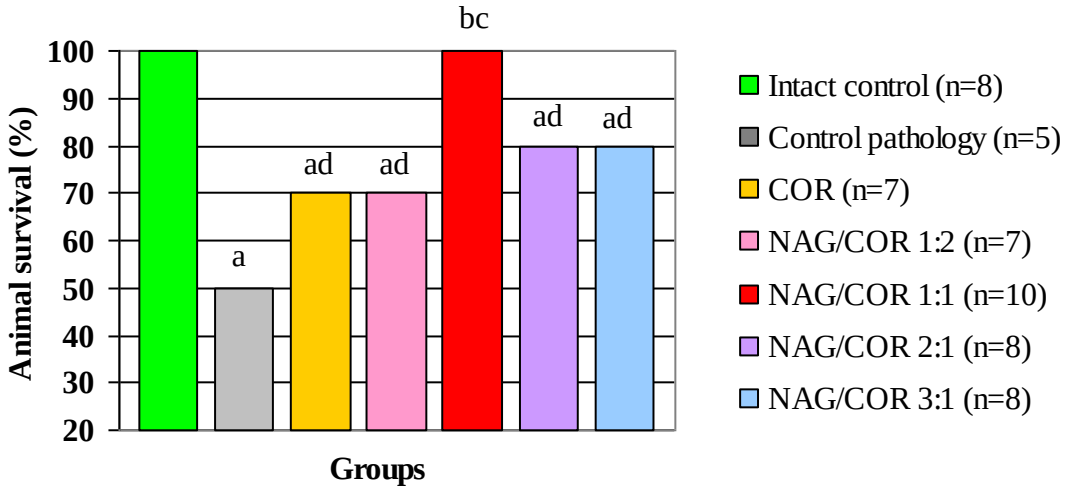

Figure 1. Influence of NAG/COR combinations on the survival of rats with AKI.

Data are presented as the percentage of animals survived in each group (n is the number of animals at the end of experiment). [a]p<0.05 compared to intact control group, [b]p<0.05 compared to control pathology group, [c]p<0.05 compared to COR-treated group, [d]p<0.05 compared to group treated with NAG/COR 1:1 (Fisher's exact test). COR: Corvitin®, NAG: N-acetylglucosamine.

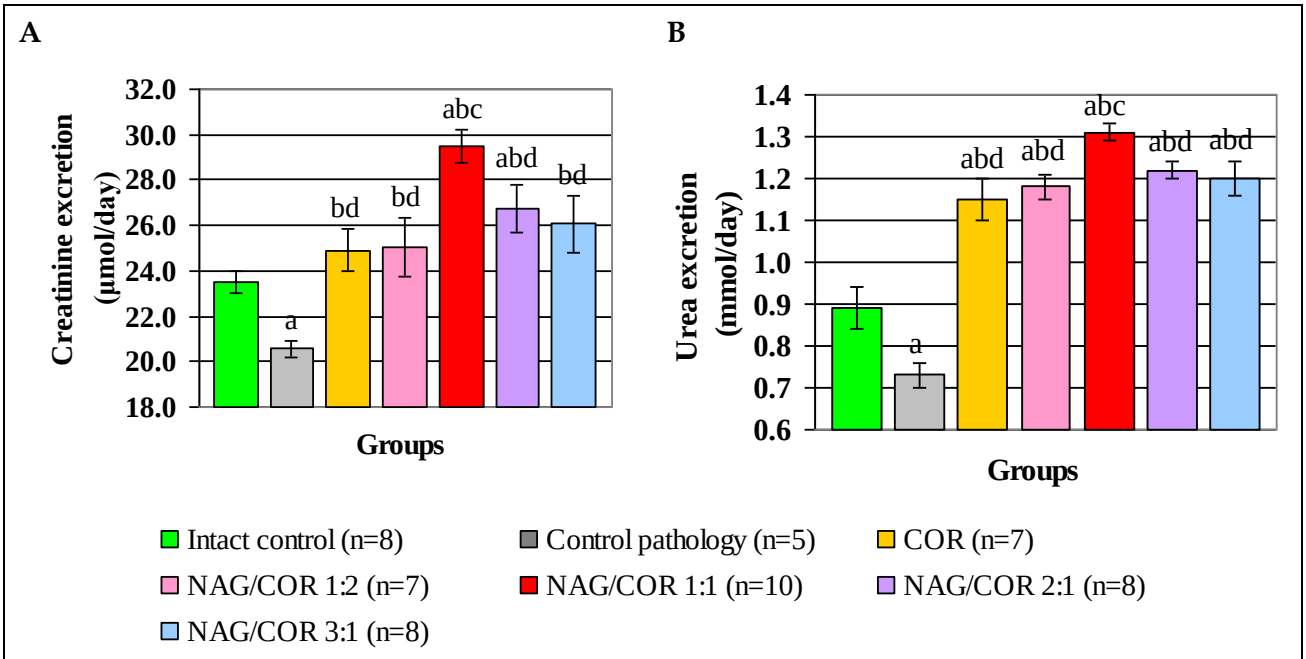

Figure 2. Urinary excretion of creatinine **(A)** and urea **(B)** under the influence of NAG/COR combinations in rats with AKI.

Data are expressed as mean ± SEM (n is the number of animals at the end of experiment). [a]p<0.05 compared to intact control group, [b]p<0.05 compared to control pathology group, [c]p<0.05 compared to COR-treated group, [d]p<0.05 compared to group treated with NAG/COR 1:1 (ANOVA, Dunnett's post-hoc test). COR: Corvitin®, NAG: N-acetylglucosamine.

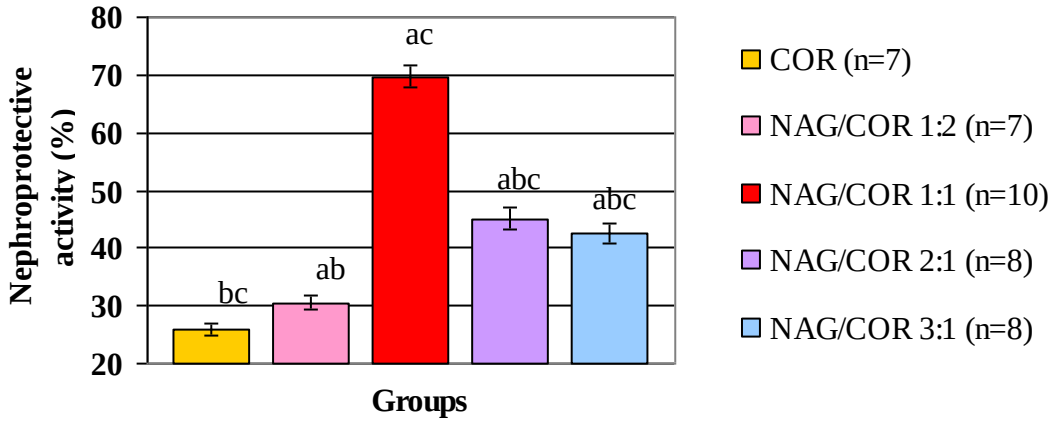

Figure 3. Total nephroprotective activity of NAG/COR combinations in rats with AKI.

Data are expressed as mean ± SEM (n is the number of animals at the end of experiment). [a]p<0.05 compared to COR-treated group, [b]p<0.05 compared to group treated with NAG/COR 1:1, [c]p<0.05 compared to group treated with NAG/COR 1:2 (ANOVA, Dunnett's post-hoc test). COR: Corvitin®, NAG: N-acetylglucosamine.

DISCUSSION

The results of experiments indicate that in the group of untreated animals severe AKI develops within a week under the influence of glycerol. This is evidenced by persistent oliguria, water retention and a sharp decline in excretory renal function. It results in the deterioration of the residual nitrogen elimination, development of azotemia and autointoxication in rats. This leads to a high mortality rate up to 50%.

The described state is typical to acute glycerol nephrosis. The key element for its induction is the i.m. administration of glycerol in the setting of water consumption limiting, which leads to severe rhabdomyolysis. As a result, a large amount of myoglobin reaches the kidneys and causes toxic and ischemic renal injury (Bao et al., 2018; Feehally et al., 2019).

Under the influence of the quercetin, which was used as COR, there was a positive effect on the course of AKI. There was an increase in the excretory renal function, elimination of nitrogen compounds and decrease in their blood level. This led to an improvement in the physiological state of rats and reduced their mortality to 30%. These results are expected, because the efficacy of quercetin has been confirmed in experimental studies on various models of kidney injury in different dosage forms, including those for injections (Layal et al., 2017; Shebeko et al., 2018; Yang et al., 2018; Vargas et al., 2018).

But the degree of COR efficacy should be considered inadequate, since the NA was only 25.8%, and it was the lowest result in the study. The insufficient efficacy of quercetin is due to the fact that its influence on the kidneys are indirect and develops as a result of antioxidant, antihypoxic, anti-inflammatory and other effects (Anand et al., 2016; Li et al., 2016). Quercetin does not have a direct protective influence on the damaged structures of the renal tissue. In this regard, it exhibits insufficient hypoazotemic action and a poor effect on glomerular hemodynamics. But despite this, its pharmacodynamics is definitely beneficial in the treatment of renal diseases (Shebeko et al., 2018).

All mentioned above resulted in the necessity for correction of the pharmacological effects of quercetin to increase the possibility of its use in kidney diseases. In this case, it was reasonable to increase the nephroprotective and hypoazotemic activity of the new drug by the expansion of the mechanisms of action and a wider range of phar-

macodynamics, which is useful in the renal diseases treatment. Among the required properties of the medication, the direct effect on the structure of the renal tissue, the ability to compensate for the deficiency of the damaged basement membranes macromolecules and the increase of nephrocytes survival under pathologically altered kidney should be presented.

As a result of the analysis of the pharmacological properties of substances of natural origin, it was proposed to improve the pharmacodynamics of quercetin by creating a combined medication with NAG. This amino sugar has a direct protective influence on membrane structures and intercellular substance of the kidneys (Morita et al., 2008; Dalirfardouei et al., 2016). The nephroprotective properties of glucosamine derivatives were proved in the experimental studies (Shebeko and Zupanets, 2006; Zupanets and Shebeko, 2006a;b). It was shown that glucosamine is embedded into the damaged structures of the renal tissue and increases the content of endogenous hexosamines. These results correlate with another studies, which showed the efficacy of glucosamine in the treatment of kidney fibrosis in mice (Park et al., 2013), contrast-induced acute kidney injury in rats (Hu et al., 2017) and the efficacy of its conjugates in rats with renal ischemia/reperfusion injury (Wang et al., 2014; Fu et al., 2016).

The experimental data have shown that combining NAG with quercetin increases the efficacy of the last one in AKI, which depends on the ratio of active ingredients of the combination. The most expressed nephroprotective and hypoazotemic properties were observed in the NAG/COR combination in ratio of 1:1. Under the influence of this combination, the functional state of rats was restored, cases of mortality disappeared, GFR and urea clearance were reliably increased and the nitrogenous substances blood level was decreased compared to untreated animals. This indicates the normalization of excretory renal function and nitrogen metabolism in rats. In this case, the NA of the combination was 69.7%, which was the highest value in the presented study. It should be noted that this combination was significantly superior to all others, as well as the comparator COR, in particular, the NA of the combination was 2.7 times higher than in COR-treated group.

The high efficacy of the proposed combination is due to the fact that quercetin and NAG have nephroprotective effects with different mechanisms of action. This is the basis of their potentiation effect, as proved by the analysis of NA indices. The injection route of administration for both components adds advantages as well, since it allows to neutralize the effect of first-pass metabolism and ensure that the total dose of the active substances inflow to the blood circulation and renal tissue in the unchanged form.

In addition to expressed nephroprotective action, the new combined medication is characterized by high hypoazotemic activity. This is due to the improvement of intraglomerular hemodynamics under the influence of both components – quercetin and NAG, which leads to increased excretion of nitrogen metabolism products with urine as a result of filtration processes. All mentioned above proved the positive effect of the proposed combination on the course of renal failure.

CONCLUSIONS

This study shows that the combining of NAG and quercetin in the solution for injections results in a significant increase in nephroprotective and hypoazotemic activities under the development of AKI and provides an expressed positive effect on the course of nephropathy.

The combination of NAG and COR in ratio of 1:1 credible exceeds the COR effect for a separate administration and the efficacy of combinations with another ratios. This combination in injectable dosage form is a very promising medication for the treatment of AKI, as well as exacerbations of chronic kidney disease. It is expedient to study this combination in further experiments at i.m. administration to ground the use in the treatment of renal diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Shebeko SK	Zupanets IA	Propisnova VV
Concepts or ideas	x	x	
Design	x	x	
Definition of intellectual content	x	x	x
Literature search	x		x
Experimental studies	x	x	
Data acquisition	x		x
Data analysis	x	x	
Statistical analysis	x		x
Manuscript preparation	x		x
Manuscript editing	x	x	
Manuscript review	x	x	x

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