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Research Article

Assessment of pharmacological activity and bioavailability of the new derivative 1,3,4-thiadiazole

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Abstract

Introduction: Acexazolamide is a new derivative of 1,3,4-thiadiazole and acexamic acid.

Materials and methods: In animal experiments, acute toxicity, pharmacological activity and bioavailability of acexazolamide were evaluated. Anti-inflammatory activity was assessed on the model of formalin edema of paw and cotton pellet granuloma in rats. Assessment of analgesic activity was carried out in a hot plate test in mice, chemical pain stimulation of the peritoneum and inflammatory hyperalgesia in rats. The antipyretic activity of acexazolamide was evaluated in a model of yeast-induced hyperthermia in rats. The anti-burn activity of acexazolamide was evaluated in a thermal skin burn model in rats. Bioavailability of acexazolamide with intragastric administration was determined in rabbits. The content of acetaxazolamide in blood plasma was determined by HPLC-MS/MS method.

Results and discussion: DL_{50} of acexazolamide after intragastric administration to mice was 860.99 (95% CIs, 462.2 to 1259.8) mg/kg. The anti-inflammatory activity of acexazolamide (21.5 mg/kg) with formalin-induced paw edema and fetal granuloma in rats was higher than that of ketoprofen (23.0 mg/kg). ED_{50} value for analgesic activity with acetic acid induced cortex was 24.99 (95% CIs: 15.31–34.68) mg/kg. With thermal stimulation of the paw in mice, the ED_{50} value was 25.56 (95% CIs: 15.13 to 35.98) mg/kg. ED_{50} value for antipyretic activity was 31.85 (95% CIs: 19.22–44.47) mg/kg. Acexazolamide (21.5 mg/kg) had a stimulating effect on the regeneration of damaged tissues in case of thermal skin burns. Bioavailability of acexazolamide (1 mg/kg) with intragastric administration was 37%.

Conclusion: Acexazolamide has the properties of a non-steroidal anti-inflammatory agent.

Keywords

nonsteroidal anti-inflammatory agents, 1,3,4-thiadiazole derivatives, acexamicacid.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are currently used worldwide (Serhan 2017, Rayar et al. 2017). Many NSAIDs cause a number of serious adverse reactions, including erosive and ulcerative lesions of the gastrointestinal tract, marked changes in the function of the liver, kidneys, etc. (Weintraub 2017). The emergence of combined NSAIDs with components of other pharmacological groups (proton pump inhibitors, H_2-histamine receptor blockers, prostaglandins, etc.) made it possible to reduce the damaging effect on the gastrointestinal mucosa. However, such combination drugs had a number of side effects, non-specific for NSAIDs. The appearance of specific

inhibitors of cyclooxygenase-2 could not completely solve the problem of the safety of NSAIDs either (Serhan 2017, Bjarnason et al. 2018, Grosser et al. 2017). In connection with this, it is now urgent to search for new anti-inflammatory drugs with an improved safety profile, including those with nonulcerogenicity. The properties of NSAIDs can be found in drugs with different chemical structures. Among them are acid derivatives (indoleacetic, phenylacetic, propionic, salicylic), oxycam, coxibes, and sulfoanilides. Thiadiazole derivatives are one of the promising groups for creating new drugs (Aday et al. 2018, Aliabadi et al. 2017, Djukic et al. 2018, Gomha et al. 2017, Haider et al. 2015, Kumari et al. 2017, Matysiak 2015, Pal et al. 2014, Zhong et al. 2017), including non-steroidal anti-inflammatory medicines (Altıntop et al. 2016, Karki et al. 2015, Sharma et al. 2008). It is known that replacement of the carboxyl group in the structure of a number of NSAIDs (indomethacin, ketoprofen, ibuprofen) with thiadiazole leads to a significant decrease in ulcerogenicity without a change in the pharmacological activity (Kazaishvili and Popov 2013). In addition, presence of thiadiazole nucleus increases lipophilicity of the compounds, thereby improving their pharmacokinetic properties, as well as increasing their selectivity towards cyclooxygenase-2 (Raj et al. 2018, Altıntop et al. 2016, Banerjee et al. 2016). In animal experiments, it was demonstrated that a number of new derivatives of 1,3,4-thiadiazole and organic acids (picoline, oxoethane, propionic and others) exhibit properties of non-steroidal anti-inflammatory medicines, while with low toxicity and low ulcerogenicity. However, in the literature there are no references to the pharmacological activity of amino acid derivatives of thiadiazole. In this regard, it is relevant to study pharmacological activity of thiadiazole derivatives with acexamic acid, known for its healing and minor anti-inflammatory properties (Kim et al. 2013, Popov et al. 2017).

Materials and methods

Experimental animals

A total of 312 male and female Wistar rats, weighing 180–220 g; 72 male and female SHK mice, weighing 19–23 g; 16 inbred male Balb/C mice, weighing 21–23 g; 6 male chinchilla rabbits weighing 3.2–3.4 kg. The animals were quarantined and acclimatized to the laboratory conditions for 14 days prior to the start of the experiment. The animals were observed for their general health condition and suitability for testing during this period. They were maintained at a constant room temperature (22±2 °C) under a 12-h light-dark cycle (light on from 8 am to 8 pm). Food and water were available ad libitum. The animals were kept under the standard conditions corresponding to "The Sanitary Regulations on Organizing, Equipping and Maintaining Experimental Biological Clinics (Vivariums) "No. 1045–73, approved by the USSR Chief State Sanitary Officer on 06.04.73 and GOST R 53434–2009. All the experiments were carried out in compliance with "The European Convention on Protection of Vertebrates Used for Experimental and Other Scientific Purposes" (Directive 2010/53/EU). All the protocols were approved by the Ethics Committee of Tver State Medical University of the Russian Ministry of Healthcare.

Chemicals and drugs

Acexazolamide (2-(5-aethyl-1,3,4-thiadiazolil) amide of acexamic acid) (All-union Scientific Center for the Safety of Biologically Actice Substances, Staraya Kupavna, Moscow region) (Figure 1); Indomethacin (JSC Biosintez, Russian Federation), Ketoprofen (Lek, Slovenia), Prednisolone (Agio pharmaceuticals, India), Formalin, Acetic acid (Base of chemicals № 1, Russian Federation).

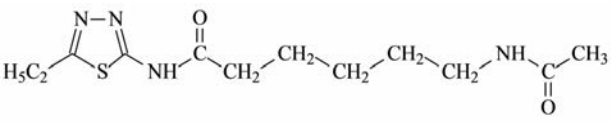


Figure 1. Chemical structure of acexazolamide (2-(5-aethyl-1,3,4-thiadiazolil) amide of acexamic acid)

The structure of acexazolamide is confirmed by spectral characteristics, including mass spectra of the I (Figure 2) and II orders (Figure 3).

The individuality of acexazolamide is confirmed by the results of high-performance liquid chromatography.

Synthesis of acexazolamide

A 100 ml of acetonitrile, 12.91 g (0.1 m) of 2-amino-5-ethyl-1,3,4-thiadiazole was charged into a three-necked flask equipped with a stirrer, thermometer and condenser. The mass was stirred, gradually adding 16.65 g (0.1 m) of N-acetylaminohexanoic acid chloride; then it was heated to boiling. Three 3 hours later, acetonitrile was distilled off; 150 ml of distilled water was added, mixed thoroughly, and heated to 80 °C; 15–20 minutes later, the precipitate was filtered off, washed on the filter by distilled water to reach pH 6–7 of washing water, and then dried until it reached its constant weight at 100–105 °C.

Drug administration

Acexazolamide was administered to the experimental animals as an aqueous suspension of Tween-80 intragastrically through a metal probe. The volume of the administered drug did not exceed 3 ml for rats and 1 ml for mice. The concentration of the suspension provided the administration of the drug in the required dose at the optimum volume.

Acute toxicity evaluation

The groups of 6 mice received doses of 500, 1000, 1500 and 2250 mg/kg of acexazolamide intragastrically. The groups were observed for 14 days and at the end of this

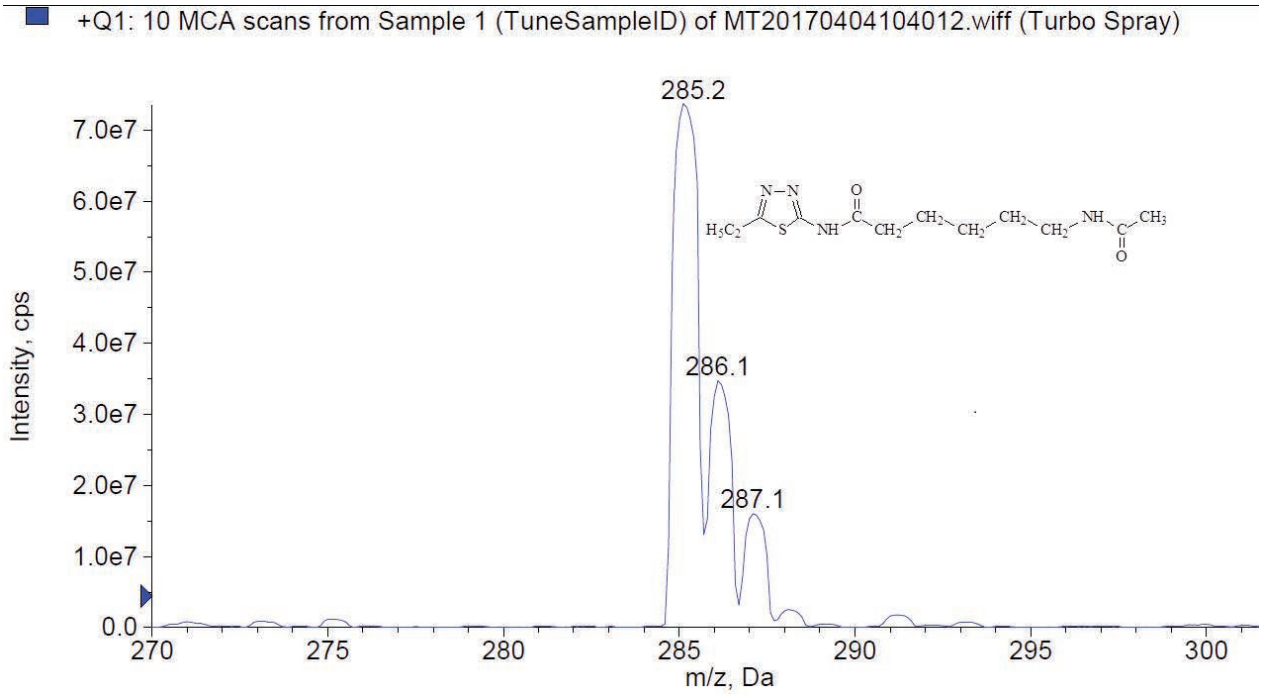
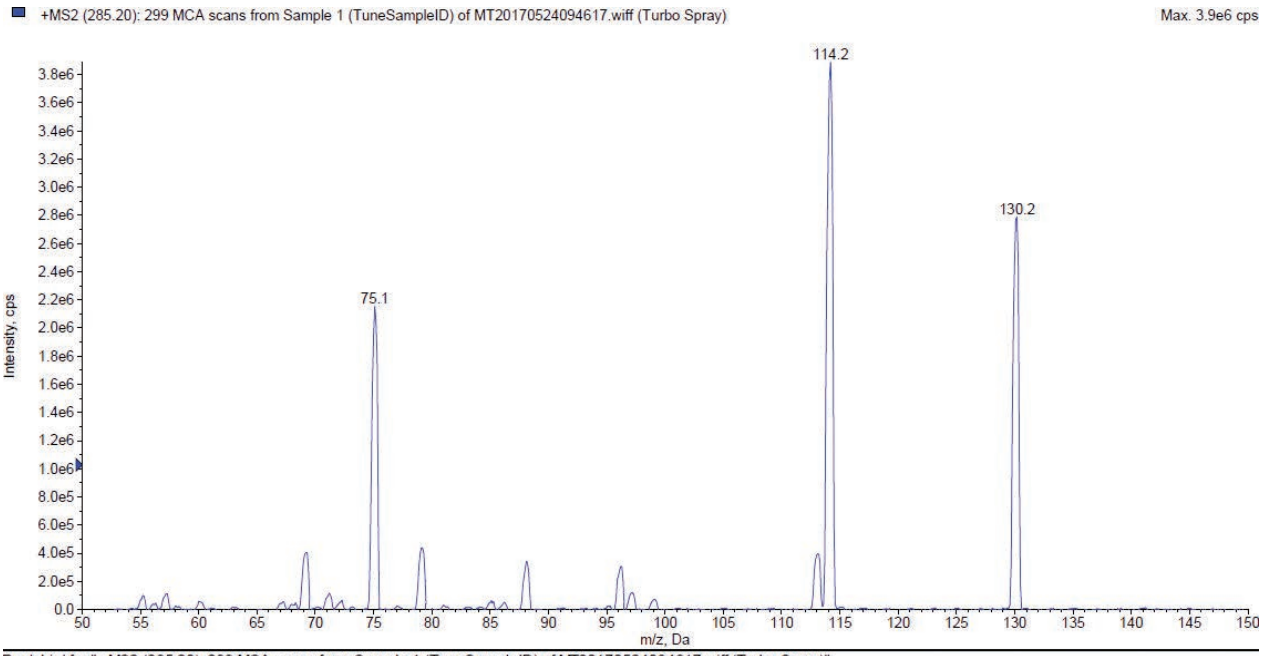


Figure 2. Mass spectrum (I order) of the protonated molecule of acexazolamide (in the positive ion scan mode $[M+H]^+$)



Peak List for " +MS2 (285.20): 299 MCA scans from Sample 1 (TuneSampleID) of MT20170524094617.wiff (Turbo Spray)"

	m/z (Da)	Centroid mass (Da)	Resolution	Intensity (cps)	Peak area (counts)	Charges	Peak start (Da)	Peak end (Da)	Width (Da)	%Intensity
1	75.1000	75.1129	137.4186	2.1532e6	1.1544e7	Undefined	74.4000	75.5000	0.5466	55.3811
2	114.2000	114.1591	190.5049	3.8879e6	2.2197e7	Undefined	113.5000	114.6000	0.5992	100.0000
3	130.2000	130.1177	227.1122	2.7868e6	1.5555e7	Undefined	129.2000	130.5000	0.5729	71.6787

Figure 3. Mass spectrum (I order) of the ion products of acexazolamide (in the positive ion scan mode, precursor ion *m/z* 285.2 Da)

period mortality was recorded for each group. DL_{50} value was determined by means of Litchfield and Wilcoxon probit analysis method modified by V.B. Prozorovsky. The hazard class of the studied compound was determined by the value of DL_{50} in accordance with GOST 12.1.007–76 and the classification of Hodge and Sterner.

Ulcerogenic activity

Acute ulcerogenic activity

The experiments were performed on Wistar rats weighing 194.4±6.5 g, which were divided into 2 groups of 8 animals each. Acexazolamide (86.1 mg/kg) and indometha-

cin (10.0 mg/kg) were administered as water suspension intragastrically to the animals which had been kept unfed prior to the trial. Three hours later, rats were euthanized; and their stomachs were removed and opened along the lesser curvature, washed with normal saline to remove the contents. Then visual evaluation of the gastric musoca condition was carried out. The ulcerogenic activity of the compounds studied was evaluated by Pauls index (PI).

$$PI = M \times N/100$$

PI – Pauls index; M – the mean number of ulcers in the group per one rat; N – the percentage of animals with ulcers.

Subchronic ulcerogenic activity

The subchronic ulcerogenic of acexazolamide and indomethacin was determined using Kulkarni's method (1993). The experiments were performed on Wistar rats weighing 194.4±6.5 g, which were divided into 2 groups of 8 animals each. The rats were intragastrically given acexazolamide (21.5 mg/kg) or indomethacin (2.5 mg/kg) for 4 days. On day 5, the rats were anaesthetized with ether and killed by decapitation, then their stomachs were removed and opened along the lesser curvature according to the method given. The opened stomach was washed with normal saline, and damage to the gastric mucosa was studied. The ulcerogenic activity of the compounds under study was evaluated by Pauls index.

Anti-inflammatory activity

Formalin-induced edema

The experiments were performed on male and female Wistar rats weighing 190–200 g, which were divided into groups of 8 animals each. Anti-inflammatory activity was assessed by the degree of inhibition of paw edema induced by the injection of 0.1 ml of 2% formalin-water solution (an edematogenic agent) into the subplantar region of the right hind paw of the rat. Edema intensity was evaluated by measuring paw thickness of the experimental animal prior to the formalin injecton and at 2, 4, 6, 24 and 48-h intervals after the administration of formalin using an electronic calliper (Vorel 15240, Poland). Acexazolamide at different doses (4.3 mg/kg, 21.5 mg/kg, 43.0 mg/kg and 86.1 mg/kg) and ketoprofen (23.0 mg/kg) were administered intragastrically 1 h before formalin injection.

Cotton pellet granuloma

In this experiment, the effect of acexazolamide and ketoprofen on proliferative and exsudative phases of inflammation was investigated employing cotton pellet granuloma method. The experiments were performed on outbred male and female Wistar rats weighing 194.4±6.5 g. The rats were anesthetized with diethyl ether. Then cotton pellets, weighing 10±1 mg each, were implanted on both sides in scapular region under sterile condition. Acexazolamid (21.5 mg/kg) and ketoprofen (23.0 mg/kg) were administered daily for seven consecutive days as Tween-80 suspension. On the 8th day, the rats in all groups were sacrificed with a high dose of anesthesia. The pellets surrounded by granuloma tissues were dissected out and kept overnight for incubation at 37 °C. The pellets were then dried at 60 °C until they reached constant weight. The weight of wet pellets was also recorded after sacrificing the rats. The average weights of the granulomas were calculated for the rats of the control group and of other treated groups. The percentage change of granuloma weights was calculated for all the test groups by comparing with that of the control group.

Analgesic activity

Acetic acid-induced abdominal constrictions

Analgesic activity of acexazolamide was evaluated by the test of abdominal writhing induced by acetic acid in rats. The experiments were performed on outbred male and female Wistar rats weighing 190–200 g, which were divided into 6 groups of 8 animals each. The experimental animals were injected intraperitoneally with 0.1 ml of 0.75% acetic acid, after which the number of writhings was counted for 15 minutes. Acexazolamide (4.3 mg/kg, 21.5 mg/kg, 43.0 mg/kg and 86.1 mg/kg) and ketoprofen (23.0 mg/kg) were administered intragastrically 1 hour prior to the injection of acetic acid. The control group animals received an isotonic sodium chloride solution.

Hot-plate model

The experiments were performed on white non-inbred male and female SHK mice weighing 21.5±2.0 g. The mice were divided into 6 groups of 8 animals each. The experimental animals were placed on a hot aluminum plate at a temperature of 55±0.5 °C for a maximum time of 30 s. The latent period was recorded before mice showed any pain reaction (jumping or licking limbs) 30, 60 and 120 minutes after the administration of acexazolamide (8.6mg/kg, 43.1mg/kg, 86.1mg/kg, 172.2 mg/kg) and ketoprofen (46.0 mg / kg).

Randall & Selitto test (paw-pressure test)

Hyperanalgesia was induced by the subplantar administration of 0.1 ml of 2% formalin into the rat's hind paw, after which a pain threshold was measured by putting pressure on the paw (Randall and Selitto 1957). In the Randall & Selitto test, an analgesy-meter with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the plantar surface of the paw, was used. The pain threshold was expressed in grams. The moment of a pain reaction was determined by pulling the animal's paw from the analgesimeter. A cut-off value

of 300 g was used to prevent damage to the paws. Rats were divided into 6 groups of 8 animals in each. Acexazolamide at different doses (4.3, 21.5, 43.0 and 86.1 mg/kg) and ketoprofen (23.0 mg/kg) were administered intragastrically 2 h after formalin injection.

Antipyretic activity

Yeast-induced hyperthermia model was performed in male and female Wistar rats weighing between 204.1±9.2 g following the method of Teotino et al. (1963). The rats were divided into 3 groups of 8 animals each. The rectal temperatures were recorded with an electric thermometer (Citizen CT461C). Hyperthermia was induced by subcutaneous injection of yeast (20% water suspension). The body temperatures were recorded again 18 h later. The rats with the temperature increasing over 1 °C were orally administered acexazolamide at different doses (8.6mg/kg, 43.1mg/kg, 86.1mg/kg, 172.2 mg/kg) and ketoprofen (23.0 mg/kg). The rectal temperatures were then recorded 1, 2, 3, 4, 5, 6 and 7 h after the administration of the drugs.

Active Cutaneous Anaphylactic (ACA) Reactions

Balb/C mice were sensitized by subcutaneous injection of 1 ug of ovalbumin in 0.2 ml of normal saline with vaseline oil. Twenty-one days later, ACA reactions were induced by intradermal injection of 0.05 ug of ovalbumin. Simultaneously, 0.25 ml of 1.6% Evans blue solution was administered intravenously. Twenty min later, acexazolamide and prednisolone were administrated as well. One hour later, 0.05 ug of ovalbumin was injected intradermally into a different skin area. The mice were euthanized 20 min later, after which the area of the spots on the skin internal surface caused by Evans blue diffusion was measured.

Anti-burn activity

Evaluation of the anti-burn activity of acexazolamide was carried out on the model of thermal skin burn in rats. A thermal injury was caused with a steel stencil (surface area – 225 mm^2, incandescence temperature – 240 °C, exposure time –14 s, force – 1.6 N). The area of the skin defect was daily determined; the burns were evaluated visually; the presence and nature of the scab, as well as an eschar were examined, and the complete healing of the defects was timed. Prior to the beginning of the study, and also on the 5^{th}, 10^{th} and 15^{th} day, biopsy of the wound edges with the areas of intact skin adjacent to the defect area was performed. The histological sections were stained with hematoxylin and eosin. With the help of an eyepiece micrometer, the biometrics of the sections of the resultant tissues was carried out: the thickness of the eschar, of the granulation tissue, of the border zone of the epithelium, of the leukocyte shaft, and the length of the epithelial wedge.

Determination of acexazolamide in blood plasma by HPLC-MS/MS method

Determination of acexazolamide in blood plasma of rabbits was carried out by HPLC-MS/MS method (Popov et al. 2017, Demidova et al. 2018). Chromatography was performed with a high-performance liquid chromatograph Agilent 1260 Infinity II (Agilent Technologies, Germany) and an analytical column Agilent InfinityLab Poroshell 120 EC-C18 2.7 μm 4.6×100 mm. As a mobile phase, a mixture of acetonitrile and deionized water at the ratio 30:70 with adding 0.1% formic acid in the isocratic mode; the flow rate of the mobile phase was 0.6 ml/min. For mass spectrometry, an AB SciexQTrap 3200 MD triple quadrupole mass spectrometer (AB Sciex, Singapore) was used with an electrospray ion source (Turbo V with a TurboIonSpray probe). Calibration of the mass spectrometer was carried out using a test solution of reserpine at a concentration of 6.1×10^{-2} mg/L. For mass spectrometric identification of acexazolamide, MRM transitions were used in the positive ion recording mode. The MRM values were *m/z* 285.2 → *m /z* 75.1; *m/z* 114.2 and *m/z*130.2. The detection limit of acexazolamide in rat plasma was 0.25 ng/ml. The application range of the procedure was from 1 ng/ml to 1000 ng/ml.

Validation of liquid chromatography-tandem mass spectrometry (HPLS-MS-MS) method for detection of acexazolamide in blood plasma

The HPLS-MS-MS method was validated by the parameters of selectivity, accuracy, precision, linearity, cross-transfer, and stability (EMA 2010).

Evaluation of bioavailability

Pharmacokinetic studies were performed using 6 rabbits of the Chinchilla breed. The study was conducted using an open, randomized, cross-sectional scheme. The washout period for acexazolamide between the stages was 7 days. Acexazolamide was administered intravenously at a dose of 1 mg/kg in a 0.33% solution of dimexide or intragastrically at a dose of 1 mg/kg in 20 ml of 2% starch mucus. The duration of the monitoring of the concentration of acexazolamide in blood plasma exceeded the elimination half-life period ($T_{1/2}$) 5 times on average. The blood was centrifuged for 10 minutes at a rate of 3000 rpm; the resulting blood plasma was stored at -40 °C. Sample preparation was carried out by the method of blood plasma protein precipitation by acetonitrile. The maximum concentration of acetaxazolamide in the blood (Cmax) and the mean time to reach it (t_{max}) after a single intragastric administration to rabbits were determined. The values of the area under the pharmacokinetic curve $AUC_{0\to\infty}$ (from the moment of administration of the test compound to infinity) and $AUC_{0\to36}$ (from the moment of administration of the test compound to 36 hours) were calculated for intragastric and intravenous administrations. The calculation of the bioavailability of the tested drug with intragastric administration was carried out according to formula (1):

$$Fa = \frac{AUC\ i.g.}{AUC\ i.v.} \times 100\%$$

Fa – bioavailability (%); AUC i. g. – area under the pharmacokinetic curve with intragastric administration; AUC i.v. – area under the pharmacokinetic curve with intravenous administration.

Statistical analysis methods

For all of the data, the descriptive statistics methods were used. The obtained data were checked for normality of distribution by using Shapiro-Wilk test. The data are represented as the mean±standard error of the mean (S.E.M.) (in the case of the normal distribution). In cases of abnormal distribution, the median and the quartile range were calculated. The statistical analysis was performed using the Student's t-test or Mann-Whitney U-test. The P-values less than 0.05 were considered significant. The statistical analysis was performed using software BioStat –2009 software by AnalystSoft Inc.

Results and discussion

Acute toxicity

The mortality of mice after single intragastric administration of acexazolamide at doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2250 mg/kg is shown in Table 1.

The mortality (in probits) – dose (in logarithms) relationship with a single intragastric administration of acexazolamide in acute toxicity test in mice is shown in Figure 4.

The DL_{16}, DL_{50}, DL_{84} for mice after intragastric administration were 645.7 mg/kg, 861.0 mg/kg (CIs 754.9–967.1) and 1148.2 mg/kg, respectively.

Basing on the results of the study in accordance with GOST 12.1.007-76, acetaxazolamide was classified as a 3rd hazard class substance, and as moderately toxic substance according to the classification of Hodge and Sterner.

Ulcerogenic activity

During safety assessment of acexazolamide, the subject of study was its ulcerogenic activity at a single (1/5 DL_{50}) and subchronic (1/20 DL_{50}) instragastric administration in rats compared to that of indomethacinin at equitoxic doses.

Acute ulcerogenic activity

Three hours after a single administration of indometacin (10 mg/kg, 1/5 DL_{50}), all the experimental rats developed destructive changes in the gastric mucosa and serosa. Pauls Index (PI) was 11.68.

At a single intragastric administration of acexazolamide (86.1 mg/kg, 1/5 DL50) no obvious ulceric defects or extensive bleeding in the gastric mucosa were detected. Only 35% of the experimental animals had insignificant destructive changes. PI in rats which received a single dose of acexazolamide was 5.7% of the PI in the comparison group. The values of the Pauls index and the number of destructive changes in the rats' stomach at a single intragastric administration of indomethacin and acexazolamide at a dose of 1/5 DL50 are shown in Table 2.

The destructive changes (erosion, ulcers and a significant number of petechiae) of the gastric mucosa of rats 3 hours after a single intragastric administration of indomethacin (10 mg/kg) are shown in Figure 5.

Three hours after a single intragastric administration of acexazolamide (86.1 mg/ kg), no ulcerative defects in the stomachs of the experimental rats were registered; there were individual erosions and petechiae (Figure 6).

Subchronic ulcerogenic activity

At a subchronic intragastric administration of indometacine (2.5 mg/kg, 1/20 DL50) on the 5th day of the study, all the experimental rats had obvious destructive changes in the gastric mucosa.

At an intragastric subchronic administration of acexazolamide (21.5 mg/kg, 1/20 DL_{50}), the destructive chan-

Table 1. The mortality of mice after single intragastric administration of acexazolamide

Run of tests	N	Dose (mg/kg)	Number of dead mice					Mortality rate (%)
			1 day	2 day	3 day	4–14 days	Total	
Acexazolamide	6	500	0	0	0	0	0	0
	6	1000	0	0	4	0	4	66.7
	6	1500	0	0	6	0	6	100
	6	2250	3	3	0	0	6	100

Table 2. The values of the Pauls index and the number of destructive changes in the rats' stomach with a single intragastric administration of indomethacin and acexazolamide at a dose of 1/5 DL_{50}

Run of tests	N	Single-dose (mg/kg body wt.)	Number of destructions per 1 rat			Average number of destructions per 1 rat	Pauls index
			Petechiae	Erosions	Ulcers		
Acexazolamide	8	86.1	2.12±0.34*	0.25±0.11*	0	2.37±0.44*	0.83*
Indomethacin	8	10.0	8.31±0.91	1.21±0.39	2.16±0.45	11.68±1.25	11.86

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group (indomethacin).

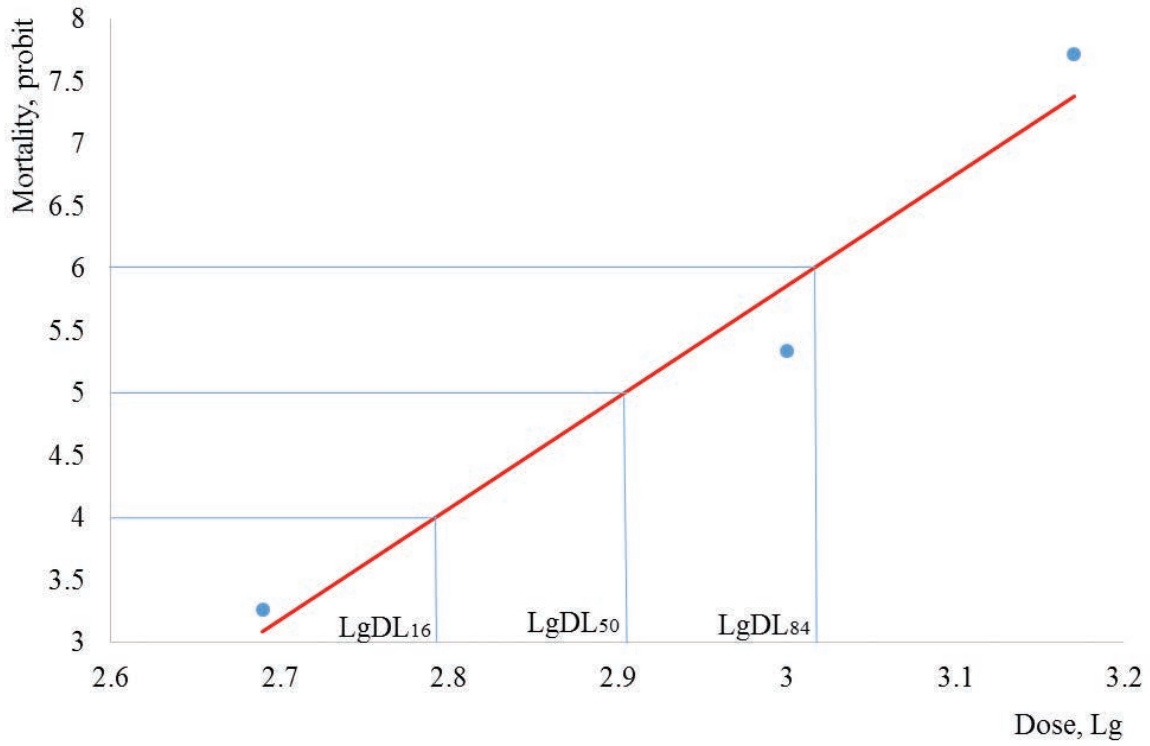


Figure 4. The mortality (probit) – dose(Lg) relationship with a single intragastric administration of acexazolamide at doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2250 mg/kg in acute toxicity test in mice

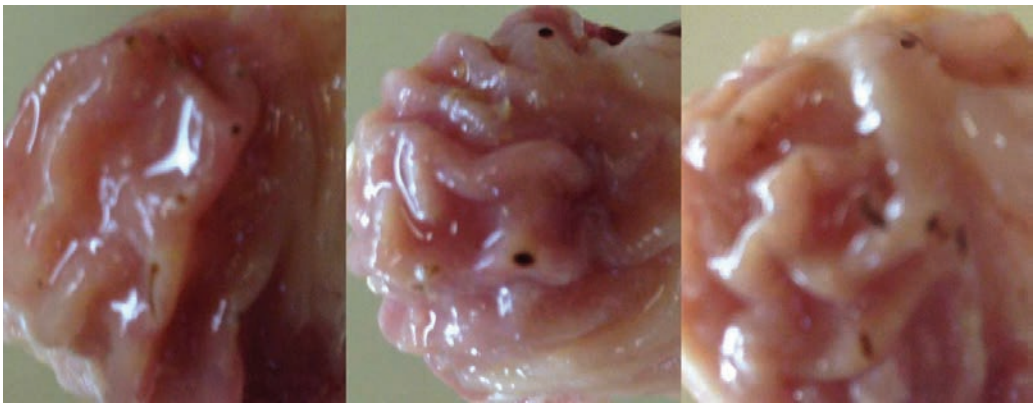


Figure 5. Destructive changes (erosion, ulcers and a significant number of petechiae) of the gastric mucosa of rats 3 hours after a single intragastric administration of indomethacin (10 mg/kg)

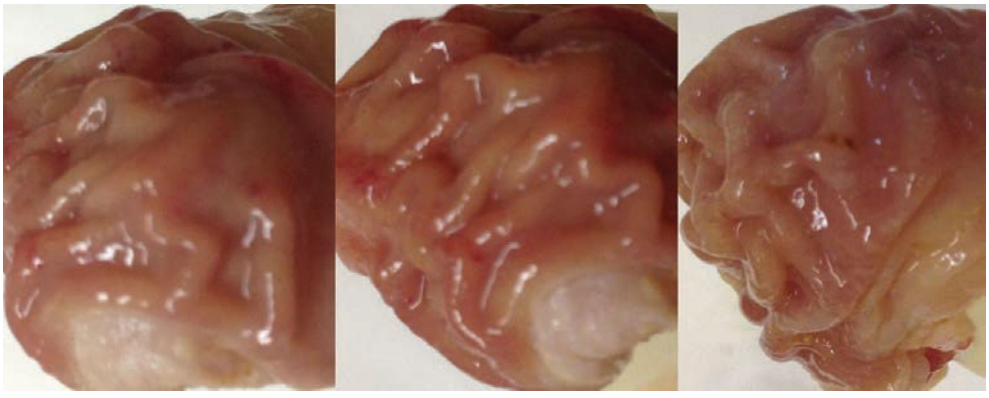


Figure 6. Gastric mucosa of rats 3 hours after a single intragastric administration of acexazolamide at a dose of 86.1 mg/kg

ges were found in 15% of the experimental animals. The destructions found were in the form of insignificant petechiae, the average number of which per animal was 1.33±0.34, which is 88.8% less than that of the animals which had received indometacine intragastrically. IP based on the overall number of destructions was 0.19 in the tested group, which is 98.4% less than that in the comparison group.

The value of the Pauls index and the number of destructive changes in the rats'stomach with a subchronic intragastric administration of indomethacin and acexazolamide at a dose of 1/20 DL_{50} are shown in Table 3.

Figure 7 shows the destructive changes (erosion, ulcers and petechiae) of the gastric mucosa of rats after subchronic intragastric administration of indomethacin at a dose of 2.5 mg/kg (1/20 DL50).

There were no gross destructive changes in the gastric mucosa with subchronic intragastric administration of acexazolamide (21.5 mg/kg) (Figure 8).

The data obtained indicate low ulcerogenicity of acexazolamide.

Anti-inflammatory activity

At the next stage of experimental research, the anti-inflammatory, analgesic and antifebrile effects of acexazolamide were assessed in comparison with the effects of non-steroidal anti-inflammatory ketoprofen. On models of acute exudative and chronical proliferative inflammation in rats, acexazolamide was found to have an obvious anti-inflammatory effect.

Formalin-induced edema

At an intragastric administration of acexazolamide (21.5 mg/kg, 1/20 DL_{50}), intensity of formalin-induced edema in rats 2 hours after induction of inflammation was 1.4 times less than that in the control and 1.3 times more than in the animals which had received ketoprofen in an equitoxic dose. Twenty-four hours after administration of formalin, the experimental animals whihc had received acexazolamide did not have edema of the paw, whereas in the experimental rats which had received ketoprofen, edema remained and was less than in the control (Table 4).

Table 3. The value of the Pauls index and the number of destructive changes in the stomach in rats with a subchronic intragastric administration of indomethacin and acexazolamide at a dose of 1/20 DL_{50}

Run of tests	N	Dose (mg/kg body wt.)	Number of destructions per 1 rat			Average number of destructions per 1 rat	Pauls index
			Petechiae	Erosions	Ulcers		
Acexazolamide	8	21.5	1.33±0.34*	0	0	1.33±0.34*	0.19*
Indomethacin	8	2.5	6.41±0.84	2.11±0.41	3.24±0.44	11.76±1.56	11.76

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group (indomethacin).

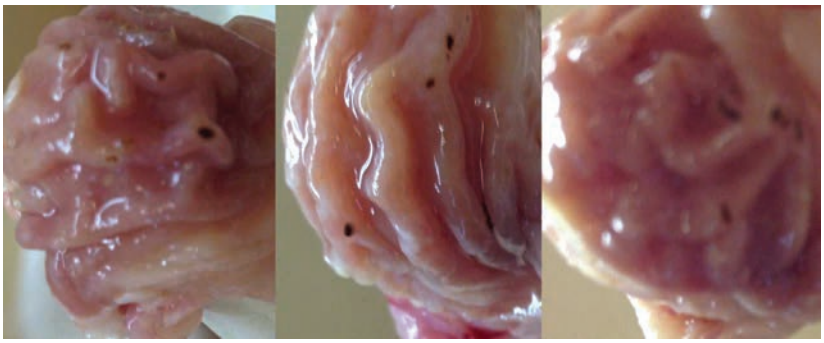


Figure 7. Destructive changes (erosion, ulcers and petechiae) of the gastric mucosa of rats after subchronic intragastric administration of indomethacin (2.5 mg/kg)

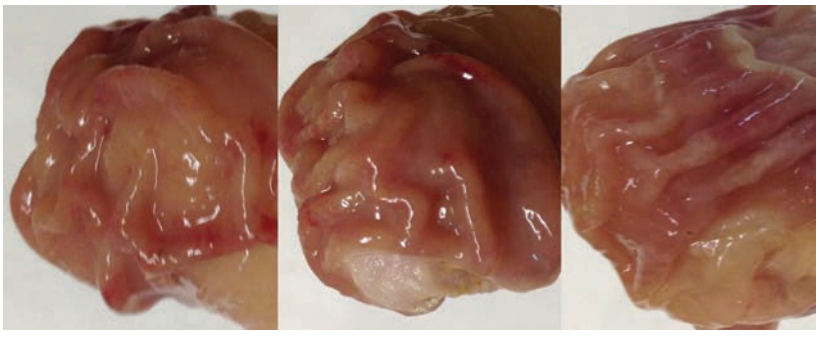


Figure 8. Gastric mucosa of rats after subchronic intragastric administration of acexazolamide at a dose 21.5 mg/kg

At an intragastric administration of acexazolamide, the highest degree of inhibiting edema of the paw was recorded 2 hours after its induction, whereas at an administration of ketoprofen it was 4 hours later.

The dependence of anti-inflammatory effect of acexazolamide (in probits) on a dose (in decimal logarithms) in case of a formalin-induced edema of the paw in rats is shown in Figure 9.

Based on the analysis of dose-effect relationship between anti-inflammatory properties of acexazolamide in formalin-induced edema in rats from dose (1/5 DL_{50}, 1/10 DL_{50}, 1/20 DL_{50}, 1/100 DL_{50}) ED_{50}equaled13.8 (95% CIs: 8.2–19.4) mg/kg.

Cotton pellet granuloma

Ani-inflammatory properties of acexazolamide were proven ШТ case of chronical proliferative inflammation in rats when modelling a cotton pellet granuloma. Reduction in both exudative and proliferative inflammation phases was registered in a number of tests with using acexazolamide. The exudative reaction in the experimental rats which had receieved acexazolamide (21.5 mg/kg, 1/20 DL_{50}) was 1.2 times less than in the control and 1.1 times ($p<0.05$) than when using ketprofen in an equitoxic dose. The proliferative reaction in the experimental rats which had received acexazolamide (21.5 mg/kg, 1/20 DL_{50}) was, respectively, on average 6.0 and 2.0 times less than that in the control and when using ketoprofen (Table 5).

Analgesic activity

On the models of thermal irritation of the inflammatory paw edema in mice (hot-plate test), chemical pain irritation of peritoneum in rats, mechanic irritation of inflammatory paw edema in rats, it was determined that acexazolamide had a pronounced analgesic effect when administered intragastrically.

Table 4. Effects of acexazolamide and ketoprofen on formalin-induced paw edema in rats

Run of tests	N	Dose (mg/kg body wt.)	Paw size before inflammation induction (mm)	Difference between pawsize (mm) 4 hours after drug administration	Edema inhibition (%)
Acexazolamide	8	4.3	5.59±0.15	6.95±0.15	27.1
	8	21.5	5.62±0.10	6.74±0.10	38.3
	8	43.0	5.58±0.10	6.18±0.09	59.9
	8	86.0	5.22±0.09	5.74±0.10	62.8
Ketoprofen	8	23.0	5.46±0.06	6.47±0.11	31.2
Formalin (control)	8	–	5.83±0.15	7.40±0.07	–

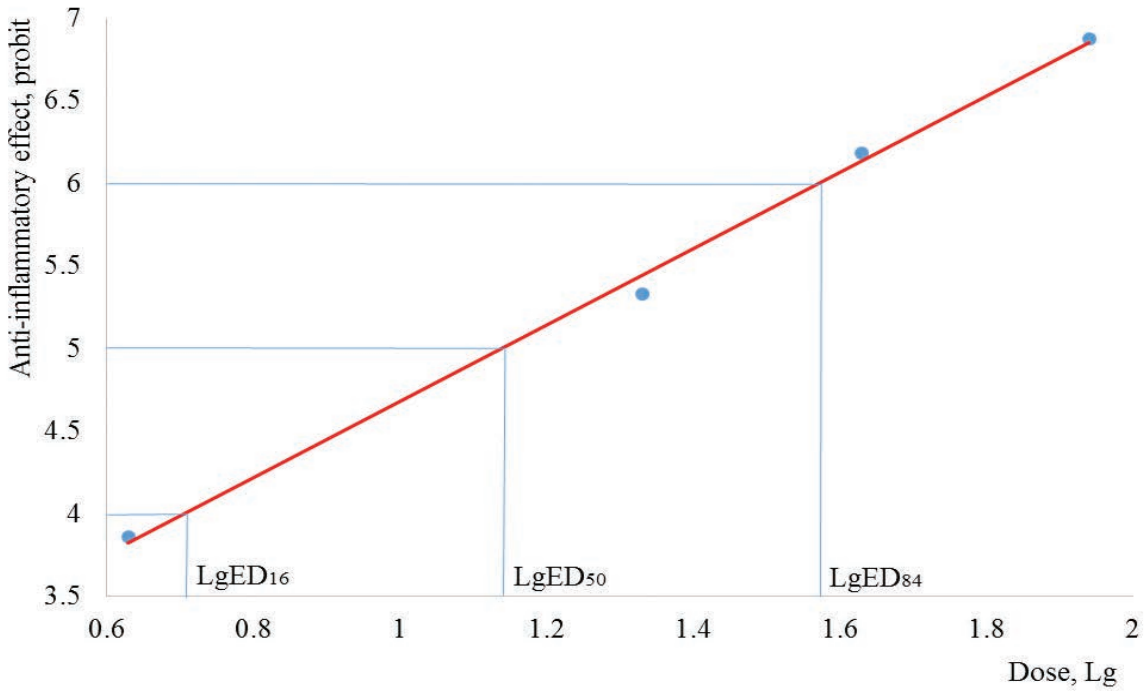


Figure 9. The dependence of anti-inflammatory effect of acexazolamide (in probits) on a dose (in decimal logarithms) in case of a formalin-induced edema of the paw in rats

Table 5. Effects of acexazolamide and ketoprofen on chronic proliferative inflammation in rats

Run of tests	N	Dose (mg/kg body wt.)	Weight of cotton pellet (mg)	Weight of wet granuloma (mg)	Weight of dry granuloma (mg)	Exudativereaction (mg)	Proliferative reaction (mg)
Acexazolamide	8	21.5	11.55±0.31	139.25±2.43*	16.17±0.39*	123.07±2.37*	4.63±0.38*
Ketoprofen	8	23.0	12.08±0.29	154.0±2.11*	21.33±1.04*	132.66±2.52*	9.25±0.78*
Control	8	–	12.82±0.47	190.87±3.19	40.50±1.29	150.37±3.45	27.67±1.28

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

Model of mechanic irritation of inflammatory paw edema (Randall & Selitto test)

When mechanically irritating the inflammatory paw in rats before administration of formalin, the load on the inflamed paw causing pain in the animal (week, limb withdrawal) was on average 122.5 g (p<0.05). However, 3 hours after administration of formalin, pain limit reduced to 66.7 g (p<0.05) on average, which is 45.5% less than that before induction of inflammation (Table 6).

When administering acexazolamide (21.5 mg/kg, 1/20 DL_{50}) and ketoprofen (23.0 mg/kg, 1/20 DL_{50}) intragastrically, a significant reduction in the intensity of inflammatory hyperalgesia was recorded. Three hours after administration of formalin, pain limit in rats which had received acexazolamide (21.5 mg/kg, 1/20 DL_{50}) and ketoprofen (23.0 mg/kg, 1/20 DL_{50}) reduced to 19.7% and 21.7% respectively. The assessment of pain limit on an intact paw did not reveal any significant differences before and after administration of acexazolamide.

The dose-effect relationship between analgesic effect (in probits) of acexazolamide and a dose (in decimal logarithms) in case of inflammatory hyperalgesia in rats is shown in Figure 10.

Based on dose-effect relationship between analgesic effect of acexazolamide in the test of mechanic irritation of an inflammatory paw in rats and a dose (1/5 DL_{50}, 1/10 DL_{50}, 1/20 DL_{50}, 1/100 DL_{50}) (Figure 10), ED_{50} was calculated, which was 14.0 (95% CIs: 8.3–19.6) mg/kg.

Abdominal constriction test induced by acetic acid (writhings)

Analgesic properties of acexazolamide were proven when giving rats chemical pain irritation of the peritoneum with 0.75% vinegar acid solution (0.1 ml/10 g of body mass). The test results showed that in the control group the number of specific nociceptive responses in abdominal constriction test was 26.8±1.23 within 15 minutes (Table 7).

When using acexazolamide (21.5 mg/kg, 1/20 DL_{50}), the number of specific nociceptive responses was 51.7% less than that in the control. It was also noted that analgesic activity of ketoprofen (23.0 mg/kg, 1/20 DL_{50}) in abdominal constriction test in rats had no significant difference from that of the compound studied.

The dose-effect relationship between analgesic effect of acexazolamide (in probits) and a dose (in decimal logarithms) in abdominal constriction test induced by acetic acid in rats is shown in Figure 11.

Based on the dose-effect relationship between analgesic effect of acexazolamide and a dose (1/5 DL_{50}, 1/10 DL_{50}, 1/20 DL_{50}, 1/100 DL_{50}) in abdominal constriction test (Figure 11), ED_{50} was calculated, which amounted to 25.0 (95% CIs: 15.3–34.7) mg/kg.

Hot-plate model

The results of hot-plate model test on mice proved analgesic effect of acexazolamide. When placed on 55 °C hot surface the experimental mice of the control group started showing defensive reflex after 11.3 seconds on average (p<0.05). Thirty minutes, 1 and 2 hours after administration of acexazolamide (43.0 mg/kg, 1/20 DL_{50}) the time before defensive reflex appeared was, respectively, 17.0%; 61.9% and 46.4% longer than in the control. However, in the hot-plate test 1 and 2 hours after intragastric administration of acexazolamide its analgesic activity was, respectively, 9.3%, 54.1% and 70.3 % inferior to that of ketoprofen (46.0 mg/kg, 1/20 DL_{50}) (Table 8).

Table 6. Effect of acexazolamide and ketoprofen on pain threshold when mechanically irritating the inflammatory paw in rats

Run of tests	N	Dose (mg/kg body wt.)	Pain thresholds (g)					
			Before inflammation induction	1 h	2 h	3 h	4 h	5 h
Acexazol-amide	8	4.3	125.2±2.7	89.4±3.5*	–	–	–	–
	8	21.5	127.4±3.5	105.0±3.1*	100.0±2.2*	102.31.7*	103.4±3.2*	103.3±2.7*
	8	43.0	134.4±4.1*	114.4±4.1*	–	–	–	–
	8	86.0	121.7±5.2	128.3±3.7*	–	–	–	–
Keto-profen	8	23.0	126.1±3.9	100.5±1.9*	99.1±2.5*	98.7±2.4*	99.2±2.5*	99.1±1.9*
Control	8	–	122.5±6.8	66.7±3.1	69.3±3.1	73.8±2.6	79.6±3.3	84.4±3.4

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

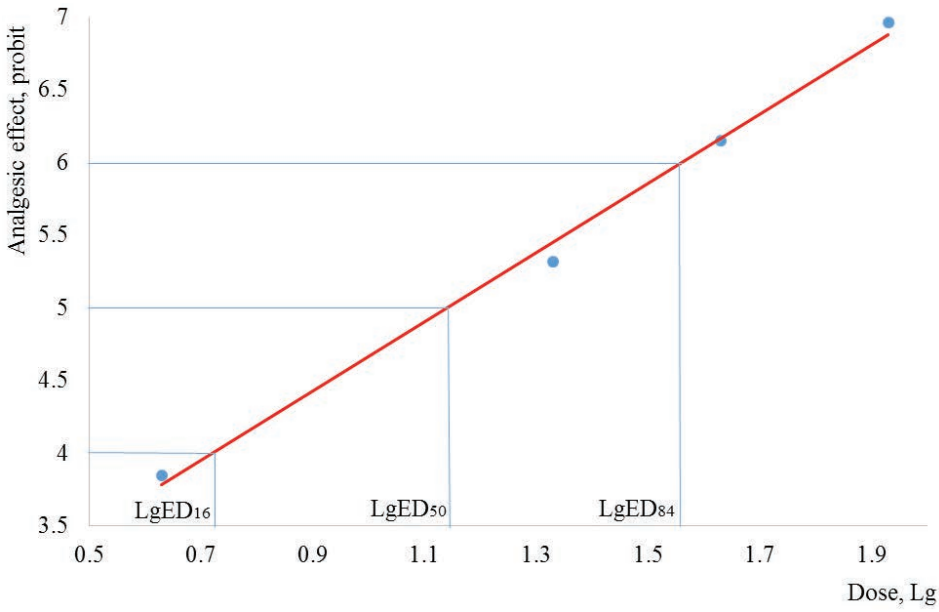


Figure 10. Dose-effect relationship between analgesic effect (in probits) of acexazolamide and a dose (in decimal logarithms) in case of inflammatory hyperalgesia (Randall & Selitto test) in rats

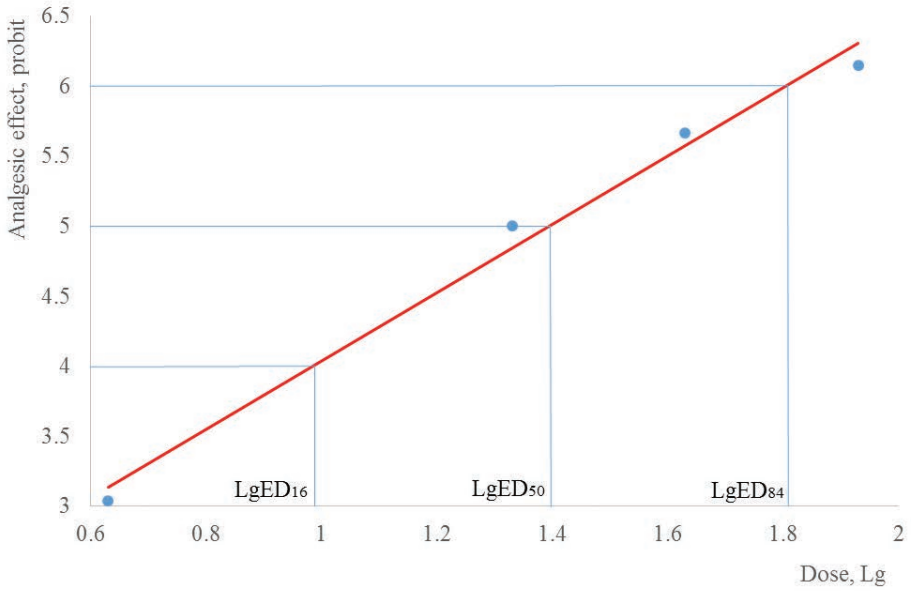


Figure 11. The dose-effect relationship between analgesic effect (in probits) and a decimal logarithm of a dose of of acexazolamide in abdominal constriction test in rats

Table 7. Effects of acexazolamide and ketoprofen on the number of specific nociceptive responses (writhings) in abdominal constriction test in rats

Run of tests	N	Dose (mg/kg body wt.)	Number of writhing over a period of 15 min	Inhibition of pain reaction (%)
Acexazolamide	8	4.3	22.75±1.06*	15.4
	8	21.5	13.0±0.72*	51.7
	8	43.0	11.87±0.95*	55.9
	8	86.0	11.0±0.71*	59.1
Ketoprofen	8	23.0	14.0±0.69*	47.9
Control	8	–	26.9±1.23	–

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

Table 8. Effect of acexazolamide and ketoprofen on the latent period before mice exposed to the hot plate test showed pain reaction

Run of tests	N	Dose (mg/kg body wt.)	Reaction time to pain(s)		
			0.5 h	1 h	2 h
Acexazolamide	8	8.6	–	13.86±0.63*	–
	8	43.0	13.8±0.5*	18.3±0.50*	18.3±0.6*
	8	86.0	–	19.88±0.70*	–
	8	172.0	–	24.04±1.02*	–
Ketoprofen	8	46.0	14.9±0.6*	24.4±0.70*	27.2±1.0*
Control	8	–	11.8±0.8	11.3±0.90	12.5±0.4

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

The dose-effect relationship between analgesic effect of acexazolamide (in probits) and a dose (in decimal logarithms) with thermal irritation of paw in mice in the hot-plate test is shown in Figure 12.

Based on dose-effect relationship between analgesic effect of acexazolamide on a dose (1/5 DL_{50}, 1/10 DL_{50}, 1/20 DL_{50}, 1/100 DL_{50}) with thermal irritation of paw in mice in the hot-plate test, ED_{50} was calculating, which amounted to 25.6 (95% CIs: 15.1–36.0) mg/kg.

Antipyretic activity

In a yeast-induced hyperthermia model in rats, acexazolamide when administered intragastrically showed obvious antipyretic activity. Maximal hyperthermic reaction in rats developed 18 hours after administration of 20% suspension of baker's yeast; and in the control group it amounted to 0.95 °C (p<0.05) on average. Intragastric administration of acexazolamide (21.5 mg/kg, 1/20 DL_{50}) and ketoprofen (23.0 mg/kg, 1/20 DL_{50}) with a background of maximum temperature rise led to reduction of hyperthermic reaction. For example, 2 hours after intragastric administration of acexazolamide (21.5 mg/kg), the temperature of the test rats reduced by 0.83 °C (p<0.05) on average. Yet, antipyretic activity of the tested chemical was inferior to that of ketoprofen, 2 hours after using which the hyperthermic reaction lowered by 1.0 °C (p<0.05) on average. Further observation revealed better antipyretic properties of ketoprofen in comparison with that of acexazolamide when used in equitoxic doses (Table 9).

The relation of antipyretic effect of acexazolamide (in probits) and a dose (in decimal logarithms) in a yeast-induced hyperthermia test in rats is shown in Figure 13.

Based on the dose-effect relationship between antipyretic effect of acexazolamide in the yeast-induced hyperthermia test in rats and a dose (1/5 DL_{50}, 1/10 DL_{50}, 1/20 DL_{50}, 1/100 DL_{50}) (Figure 13), ED_{50} was obtained, amounting to 31.9 (95% CIs: 19.2–44.5) mg/kg.

Antiallergic activity

When given to the experimental mice intragastrically, acexazolamide (43.0 mg/kg, 1/20 DL_{50}) reduced reaction of active cutaneous anaphylaxis.All ovalbumin-sensibi-

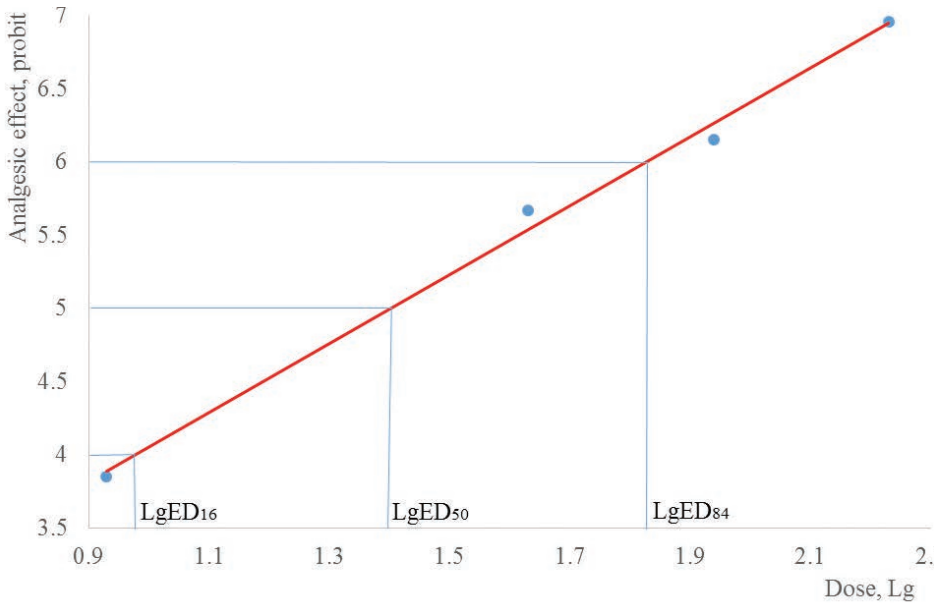


Figure 12. The dose-effect relationship between analgesic effect (in probits) and a decimal logarithm of a acexazolamide dose in the hot-plate test

Table 9. Effects of acexazolamide and ketoprofen on intensity of hyperthermic reaction in rats in a yeast-induced hyperthermia test

Run of tests	N	Dose (mg/kg body wt.)	Rectal temperature 18 h after yeast injection, °C						
			0 h	2 h	3 h	4 h	5 h	6 h	7 h
Acexazol-amide	8	4.3	37.93±0.04*	37.27±0.08*	–	–	–	–	–
	8	21.5	37.91±0.07*	37.08±0.07*	36.88±0.06*	36.95±0.06*	37.04±0.07*	36.98±0.05*	37.06±0.04*
	8	43.0	37.91±0.05*	36.81±0.07*	–	–	–	–	–
	8	86.0	37.96±0.06*	36.81±0.07*	–	–	–	–	–
Keto-profen	8	23.0	37.88±0.05	36.88±0.05*	36.85±0.03*	36.67±0.05*	36.69±0.02*	36.69±0.03*	36.83±0.03*
Control	8	–	37.66±0.11	37.86±0.04	37.61±0.04	37.51±0.03	37.51±0.05	37.51±0.04	37.53±0.03

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

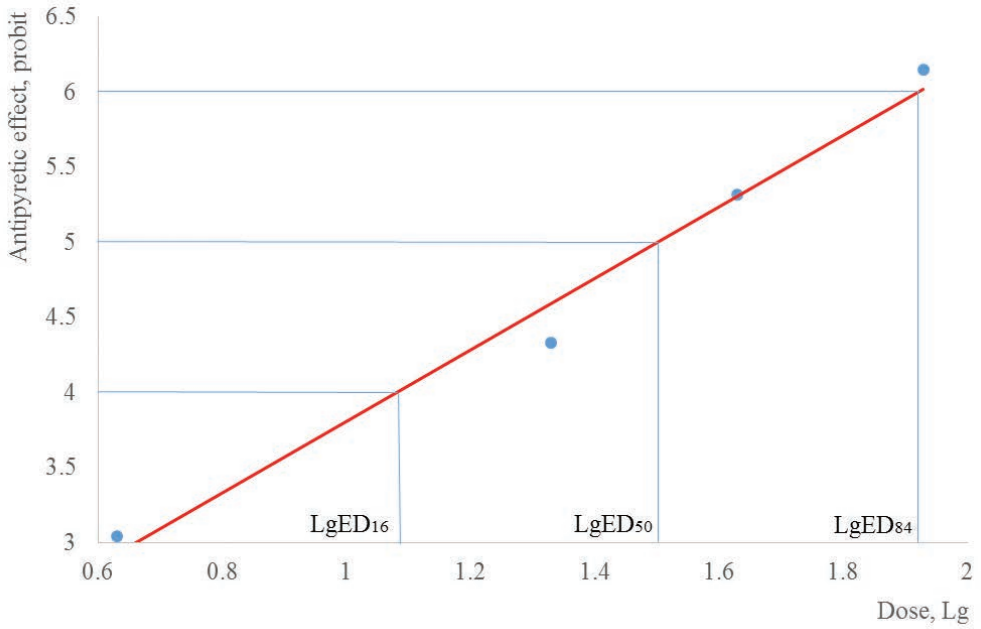


Figure 13. The relation of antipyretic effect (in probits) and a decimal logarithm of a dose of acexazolamide in yeast-induced hyperthermia test in rats

lized experimental mice developed local allergic reaction on the 21st day of latent sensibilization period when the antigen was adminiatered intradermally. In the mice which had received prednisolone at a dose of 0.5 mg/kg, the size of the test spot was 53.9±1.7 mm^2, which on average is 81.2% less than the area of the control colored spot of the skin. The group of animals which had received acexazolamide intragastrially (43.0 mg/kg, 1/20 DL_{50}) also demonstrated a lowered reaction of active cutaneous anaphylaxis (Table 10).

The area of the control spots in mice was 314.8±8.6 mm^2, whereas intragastric administration of acexazolamide at a dose of 43.0 mg/kg (1/20 DL_{50}) reduced the area of coloured skin spots by 36.4% in comparison with that in the control (Figure 14).

Results of the experimental study revealed antiallergic activity of acexazolamide. Inhibition of the reaction of active cutaneous anaphylaxis in mice when administering acexazolamide (43.0 mg/kg) was on average by 44,8% lower than that of prednisolone (0.5 mg/kg).

Anti-burn activity

Considering the fact that in the chemical structure of acexazolamide there is a fragment of acexamic acid, having pronounced reparative properties, the next stage of the study was to evaluate the anti-burn activity of acexazolamide.

The results of the study showed that daily intragastric administration of acexazolamide to rats (21.5 mg/kg, 1/20 DL_{50}) ($p<0.05$) reduced terms of full epithelialization of the burn. Its full epithelialization with cicatrization in the rats of the experimental group happened on average on the 13.3th day ($p<0.05$), which was 1.3 times ($p<0.05$) faster than that in the rats which had received normal saline per os. Starting on average from the 4th day ($p<0.05$), the area of the burn in the experimental group was positively smaller than that in the control (Figure 15).

Daily intragastric administration of acexazolamide at a dose of 21.5 mg/kg activated processes of regeneration and growth of new connective tissue and epithelium over the defected zone.

Table 10. Effect of acexazolamide and prednisolone on the intensity of reaction of active cutaneous anaphylaxis (ACA) in mice

Run of tests	N	Dose (mg/kg body wt.)	Area of coloured spot, mm²		Inhibition of ACA, %
			Experiment	Control	
Acexazolamide	8	43.0 (i.g.)	200.2±8.0*	314.8±8.6	36.4
Prednisolone	8	0.5 (i.p.)	53.9±1.7*	286.9±8.0	81.6

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group (prednisolone).

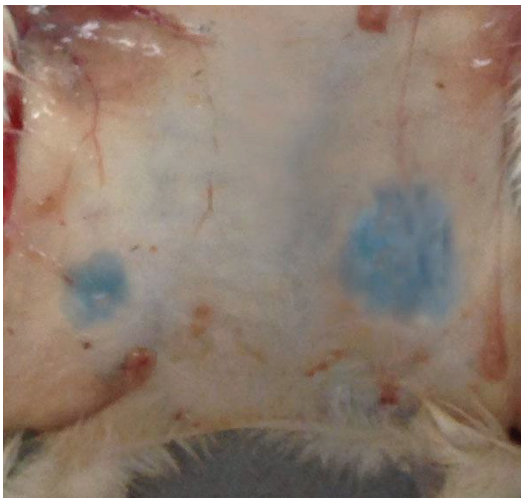


Figure 14. The reaction of active cutaneous anaphylaxis in mice when administering acexazolamide at a dose of 43.0 mg/kg: on the left – an experimental spot, on the right – a control spot

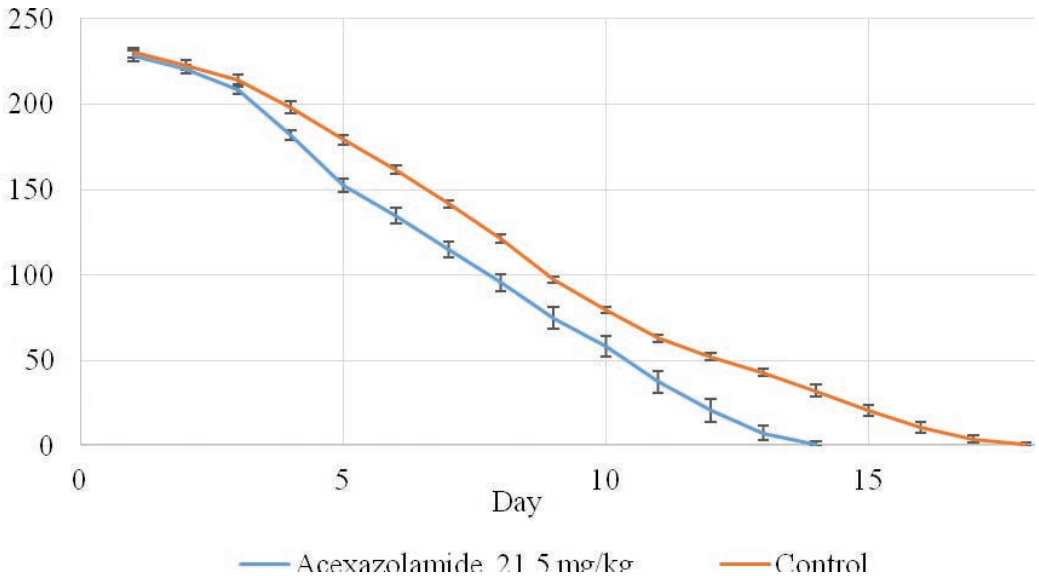


Figure 15. Area of wound defect at different periods after the thermal burn in rats receiving acexazolamide (21.5 mg/kg)

On the 5th day of the experiment, the rats of the control group had developed a tight eschar from necrotized tissue, curdled plasm and degeneratively modified leukocytes 310.8±10.1 μm thick. A thick 113.0±5.9 μm leukocyte crust consisting of 2 layers spread over the whole wound. The surface had variously shaped cells with hyperchromatic nuclea. The bottom layer consisted of cell elements of a typical structure with no signs of degeneration. Local granulation tissue formed slowly and consisted of macrophages, neutrophilic leukocytes and histocytes. Individual vertical capillaries of various diameters were located in granulation tissue. Deep layers of the wound had horizontal poorly differentiated fibroblasts of typical structure. The fibroblasts were pointed in different directions in the 662.2±21.4 μm thick granulated tissue, which had edema with leukocytal infiltration. Epithelium regeneration (5–6 layers of cells) had a wedge shape 405.5±15.2 μm long. Epithelium hypertrophy on the boundary of intact skin was ill-defined (114.0±8.8 μm) (Table 11).

Table 11. Morphological changes after thermal burn of rat skin (day 5)

Run of tests	N	Dose (mg/kg body wt.)	Thickness (μm)				Length of regenerate (μm)
			Eschar	Leukocyte infiltration	Granulation tissue	Epithelium at the edge	
Acexazolamide	8	21.5	218.2±8.5*	112.3±3.0	966.2±11.7*	200.2±5.4*	699.3±27.2*
Control	8	–	310.8±10.1	113.0±5.9	662.2±21.4	114.0±8.8	405.5±15.2

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

The rats which received acexazolamide intragastrically (21.5 mg/kg, 1/20 DL_{50}) on a daily basis on the 5th day developed a 218.6±8.5 μm thick eschar which is 29.8% less than that in the control. The thickness of the leukocytal crust did not have definable changes from that of the control. The leukocytal crust consisted of fibrin and degeneratively modified leukocytes. The granulation tissue was well-developed and had multiple parallel vertical blood vessels in its structure. Its thickness was 966.2±11.7 μm, which is 45.9% more than that in control. Also there was active proliferation of fibroblasts. Developed granulation tissue was a perfect breeding ground for new epithelium which along the defect edges had grown into the neighbouring tissues. The zones of ingrowths were marked with high quantity of cells with mitosis figures. Hypertrophied epithelium was 200.2±5.4 μm on the boundary, which is 75.6% more than that in rats of the control group. The length of epithelium regenerate (699.3±27.2 μm) was 72.5% more than that in control.

On the 10th day of observation, the control group animals had a thin leukocytal necrotic layer. The 206.0±5.8 μm thick eschar was partially fragmented and tightly sealed with the neighbouring tissues. The leukocytal crust was a 76.2±2.5 μm thin line. It had fagocytal macrophages and neutrophilic leukocytes of normal structure. The granulation tissue fully covered the burn and had a typical structure and thickness of 832.7±11.3 μm. Its surface layer had vertically oriented blood vessels. Granulation had a layer of fibroblasts chaotically-oriented around the vessels and much collagen fiber. Granulation tissue rearranged into new connective tissue. Most of the wound surface was covered with newly-produced epithelium of 5–6 layers of cells. The regenerate length was 650.±50.1 μm. Basal membrane of the epithelium regenerate was even. On the edges it formed growths into the neighbouring tissues. The epithelium thickness on the boundaries of the defect was 141.8±3.6 μm (Table 12).

Figure 16 shows the histological picture of the wound defect in the rats of the control group on the 10th day after thermal damage to the skin.

The animals which daily received acexazolamide intragastrically (21.5 mg/kg, 1/20 DL_{50}) had epithelium regenerate of a substantial length – 992.5±8.1 μm, which was 52.6% more than that in the control. In the structure of regenerative epithelium, vertical differentiation with skin derivatives was found. On the edges of the defect, epithelium was 232.5±10.0 μm thick, which was 64.0% more than that in the control. The eschar thickness was 60.0% less in comparison with that in the control. The eschar was fragmentally present in the center of the defect. Under it was a leukocytal crust 16.6±4.5 μm thick, which was 78.2% less than that in the control. In small patches of the wound not covered with epithelium, partial differentiation of granulated tissue was noted. On its surface was a layer of vertically oriented blood vessels. Most of the defect was filled with horizontally oriented fibroblasts. Among fibroblasts there were multiple collagen fiber bundles. Granulation tissue had significant thickness of (1309.8±19.7 μm against 832.7±11.3 μm in control). On the edges of the wound defect, young connective tissue was being formed (Figure 17).

The rats which had daily received acexazolamide intragastrically (21.5 mg/kg, 1/20 DL_{50}) developed a scar of a typical structure over the wounded surface on the 15th day. Regenerate resembled a thin strip differentiated by layers. The defect was filled with mature connective tissue with horizontally oriented fibroblasts and multiple collagen fiber bundles. All over the new epithelium, the basal membrane formed multiple growths into the neighbouring tissue with signs of hair follicle and oil gland formation (Figure 18 B). The majority of the experimental group animals developed organo-specific regenerate with all characteristics of normal skin (Figure 18 A).

Daily intragastric administration of 21.5 mg/kg of acexazolamide promotes epithelization of the 3rd degree burns in rats.

Table 12. Morphological changes after thermal burn of rat skin (day 10)

Run of tests	N	Dose (mg/kg body wt.)	Thickness (μm)				Length of regenerate (μm)
			Eschar	Leukocyte infiltration	Granulation tissue	Epithelium at the edge	
Acexazolamide	8	21.5	81.3±4.0*	16.6±4.5*	1309.0±19.7*	232.5±10.0*	992.5±8.1*
Control	8	–	206.0±5.8	76.2±2.5	832.7±11.3	141.8±3.6	650.5±50.1

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.

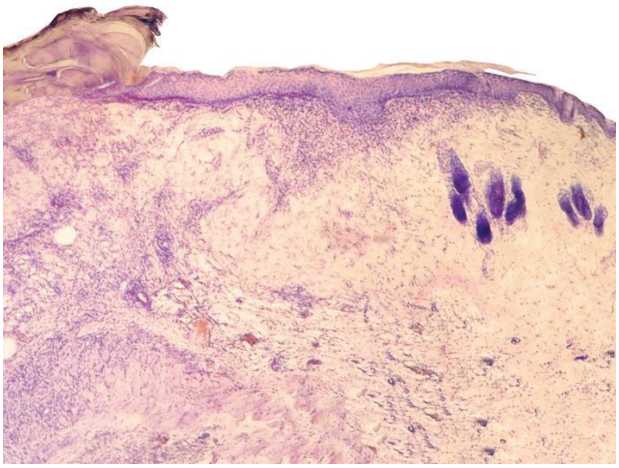


Figure 16. Histological picture of the wound defect in rats of the control group, 10th day after thermal injury of skin. Coloured with haeatoxylin and eosin. Zoom 400×

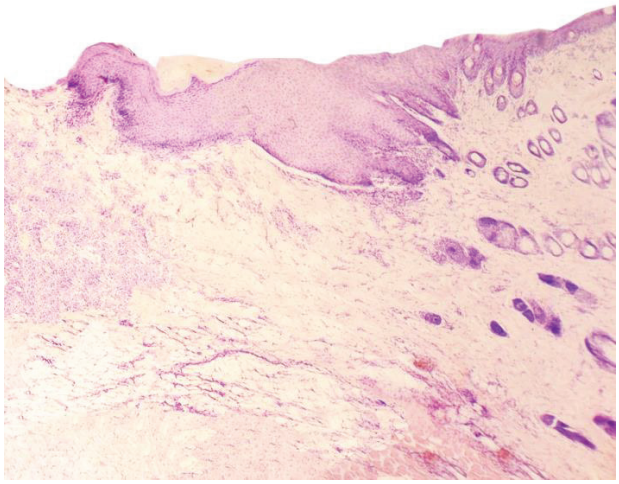


Figure 17. Histological picture of the wound defect in rats which received acexazolamide intragastrically (21.5 mg/kg), the 10th day after thermal injury of skin. Coloured with haeatoxylin and eosin. Zoom 400×

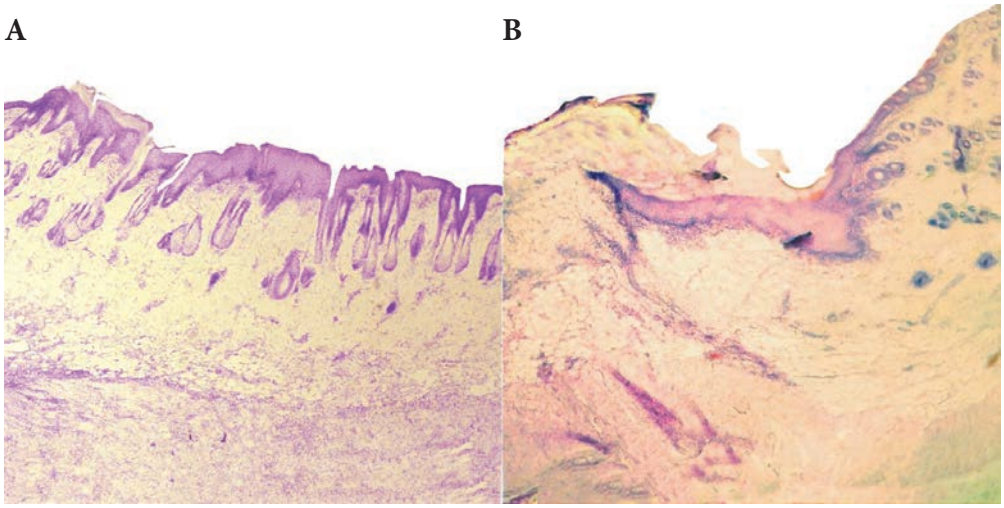


Figure 18. Histological picture of the wound defect in rats which received acexazolamide intragastrically (21.5 mg/kg) (**A**) and in the control group (**B**), the 15th day after thermal injury of skin. Coloured with hameatoxylin and eosin. Zoom 400×

Bioavailability of acexazolamide

The analysis of the results of the experimental study showed that 1 minute after intravenous (i.v.) administration of acexazolamide (1 mg/kg), its content in blood plasm of rabbits was 20323±1136 ng/ml. The value obtained was equal to the theoretical concentration calculated by means of dividing the quantity of the medicine administered to the experimental rabbits by the assumed volume of circulating blood. At intragastric (i.g.) administration of 1 mg/kg maximum content of acexazolamide was 806.8±65.6 ng/ml, which is 96% less than the maximum concentration at its itravenous administration (Table 13).

Based on the results of the pharmacokinetic study, the graphs of concentration/time relation (pharmacokinetic curves) were made for intravenous (Figure 19) and intragastric (i.g.) administration (Figure 20).

At intravenous administration, reduction in acexazolamide concentration in blood plasma of the experimental rabbits had a 2-phase character (α and β phases). The α phase was characterized by quick concentration decrease in the tested medicine in blood plasma within the first 45 minutes, perhaps on the account of its redistribution in tissue (allocation phase). The β phase was marked by a slow decrease in concentration of acexazolamide. Thirty-six hours after intravenous administration of the tested medicine, its content in blood plasma was reaching its low quantitation limit. The area under the pharmacokinetic curve $AUC_{0\rightarrow36}$ was 13531±1478 ng×h/ml, while the ratio $AUC_{36\rightarrow\infty}/AUC_{0\rightarrow36}$ was 2.96±1.59%, which proved

Table 13. Pharmacokinetic parameters of acexazolamide (1 mg/kg) in rabbit

Parameter	Acexazolamide (i.g.)		Acexazolamide (i.v.)
	C_{max}(ng/ml)	t_{max}(h)	C_{max}(ng/ml)
1	860	1.385	21454
2	890	1.323	20621
3	804	1.258	19108
4	784	1.317	21411
5	701	1.299	20560
6	802	1.487	18784
Mean	806.8	1.345	20323
Gmean	804.6	1.343	20296
SD	65.6	0.081	1136
CV, %	8.1	6.02	5.6
Median	803	1.32	20590.5
min	701	1.258	18784
max	890	1.487	21454
Confidence interval (L-95%; Up-95%)	754.4–859.3	1.28–1.41	19130–21515

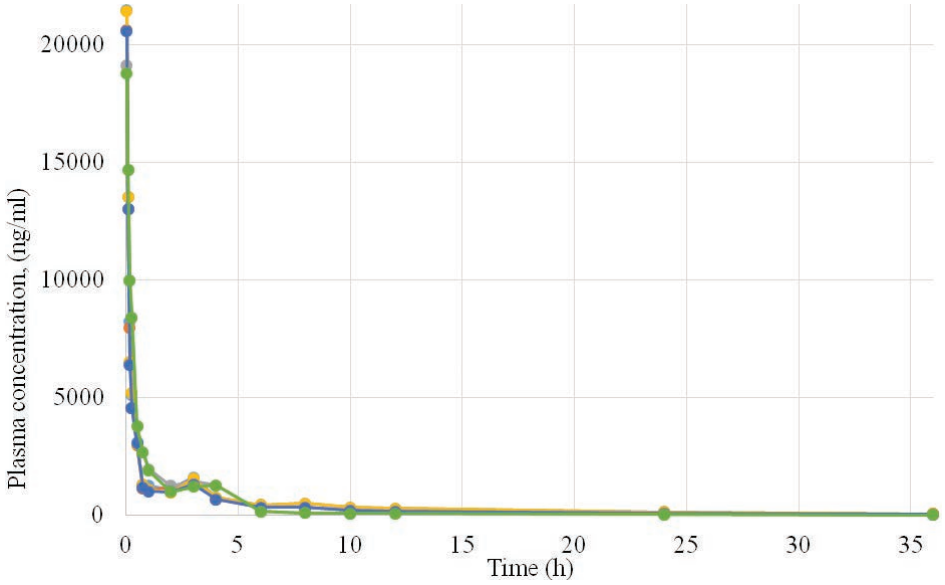


Figure 19. Pharmacokinetic curve of acexazolamide (1 mg/kg) observed in rabbit plasma (i.v.)

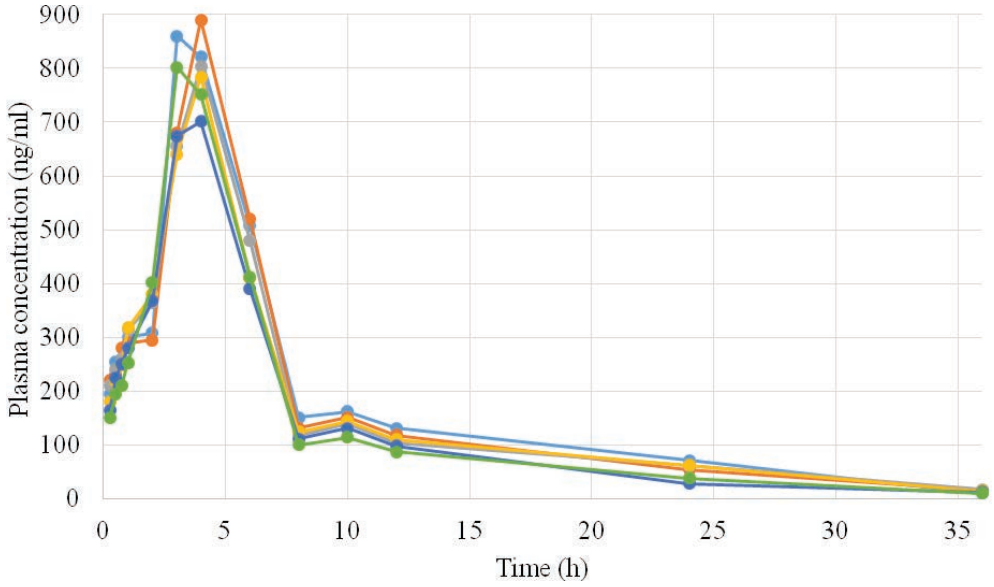


Figure 20. Pharmacokinetic curve of acexazolamide (1 mg/kg) observed in rabbit plasma (i.g.)

adequate length of monitoring the tested drug in blood plasma (Table 14).

Based on the results of the pharmacological study conducted, a value of absolute bioavailability of acexazolamideat was calculated with intragastric administration of 2% starch mucus to rabbits at a dose of 1 mg/kg. This indicator is on average 37%.

Thus, the new amino acid derivative of thiadiazol under study possesses definable anti-inflammatory, antipyretic and analgesic properties combined with low ulcerogenicity, which makes it promising as a non-steroidal anti-inflammatory medicine.

Table 14. The areas under the pharmacokinetic curve ($AUC_{0 \to 36}$ and $AUC_{0 \to \infty}$), with intravenous injection of acexazolamide at a dose of 1 mg/kg

Parameter	$AUC_{0 \to 36}$, ng×h/ml	$AUC_{0 \to \infty}$, ng×h/ml	$AUC_{36 \to \infty}/AUC_{0 \to 36}$, %
1	15642	16373	4.67
2	12761	13216	3.56
3	13168	13307	1.05
4	15061	15766	4.69
5	11905	12196	2.44
6	12650	12821	1.35
Mean	13531	13946	2.96
Gmean	13466	13863	2.54
SD	1478	1701	1.59
CV, %	10.9	12.20	53.90
Median	12965	13262	3.00
Min	11906	12196	1.05
Max	15642	16373	4.68
Confidence interval (L-95%; Up-95%)	11979–15083	12161–15732	1.28–4.63

Conclusion

DL_{50} of acexazolamide at intragastrtic administration in mice was 861.0 (95% CIs, 754.9–967.1) mg/kg, which in accordance to GOST 12.1.007–76 makes it possible to classify it as a 3rd hazard class chemical and as a moderately toxic substance in accordance with the classification of Hodge and Sterner.

Ulcerogenicity of acexazolamide at single intragastric administration in rats at a dose of 1/5 DL_{50} (86.1 mg/kg) was 14 times ($p<0.05$) lower than that of indometacine in an equitoxic dose (10.0mg/kg). At its subchronic administration, the ulcerogenicity Pauls Index for acexazolamide at a dose of 1/20 DL_{50} (21.5 mu/kg) was 0.19, which was 61.9 times ($p<0.05$) lower than that of indometacine in an equitoxic dose (2.5mg/kg).

On the models of acute exudative and chronical poliferative inflammation in rats, acexazolamide demonstrated its pronounced anti-inflammatory effect. On formalin-induced edema, the index of ED_{50} at intragastric administration was 13.8 (95% CIs: 8.2–19.4) mg/kg. Anti-inflammatory effect of acexazolamide at a dose of 21.5 mg/kg (1/20 DL_{50}) at acute exudative inflammation was on average 1.25 times ($p<0.05$) higher than that of ketoprofen in an equitoxic dose (23 mg/kg). At chronical inflammatory reaction (cotton pellet granuloma), inhibition of poliferative phase under the influence of acexazolamide at a dose of 21.5 mg/kg (1/20 DL_{50}) was approximately 1.28 times ($p<0.05$) higher than that of ketoprofen in an equitoxic dose (23 mg/kg).

On models of mechanic irritation of inflammatory paw edema and abdominal constriction test in rats, thermal irritation of mice paws, acexazolamide exercised its analgesic effetc at intragastric administration. At mechanic irritation of inflammatory paw edema in rats, ED_{50} was 14.0 (95% CIs: 8.3–19.6) mg/kg. ED_{50} for analgesic effect in case of writhings caused by vinegar acid was 25.0 (95% CIs: 15.3–34.7) mg/kg. At thermal irritation of mice paws, ED_{50} was 25.6 (95% CIs: 15.1–36.0) mg/kg. On models of mechanic irritation of inflammatory paw edema and abdominal constriction test in rats, analgesic effect of acexazolamide at a dose of 1/20 DL_{50} (21.5 mg/kg) had no significant difference from that of ketoprofen in an equitoxic dose (23 mg/kg).

Antipyretic activity of acexazolamide at a dose of 1/20 DL_{50} (21.5 mg/kg) at yeast-induced hyperthermia in rats was approximately 1.2 times ($p<0.05$) lower than that of ketoprofen in an equitoxic dose (23 mg/kg). ED_{50} was t31.9 (95% CIs: 19.2–44.5) mg/kg. At intragastrically administered to mice at a dose of 1/20 DL_{50} (43.0 mg/kg), acexazolamide reduced reaction of active cutaneous anaphyaxis 1.57 times ($p<0.05$) in comparison with that of the control.

Daily intragastric administration of acexazolamide at a dose of 1/20 DL_{50} (21.5 mg/kg) reduced healing period of 3rd degree burns in rats 1.3 times ($p<0.05$) on average. Histological structure of regenerate in the experimental

animals which had received acexazolamide was close to that of normal skin on the 15th day.

Bioavailability of acexazolamide with intragastric administration to rabbits at a dose of 1 mg/kg as an aqueous suspension averaged 37%.

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