Study on assessment of pharmacological activity and bioavailability of acexazolamide, a new derivative of 1,3,4-thiadiazole andacenamic acid.

Objective: To assess the pharmacological activity and bioavailability of acexazolamide.

Materials and methods: Acexazolamide was tested in animal experiments for acute toxicity, pharmacological activity, and bioavailability. Anti-inflammatory activity was evaluated using the formalin edema and cotton pellet granuloma models in rats. Analgesic and antipyretic activities were determined in a hot plate test and yeast-induced hyperthermia model, respectively. Bioavailability was assessed in rabbits by intragastric administration.

Results: The LD50 of acexazolamide was 860.99 ± 462.2 mg/kg. Acexazolamide showed higher anti-inflammatory activity (21.5 mg/kg) compared to ketoprofen (23.0 mg/kg). Its ED50 for analgesic activity with acetic acid-induced peritonitis was 24.99 ± 15.31–34.68 mg/kg. The ED50 for antipyretic activity was 31.85 ± 19.22–44.47 mg/kg. Acexazolamide (21.5 mg/kg) stimulated tissue regeneration.

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

Keywords: nonsteroidal anti-inflammatory agents, 1,3,4-thiadiazole derivatives, acexamic acid.
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, Alibadi et al. 2017, Djukic et al. 2018, Gomha et al. 2017, Haider et al. 2015, Kumari et al. 2017, Matsysiak 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 (indometacin, 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 chin-chilla 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 Protecti-
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 mucosa 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 injection 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. Acexazolamide (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 (8.6 mg/kg, 43.1 mg/kg, 86.1 mg/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. Aceaxazolamide 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 aceaxazolamide at different doses (8.6 mg/kg, 43.1 mg/kg, 86.1 mg/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 µg 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 µg of ovalbumin. Simultaneously, 0.25 ml of 1.6% Evans blue solution was administered intravenously. Twenty min later, aceaxazolamide and prednisolone were administered as well. One hour later, 0.05 µg 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 aceaxazolamide was carried out on the model of thermal skin burn in rats. A thermal injury was caused with a steel stencil (surface area – 225 mm², 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 5th, 10th and 15th 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 aceaxazolamide in blood plasma by HPLC-MS/MS method**

Determination of aceaxazolamide 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 TurboIontSpray 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 aceaxazolamide, 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 aceaxazolamide in rat plasma was 0.25 ng/ml. The application range of the procedure was from 1 ng/ml to 1000 ng/ml.

**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 aceaxazolamide between the stages was 7 days. Aceaxazolamide was administered intravenously at a dose of 1 mg/kg in a 0.33% solution of dimexeide 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 aceaxazolamide in blood plasma exceeded the elimination half-life period (T1/2) 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 (tmax) after a single intragastric administration to rabbits were determined. The values of the area under the pharmacokinetic curve AUC0-∞, AUC0-tmax (from the moment of administration of the test compound to infinity) and AUC0-36h (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):
\[ F_a = \frac{AUC \text{ i. g.}}{AUC \text{ i. v.}} \times 100\% \]

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 acetazolamide at doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2250 mg/kg is shown in Table 1.

| Run of tests | Dose (mg/kg) | Number of dead mice | Mortality rate (%) |
|--------------|--------------|---------------------|-------------------|
| Acetzolamide | 500          | 0                   | 0                 |
|              | 1000         | 0                   | 0                 |
|              | 1500         | 0                   | 0                 |
|              | 2250         | 3                   | 100               |

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

The DL_{50}, DL_{50}, DL_{50} 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, acetazolamide 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 acetazolamide, the subject of study was its ulcerogenic activity at a single (1/5 DL_{50}) and subchronic (1/20 DL_{50}) intragastric administration in rats compared to that of indomethacin at equitoxic doses.

**Acute ulcerogenic activity**

Three hours after a single administration of indomethacin (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 acetazolamide (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 acetazolamide 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 acetazolamide 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 acetazolamide (86.1 mg/kg, 1/5 DL50) 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 indomethacin (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 acetazolamide (21.5 mg/kg, 1/20 DL_{50}), the destructive chan-

| Table 1. The mortality of mice after single intragastric administration of acetazolamide |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Run of tests & N               | Dose (mg/kg)    | 1 day | 2 day | Number of dead mice | 4–14 days | Total | Mortality rate (%) |
| Acetzolamide | 6              | 500   | 0     | 0     | 0                 | 0       | 0     | 60 |
|              | 6              | 1000  | 0     | 0     | 0                 | 0       | 0     | 60 |
|              | 6              | 1500  | 0     | 0     | 0                 | 0       | 0     | 60 |
|              | 6              | 2250  | 3     | 3     | 0                 | 1       | 0     | 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 acetazolamide at a dose of 1/5 DL_{50} |
|---------------------------------|--------------------|-----------------|-----------------|-----------------|-----------------|
| Run of tests & N | Single-dose (mg/kg body wt.) | Petechiae | Erosions | Ulcers | Average number of destructive changes per 1 rat | Pauls index |
| Acetzolamide | 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.
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...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 $1.33\pm0.34$, 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 DL$_{50}$).

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 chronic 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 which 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.) | Petechiae | Number of destructions per 1 rat | Average number of destructions per 1 rat | Pauls index |
|--------------|----|-----------------------|-----------|---------------------------------|------------------------------------------|-------------|
| Acexazolamide | 8  | 21.5                  | 1.33±0.34* | 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  |

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](image-url)
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 DL₅₀, 1/10 DL₅₀, 1/20 DL₅₀, 1/100 DL₅₀) ED₅₀ equaled 13.8 (95% CIs: 8.2–19.4) mg/kg.

**Cotton pellet granuloma**

Anti-inflammatory properties of acexazolamide were proven in 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 received acexazolamide (21.5 mg/kg, 1/20 DL₅₀) was 1.2 times less than in the control and 1.1 times (p<0.05) than when using ketoprofen in an equitoxic dose. The proliferative reaction in the experimental rats which had received acexazolamide (21.5 mg/kg, 1/20 DL₅₀) 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 paw size (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 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.) | Weight of cotton pellet (mg) | Weight of wet granuloma (mg) | Weight of dry granuloma (mg) | Exudative reaction (mg) | Proliferative reaction (mg) |
|--------------|---|----------------------|-----------------------------|-----------------------------|-----------------------------|------------------------|-----------------------------|
| Acexazolamide| 8 | 21.5                 | 11.5±±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 DL50) and ketoprofen (23.0 mg/kg, 1/20 DL50) intragastrically, a significant reduction in the intensity of inflammatory hyperalgesia was recorded. Three hours after administration of formalin, pain limit 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 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 (in probits) and a dose (in decimal logarithms) in abdominal constriction test induced by acetic acid in rats is shown in Figure 11.

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 DL50), 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 DL50) 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 DL50, 1/10 DL50, 1/20 DL50, 1/100 DL50) in abdominal constriction test (Figure 11), ED50 was calculated, which amounted to 25.0 (95% CI: 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 DL50) 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 DL50) (Table 8).
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.
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 DL50, 1/10 DL50, 1/20 DL50, 1/100 DL50) with thermal irritation of paw in mice in the hot-plate test, ED50 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), 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 DL50, 1/10 DL50, 1/20 DL50, 1/100 DL50) (Figure 13), ED50 was obtained, amounting to 31.9 (95% CIs: 19.2–44.5) mg/kg.

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.) | 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.

Antiallergic activity

When given to the experimental mice intragastrically, acexazolamide (43.0 mg/kg, 1/20 DL50) reduced reaction of active cutaneous anaphylaxis. All ovalbumin-sensibi-
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 |
| Acexazolamide | 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* | – | – | – | – | – |
| Ketoprofen    | 8  | 23.0                  | 37.88±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  | 37.36±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

Figure 14. The relation of antipyretic effect (in probits) and a decimal logarithm of a dose of acexazolamide in yeast-induced hyperthermia test in rats

Figure 15. 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 sensitization period when the antigen was administered 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 mm², 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 intragastrically (43.0 mg/kg, 1/20 DL₅₀) also demonstrated a lowered reaction of active cutaneous anaphylaxis (Table 10).

The area of the control spots in mice was 314.8±8.6 mm², whereas intragastric administration of acexazolamide at a dose of 43.0 mg/kg (1/20 DL₅₀) reduced the area of colored 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 DL₅₀) (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 13.3rd 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

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 nuclei. 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).

Figure 15. Area of wound defect at different periods after the thermal burn in rats receiving acexazolamide (21.5 mg/kg)
The rats which received acexazolamide intragastrically (21.5 mg/kg, 1/20 DL50) 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 thick, which was 52.6% more than that in the control. The thickness of 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 thick, which was 52.6% more than that in the control. In small patches of the wound not covered with epithelium, partial differentiation of granulated tissue was noted. On the surface it 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 animals which daily received acexazolamide intragastrically (21.5 mg/kg, 1/20 DL50) 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. 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 DL50) 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 11. Morphological changes after thermal burn of rat skin (day 5) |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Run of tests     | N    | Dose (mg/kg body wt.) | Eschar | Leukocyte infiltration | Granulation tissue | Epithelium at the edge | Length of regenerate (µm) |
| 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    | –               | 30.0±10.1  | 113.0±5.9          | 662.0±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.

| Table 12. Morphological changes after thermal burn of rat skin (day 10) |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Run of tests     | N    | Dose (mg/kg body wt.) | Eschar | Leukocyte infiltration | Granulation tissue | Epithelium at the edge | Length of regenerate (µm) |
| 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±30.1            |

Note: The values are expressed as mean±SEM, (n=8). The symbol * indicates statistical significance (P<0.05) compared to the control group.
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 plasma of rabbits was $20323 \pm 1136$ 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 $806.8 \pm 65.6$ ng/ml, which is 96% less than the maximum concentration at its intravenous 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 ($\alpha$ and $\beta$ phases). The $\alpha$ 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 $\beta$ 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 $\text{AUC}_{0 \to 36}$ was $13531 \pm 1478$ ng×h/ml, while the ratio $\frac{\text{AUC}_{36 \to \infty}}{\text{AUC}_{0 \to 36}}$ was $2.96 \pm 1.59\%$, 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 acexazolamide 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 ($\text{AUC}_{0 \rightarrow 36}$ and $\text{AUC}_{0 \rightarrow \infty}$), with intravenous injection of acexazolamide at a dose of 1 mg/kg |
| Parameter | $\text{AUC}_{0 \rightarrow 36}$, ng×h/ml | $\text{AUC}_{0 \rightarrow \infty}$, ng×h/ml | $\text{AUC}_{36 \rightarrow \infty} / \text{AUC}_{0 \rightarrow 36}$, % |
|------------|-----------|----------------|-----------------|
| 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           | 10.9            |
| CV, %      | 10.9      | 12.20          | 153.90          |
| Median     | 12965     | 13262          | 3.00            |
| Min        | 11906     | 12196          | 1.05            |
| Max        | 15642     | 16373          | 4.68            |
| Confidence interval | 11979–15083 | 12161–15732 | 1.28–4.63 |

Conclusion

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

On the models of acute exudative and chronic 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 chronic 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 effect 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) kg/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 acetazolamide was close to that of normal skin on the 15th day.

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

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