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The effect of an aminoguanidine derivative on adjuvant arthritis in rats

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ABSTRACT

Aim. To study anti-inflammatory, analgesic, and possible ulcerogenic effects of a novel aminoguanidine derivative in adjuvant arthritis (a model of rheumatoid arthritis) in rats.

Materials and methods. The experiments were carried out on 42 outbred male Sprague Dawley rats. After modeling arthritis (starting from day 7 after the administration of complete Freund's adjuvant), intramuscular injections of the aminoguanidine derivative (code LIS-M) at a dose of 2.5, 5, and 10 mg / kg or the reference drug diclofenac at a dose of 4 mg / kg were performed once a day for 22 days. The volume of the inflamed limb was measured twice a week, pain threshold was measured every week. After finishing the administration of the compounds, the levels of interleukin (IL) 1, IL-6, and tumor necrosis factor-α (TNFα) were measured in rat plasma, the ankle joint was histologically studied, and the gastric mucosa was studied to detect damage, ulcers, and scarring.

Results. The aminoguanidine derivative, an inhibitor of inducible nitric oxide synthase, was more effective at the dose of 10 mg / kg than diclofenac at the dose of 4 mg / kg. It had anti-inflammatory and analgesic effects in the joint affected by complete Freund's adjuvant, promoted restoration of the histologic structure in the synovial membrane and articular cartilage, and reduced the plasma concentration of IL-1, IL-6, and TNFα by 1.4–1.5 times. The LIS-M compound did not damage the gastric mucosa in rats with adjuvant arthritis.

Conclusion. The aminoguanidine derivative LIS-M exerts potent anti-inflammatory and analgesic effects in adjuvant arthritis in rats (a model of rheumatoid arthritis). LIS-M has no ulcerogenic effect on the gastric mucosa in rats.

Keywords: aminoguanidine derivative, diclofenac, adjuvant arthritis, anti-inflammatory effect, effect on the gastric mucosa, rats

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 5591 of 23.10.2017) and IPHAR LLC (Protocol No. 191 of 10.07.2017).

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Влияние производного аминогуанидина на течение адъювантного артрита у крыс

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РЕЗЮМЕ

Цель исследования – изучить при адъювантном артрите у крыс (модель ревматоидного артрита) противовоспалительное, анальгетическое и потенциальное ульцерогенное действие нового соединения – производного аминогуанидина.

Материалы и методы. Эксперименты проводили на 42 аутбредных самцах крыс стока Sprague Dawley. После развития артрита, начиная с 7-х сут после введения адъюванта Фрейнда, животным в течение 22 сут 1 раз/сут внутримышечно вводили производное аминогуанидина (шифр – LIS-M) в дозах 2,5; 5 и 10 мг/кг или препарат сравнения диклофенак в дозе 4 мг/кг. Объем воспаленной конечности измеряли 2 раза/нед, болевую чувствительность – еженедельно. После завершения введения веществ в плазме измеряли концентрацию интерлейкина (ИЛ) 1, ИЛ-6 и фактора некроза опухоли α (ФНО-α), гистологически изучали ткани заплюсневого сустава, с помощью бинокулярной лупы оценивали состояние слизистой оболочки желудка на наличие дефектов, язв и рубцов.

Результаты. Ингибитор индуцируемой синтазы оксида азота – производное аминогуанидина – в дозе 10 мг/кг эффективнее диклофенака в дозе 4 мг/кг оказывало в суставе, поврежденном адъювантом Фрейнда, противовоспалительное и анальгетическое действие, способствовало восстановлению гистологической структуры синовиальной оболочки и суставного хряща, в 1,4–1,5 раза уменьшало в плазме концентрацию ИЛ-1, ИЛ-6 и ФНО-α. Соединение LIS-M не повреждало слизистую оболочку желудка крыс с адъювантным артритом.

Заключение. Производное аминогуанидина LIS-M оказывает выраженное противовоспалительное и анальгетическое действие при адъювантном артрите у крыс α модели ревматоидного артрита. LIS-M лишен ульцерогенного действия на слизистую оболочку желудка крыс.

Ключевые слова: производное аминогуанидина, диклофенак, адъювантный артрит, противовоспалительное, анальгетическое действие, влияние на слизистую оболочку желудка, крысы

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Rheumatoid arthritis is usually treated with immunosuppressive drugs and glucocorticoids and, at earlier stages, with nonsteroidal anti-inflammatory drugs (NSAID) [1, 2]. NSAID inhibit cyclooxygenase-2 and reduce prostaglandin production in the inflamed tendon sheath. Prostaglandin E_2 at a low concentration is unable to activate the nuclear factor kB, which inhibits its activating effect on nitric oxide (NO) synthase, induced during inflammation. This results in reduced production of aggressive proinflammatory cytokines, such as prostaglandins and NO, in the joints [3]. In normal conditions, these compounds regulate many functions in the body, in particular, they exert a gastroprotective effect. Nitric oxide increases production of protective mucosa in the stomach, improves blood flow, and reduces leucocyte migration to the ulceration site [4, 5]. Therefore, development of a selective inhibitor of inducible nitric oxide synthase (iNOS), able to inhibit NO synthesis at the inflammation site without adverse ulcerogenic effect, is promising. According to prior studies, a new compound, an aminoguanidine derivative codenamed LIS-M, has these properties.

The aim of the study was to investigate the anti-inflammatory, analgesic, and potential ulcerogenic effects of the aminoguanidine derivative LIS-M in adjuvant arthritis (a model of rheumatoid arthritis) in rats.

MATERIALS AND METHODS

The aminoguanidine derivative LIS-M is ({[3-(4-nitrophenyl amino)indole-2-yl] methylene} amino)guanidine methanesulfonate (Fig. 1) synthesized at IPHAR LLC (Tomsk, Russian Federation). LD_{50} of LIS-M in intramuscular administration to male rats is 382.6 mg / kg.

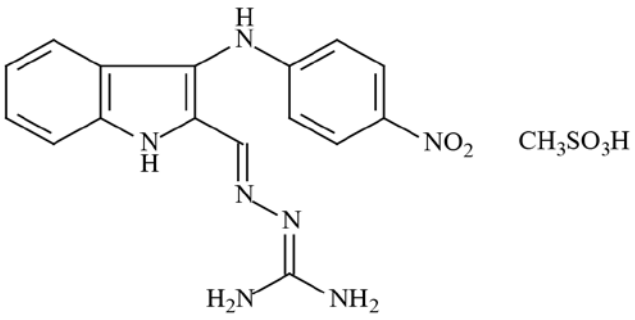

Fig. 1. Structural formula of LIS-M.

Studies were performed in the R&D center of IPHAR LLC using 42 outbred male Sprague Dawley rats weighing 230–250 g (6 groups, 7 animals per group). The animals were obtained from the laboratory animal facility of the R&D center and kept in plastic cages at 18–26 °C, relative humidity 45–65%, 10–11 air changes per hour (ACPH), and 12 / 12 h lighting regime. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 5591 of 23.10.2017) and R&D center (Protocol No. 191-OFI of 10.07.2017) and performed in accordance with the European Convention for the Protection of Laboratory Animals (Strasbourg, 1986) and principles of Good Laboratory Practice (GOST 33044–2014, Decree of the Ministry of Health of Russia No. 199n of 01.04.2016).

Adjuvant arthritis was induced by administering 0.1 ml of complete Freund's adjuvant (CFA) (Sigma, USA) under the plantar aponeurosis of the hind paw [6]. Starting from day 7 after CFA administration (after arthritis has developed), intramuscular injections of the LIS-M compound at a dose of 2.5, 5, and 10 mg / kg or the reference drug diclofenac (Sandoz, Germany) at a dose of 4 mg / kg were performed once a day for 22 days [7]. Both compounds were dissolved in 1% aqueous solution of polyvinylpyrrolidone. The animals of the control group received the equivalent volume of the solvent.

The volume of the inflamed paw was measured using a plethysmometer (Ugo Basile, Italy) before CFA administration and twice a week for 29 days after the start of the experiment. The difference between the volume of the inflamed paw in rats receiving LIS-M or diclofenac and those from the control group was expressed as a percentage [6]. Pain sensitivity in the inflamed limb, caused by sensitized mechanoreflexes, was measured once a week using von Frey filaments (Ugo Basile, Italy). Sharp withdrawal of the paw in response to sensitization with a Frey filament of a certain size was registered as a positive response, the result was expressed in grams. After the last injection of LIS-M at a dose of 10 mg / kg and diclofenac at a dose of 4 mg / kg, the levels of interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α) were measured by enzyme-linked immunosorbent assay (Vector-Best, Russian Federation).

After finishing the administration of the compounds, the animals were sacrificed in a carbon dioxide chamber. The tissues of the ankle joint, formed by the lower leg bones, tarsus, and the proximal end of the tarsal bones, were studied histologically in paraf-

fin-embedded sections stained with hematoxylin and eosin. The sections were studied using the Carl Zeiss microscope (Germany) at ×100 magnification. The degree of damage to the joint was assessed using a 5-point scale [8].

The score for the maximum severity of the pathology in one animal by all parameters (hyperplasia within the tendon sheath, inflammatory infiltrate in the periarticular tissue, joint effusion, joint space narrowing, pannus formation) was 25. The gastric mucosa of the animals was assessed for defects, ulcers, and lesions using a binocular loupe (Micromed, Russian Federation) at ×10 magnification.

Normality testing in the groups was performed using the Shapiro – Wilk test. The results of measuring the paw volume, pain threshold, and inflammation marker concentration were presented as the mean and the standard error of the mean ($M \pm m$). The one-way ANOVA method with subsequent Tukey test was used to access the intergroup differences in the variables. The results of the histological examination were presented as the total score per group, as well as the median and the interquartile range (Me (Q_1; Q_3)). The Kruskal – Wallis test was used to assess the intergroup differences in the baseline score. The differences between the groups were statistically significant at $p \leq 0.05$. Statistical processing of the data was performed using Statistica 10.0 software (StatSoft, USA).

RESULTS AND DISCUSSION

On the day of CFA administration, hyperemia and swelling of the periarticular tissues were observed in the rats. By day 7, the volume of the limb in which CFA had been injected increased by 3.1 times, reached the maximum by day 10, and was 4.4 times larger than in the intact animals by the end of the study. After 3 injections of LIS-M at the dose of 10 mg / kg, the volume of the inflamed limb decreased by 35%, after 10 injections – by 53%, and after 21 injections – by 73%, compared with the control group ($p < 0.05$). LIS-M had no anti-inflammatory effects at the dose of 2.5 and 5 mg / kg. Diclofenac at the dose of 4 mg / kg reduced limb swelling by 54% after 3 injections, and by 65% – by day 21 of the study ($p < 0.05$). LIS-M at the dose of 10 mg / kg and diclofenac at the dose of 4 mg / kg had equal anti-inflammatory effects ($p > 0.05$) (Fig.2).

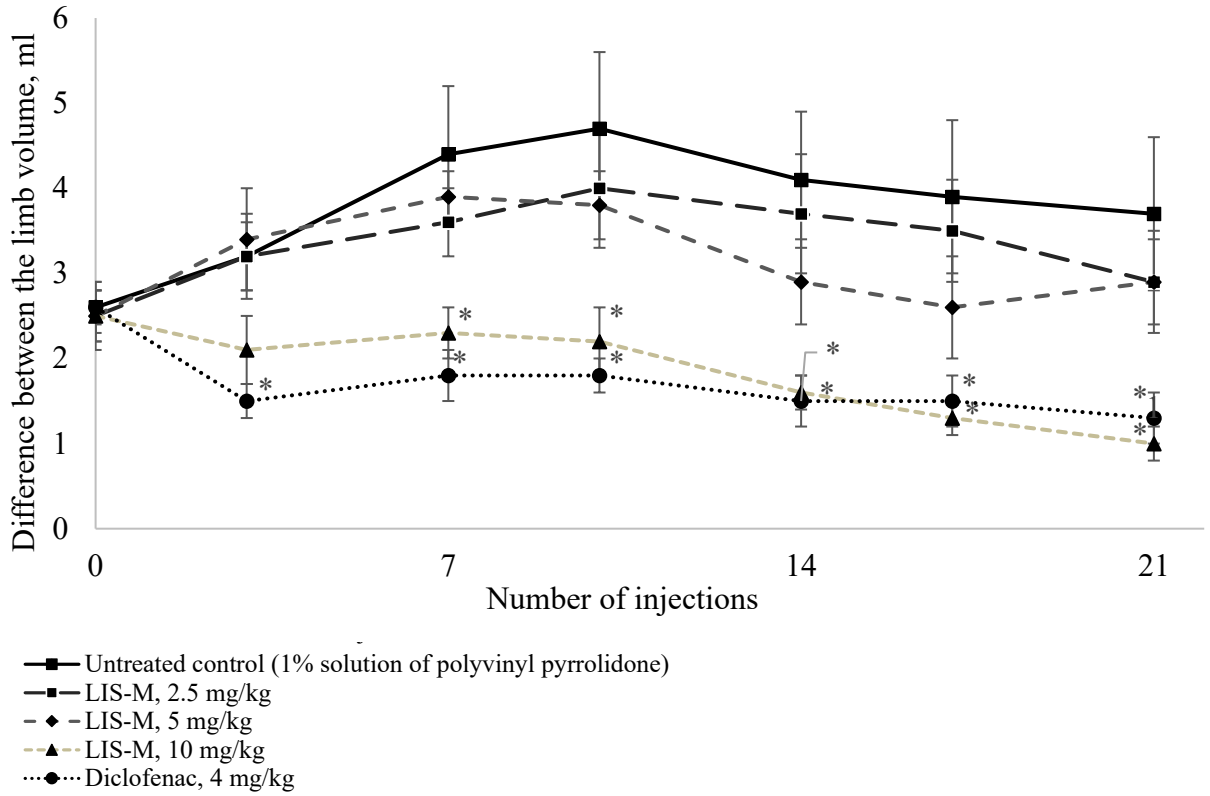

Fig. 2. Anti-inflammatory effect of LIS-M (2.5, 5, 10 mg / kg) and diclofenac (4 mg / kg) in adjuvant arthritis: * difference compared with the untreated control group, $p < 0.05$

Adjuvant arthritis resulted in a considerable increase in pain sensitivity in the inflamed limb: on day 7, it grew by 2.7 times, was increasing throughout the study, and was 3.8 times higher than that in the intact animals by day 21. LIS-M at the dose of 10 mg / kg and diclofenac at the dose of 4 mg / kg had analgesic effects after 7 days of treatment. 21 days after the start of the test compound administration, LIS-M had completely normalized pain sensitivity. With diclofenac administration, pain sensitivity reduced, compared with the values in adjuvant arthritis, but was still considerably higher than in the intact animals. The compound LIS-M had no analgesic effects at the dose of 2.5 and 5 mg / kg.

On day 28 after CFA administration, the concentration of proinflammatory cytokines in the blood plasma was elevated: IL-1 – by 2.5 times, IL-6 – by 5 times, TNF-α – by 3 times. After 21 injections of LIS-M at the dose of 10 mg / kg, the level of IL-1, IL-6, and TNF-α decreased by 1.4–1.5 times, while diclofenac at the dose of 4 mg / kg reduced their concentration less significantly, by 1.2 times ($p < 0.05$) (Table 2).

Table 1

Pain sensitivity in animals with adjuvant arthritis receiving the aminoguanidine derivative LIS-M or diclofenac, g, $n = 7$, $M \pm m$

Group	Number of injections			
	0	7	14	21
Intact animals, $n = 7$	25.5±2.1	26.3±1.7	26.1±1.4	27.3±1.0
Animals with adjuvant arthritis who received:				
1% solution of polyvinylpyrrolidone (control), $n = 7$	11.3±2.1*	4.2±1.1*	6.2±1.6*	7.2±1.4*
LIS-M, 2.5 mg / kg, $n = 7$	12.9±3.6*	5.8±1.2*	6.6±2.0*	8.1±1.0*
LIS-M, 5 mg / kg, $n = 7$	9.8±2.9*	5.7±0.7*	6.4±0.6*	8.5±1.7*
LIS-M, 10 mg / kg, $n = 7$	9.4±2.6*	15.2±4.1*^	19.9±4.0*^	26.0±1.4^#
Diclofenac, 4 mg / kg, $n = 7$	10.3±2.4*	14.9±3.0*^	16.2±3.3*^	14.7±3.6*^

* $p < 0.05$ compared with the intact animals; ^ $p < 0.05$ compared with the animals with adjuvant arthritis receiving 1% solution of polyvinylpyrrolidone on the corresponding day of the experiment (control); # $p < 0.05$ compared with the animals receiving diclofenac.

Table 2

Cytokine concentration in the blood plasma of rats with adjuvant arthritis after 21-day administration of the aminoguanidine derivative LIS-M or diclofenac, pg / ml, $M \pm m$

Experiment conditions	IL-1	IL-6	TNF-α
Intact animals	35.6 ± 4.4	25.3 ± 4.9	50.3 ± 5.5
Animals with CFA-induced arthritis who received:			
1% solution of polyvinylpyrrolidone (control), $n = 7$	87.8 ± 5.4*	127.7 ± 5.4*	148.5 ± 6.1*
LIS-M, 10 mg / kg, $n = 7$	61.8 ± 4.8*^	85.8 ± 3.8*^	101.8 ± 2.4*^
Diclofenac, 4 mg / kg, $n = 7$	76.3 ± 4.7*	102.5 ± 6.0*	122.1 ± 4.8*

* $p < 0.05$ compared with the intact animals; ^ $p < 0.05$ compared with the diclofenac group.

The histological examination of the ankle joint tissue demonstrated that adjuvant arthritis resulted in proliferative synovitis and inflammation of the periarticular tissues, joint space narrowing, formation of intraarticular pannus, and damage to the articular cartilage. Damage to the joint slightly decreased in the group of animals receiving LIS-M at the dose of 2.5 mg / kg. LIS-M at the dose of 5 mg / kg reduced hyperplasia within the tendon sheath and joint effusion and prevented joint space narrowing. LIS-M at the dose of 10 mg / kg and diclofenac at the dose of 4 mg / kg prevented synoviocyte proliferation, joint effusion, joint space narrowing, and pannus formation (Table 3). LIS-M lowers the NO concentration in the inflamed joint, which inhibits synthesis of collagen and proteoglycans and normalizes joint microstructure [9, 10].

Intramuscular administration of the aminoguanidine derivative LIS-M at the dose of 2.5, 5, and 10 mg / kg in rats with adjuvant arthritis had no ulcerogenic effect: no ulceration or erosion in the gastric mucosa was observed. Diclofenac at the dose of 4 mg / kg caused bleeding, ulcers, and erosions in the gastric mucosa.

Table 3

Severity of histologic changes in the ankle joint in rats with adjuvant arthritis after 21-day administration of the aminoguanidine derivative LIS-M or diclofenac, total score, *Me* (Q_1; Q_3)

Articular tissue damage	Animals with CFA-induced arthritis who received				
	1% solution of polyvinylpyrrolidone, $n = 7$	LIS-M 2.5 mg / kg, $n = 7$	LIS-M 5 mg / kg, $n = 7$	LIS-M 10 mg / kg, $n = 7$	Diclofenac, 4 mg / kg, $n = 7$
Hyperplasia within the tendon sheath	13 2 (1; 3)	8 1 (0; 2)	7* 1 (1; 1)	4* 0 (0; 1)	5* 1 (0; 1)
Inflammatory infiltrate in the periarticular tissue	29 4 (4; 4)	27 4 (4; 4)	23 3 (3; 3)	22 3 (3; 3)	25 4 (3; 4)
Joint effusion	10 1 (0; 1)	4* 0 (0; 1)	1* 0 (0; 0)	0* 0 (0; 0)	0* 0 (0; 0)
Joint space narrowing	16 2 (2; 2)	11 2 (1; 2)	6* 1 (0; 1)	4* 1 (0; 1)	5* 1 (0; 1)
Pannus formation	18 2 (2; 3)	13 2 (1; 2)	10 1 (1; 2)	5* 1 (0; 1)	5* 1 (0; 1)

* $p < 0.05$ compared with the animals with CFA-induced arthritis treated with 1% solution of polyvinylpyrrolidone (control).

CONCLUSION

CFA administration in rats has resulted in progressive swelling and pain hypersensitivity in the injected limb, as well as inflammation and degenerative changes in the tissues of the ankle joint. The levels of proinflammatory cytokines IL-1, IL-6, and TNF-α in the blood plasma increased by 2.5–5 times. The production of these cytokines is stimulated by NO produced in the reaction catalyzed by inducible NO synthase (iNOS) [11]. The aminoguanidine derivative LIS-M, a selective iNOS inhibitor, at the dose of 10 mg / kg was more effective in treating arthritis and alleviating pain than diclofenac at the dose of 4 mg / kg. LIS-M decreased the level of cytokines by 1.4–1.5 times compared with the untreated control, while diclofenac reduced their level by 1.2–1.3 times. An important advantage of LIS-M is the absence of ulcerogenic effects on the stomach. This compound does not inhibit the activity of cyclooxygenase and constitutive isoforms of NO synthase and does not disrupt synthesis of gastroprotective prostaglandins and NO.

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