

Antitumor activity of the novel pyridine derivative

Ekaterina V. Blinova¹, Anna V. Epishkina¹, Oksana M. Tumutolova², Olga N. Deryabina², Sofia Ya. Skachilova³, Mihail Yu. Kudriavtsev², Evgenia V. Shikh¹, Olga S. Vavilova¹, Yulia S. Gilevskaya¹, Gordey V. Brykin¹, Anna A. Makhrova⁴, Dmitry S. Blinov^{3,4}

¹ Sechenov University, 8/2 Trubetskaya St., Moscow 119991, Russia

² Ogarev National Research Mordovia State University, 68 Bolshevistskaya St., Saransk 430005, Russia

³ All-Union Research Center for Safety of Biological Active Substances, 23 Kirova St., Staraya Kupavna, Moscow region 125450, Russia

⁴ Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, 1 Samory Mashela St., Moscow 117998, Russia

Corresponding author: Ekaterina V. Blinova (bev-sechenov@mail.ru)

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Abstract

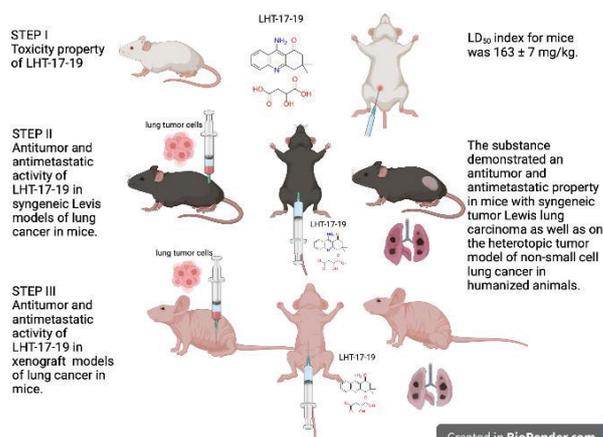
Introduction: The study aim was to explore a toxicological property and antitumor action of the novel pyridine derivative LHT-17-19 in cell culture and on experimental models of lung cancer in mice.

Materials and methods: The study was performed on male and female ICR(CD-1), male BALB/c, male BALB/c nu/nu mice. Pyridine derivative (LHT-17-19) was studied as water-soluble pharmaceutical substance. Acute toxicity was evaluated in groups of 5 animals, and the results were analyzed by Finney. Antitumor and antimetastatic activity was studied in syngeneic and xenograft models of lung cancer in mice.

Results and discussion: LHT-17-19 belongs to class 3 of the toxicity classification of chemicals in accordance with GOST 12.1.007–76. The substance demonstrated an antitumor and antimetastatic property in mice with syngeneic tumor Lewis lung carcinoma as well as on the heterotopic tumor model of non-small cell lung cancer in humanized animals.

Conclusion: LHT-17-19 belongs to class 3 of the toxicity classification of chemicals in accordance with WHO recommendation. LHT-17-19 exerts antitumor and antimetastatic property on both syngeneic and patient-derived lung cancer xenograft murine models.

Graphical abstract



Keywords

pyridine derivative (LHT-17-19), lung cancer, acute toxicity, antitumor action, antimetastatic activity, mice.

Introduction

Lung cancer for decades has taken a leading position among oncological diseases and is the most common cause of cancer-related death worldwide with five-year survival being under 20% (Bray et al. 2018; Herbst et al. 2018). Lung adenocarcinoma is the most common histological subtype of non-small cell lung cancer (NSCLC), accounting for about 40% of lung neoplasia (Chen et al. 2014). Smoking, long-term exposure to radon, influence of carcinogens, pollution and genetic susceptibility are at the top of NSCLC risk factors (Gould et al. 2013). However, in recent years, the number of cases in non-smokers has also increased, which emphasizes the importance of identifying risk factors for non-smokers in the development of NSCLC (Molina et al. 2008). The results of genetic studies have made it possible to determine the molecular drivers of NSCLC, among which the ones caused by somatic mutations in the *TP53*, *KRAS*, *KEAPI*, *STK11*, and *EGFR* genes (Campbell et al. 2016).

Current treatments for lung cancer include surgery, radiation therapy, chemotherapy, targeted or immunotherapy, or a combination of these interventions (Herbst et al. 2018). To date, targeted drugs and immunobiological therapy have made significant advances in the fight against NSCLC. EGFR and BRAF V600E mutation inhibitors, ALK and ROS1 blockers, and immune checkpoint (PD-1, PD-L1) antibodies have been approved for precision treatment of NSCLC (Peters et al. 2018). However, despite relentless progress, there remains a large population of patients with NSCLC without available targeted therapeutic options, either due to the lack of known genetic mutations in key oncogenic signaling pathways or due to the difficulty of targeting oncogenic mutations (Rotow et al. 2017). In addition, patients with lung cancer may experience congenital and acquired resistance to targeted therapy (Herbst et al. 2018). Therefore, the study aim was to explore a toxicological property and antitumor action of the novel pyridine derivative LHT-17-19 in cell culture and on experimental models of lung cancer in mice.

Materials and methods

Animals

The experimental study was performed on 50 ICR (CD-1) mice of both sexes weighing 18–20 g, 30 male BALB/c mice and 15 male BALB/c nu/nu mice weighing 18–20 g. All the animals were obtained from the Pushchino Animals Breeding Facility and were kept under natural daylight condition with standard temperature and humidity. The

study conformed to all the requirements of GLP and European Convention for the Protection of Vertebrates Animals Used for Experimental and Other Scientific Purposes regulations. The study protocols passed an ethical review at the Local Ethics Committee of Sechenov University (Moscow, Russia) meeting April 20, 2021 (Reg. No. 199-n/04-2021). The study was financially supported by the grant of the President of the Russian Federation NSh-843.2022.3 (agreement No. 075-15-2022-842 dated May 12, 2022).

Substances and drugs

Novel pyridine nucleoside compound (laboratory name LHT-17-19) was synthesized at the Department of Chemistry, Technology of Synthetic Medicines and Analytical Control of All-Union Research Center for Safety of Biological Active Substances.

We used **Cyclophosphamide** in a form of chemical substance (Merck Sigma-Aldrich, Germany, purity of 98.85%, CAS Number: 91329A) as a reference pharmacological agent.

All the studied substances after being dissolved in 0.9% saline were administered intraperitoneally in a volume of 1.0 ml. On the model of syngeneic lung carcinoma, LHT-17-19 and **Cyclophosphamide** were administered at a single dose related to 5.0% of LD₅₀ index on day 7 after tumor transplantation. On the model of patient-derived heterotopic xenograft of non-small cell lung cancer, experimental animals received the studied intervention and the comparator drug from day 7 after cancer tissue inoculation for 5 days once daily at two doses corresponding to 2.5 and 5.0% of LD₅₀ index.

Acute toxicity determination

Acute toxicity of LHT-17-19 was studied in male and female ICR (CD-1) mice. Animals were divided into groups of 5 mice each. LHT-17-19 was dissolved in 0.9% saline and administered intraperitoneally (IP) in increasing concentrations in a volume of 0.5 ml. The survival of animals was recorded for 14 days onwards. The LD₅₀ index was calculated by Finney method in accordance with the current methodological recommendations (Gad 2006).

Tumor models of mice

We used two animal models in our study. To establish syngeneic murine tumor model, we chose Lewis approach (LLC). The live tumor was obtained at the Institute of Experimental Oncology of National Medical Research Center of Oncology (Moscow, Russia). A suspension of 1×10⁶ tumor cells per 100 μL was prepared using Hanks

solution (Biolot, Russia). Tumor cells were inoculated subcutaneously at the region of the left thigh. Antitumor treatment began on day 7 after transplantation. The antitumor effect of LHT-17-19, as well as its anti-metastatic property, was evaluated in accordance with the current guidelines by measuring the size of the primary tumor node and determining its volume (V), weight (at the end of the experiment), tumor growth inhibition index (TGII), and survival analysis (Kiseleva et al. 2022). Animals were surveyed for 65 days after treatment or till death (whichever occurs first). On day 65, the survived mice were euthanized under neoflurane anesthesia, and primary tumors and lungs were extirpated for further processing. The number of lung metastases was counted immediately after fixing murine lungs in Karnoy's solution, using a binocular loupe with a magnification of 8×2.

A tumor tissue of non-small cell lung cancer (histologically confirmed adenocarcinoma) was obtained during surgery at the Cancer Clinic of Sechenov University from a 53-year-old man who gave voluntary informed consent and had not previously received chemotherapy treatment. The tumor model was reproduced by successive three-stage transplantation of tumor tissue particles in the animals on the left hind paw subcutaneously according to (Chijiwa et al. 2015). On day 22 after the cessation of treatment, half of the animals randomly selected in each group were withdrawn from the experiment under isoflurane anesthesia. The antitumor effect of the substance and its antimetastatic activity were evaluated in accordance with the current recommendations, by measuring the size of the primary tumor node, its weight (at the end of the experiment) and calculating the tumor growth inhibition index (TGII). The number of superficial metastases in the lung was counted using a binocular microscope with 8×2 magnification. The remaining animals in each group were followed up to 90 days from the point of tumor transplantation to record survival and remission rates (Blinova et al. 2018).

Statistical analysis

Statistical analysis of the obtained data was carried out using STATA version 15.3 (USA). After assessment of the normality of continuous variable distribution two-tail t-test or ANOVA with post-hoc Tukay's parametric criterion was used. For categorical variables, nonparametric exact Fisher test was applied. The survival Kaplan-Meier curve as a line indicating the number of the deceased animals at different time points after cessation of treatment was analyzed using a log-rank test (Glantz 2012).

Results and discussion

LHT-17-19 Acute toxicity

LHT-17-19 (IP) LD₅₀ index for mice was 163±7 mg/kg. This indicator was 1.5 times less than one of the reference drugs [Cyclophosphamide](#) (Table 1). LHT-17-19 belongs to class 3 (moderately toxic substances) of the toxicity classification of chemicals in accordance with WHO recommendations (Berezovskaya 2003).

Table 1. Acute toxicity of IP LHT-17-19 as pharmaceutical substance in ICR (CD-1) mice

Substance (compound)	LD ₅₀ , mg/kg, Mean±SD	95% CI
Cyclophosphamide	108±6	[96÷120]
LHT-17-19	163±7*	[153÷174]

Note: * p = 0.003 when compared with [Cyclophosphamide](#) (two-tail t-test).

Antitumor and antimetastatic effect of the LHT-17-19 on the syngeneic tumor model of Lewis lung carcinoma (LLC)

The results of the study of the antitumor effect of the compound on the syngeneic model of Lewis lung carcinoma are shown in Table 2.

Single intraperitoneal injection of the LHT-17-19 as pharmaceutical substance on day 7 after tumor cells transplantation decreased the volume of the tumor node on day 14 of the experiment by 29.6% compared with the control. Tumor volume inhibition was comparable with that following the administration of the reference drug [Cyclophosphamide](#). On day 22, the LHT-17-19 antitumor effect was maintained, and the TGII coefficient achieved a rate of 51.7. Tumor growth inhibition index of [Cyclophosphamide](#) was 31.9.

For antimetastatic property, it was shown that neither in the LHT-17-19 group, nor in the group of the reference drug [Cyclophosphamide](#), superficial lung metastases were found.

The median survival of the untreated animals with LLC tumor was 32 days. Single intraperitoneal injection of [Cyclophosphamide](#) led to an increase in survival by 27.7%, which amounted to 47 days. The median survival in LHT-17-19 group was 52 days (Fig. 1). Long-test showed significant difference between survival time in the control group and both intervention groups (p = 0.01).

Table 2. Antitumor action of IP LHT-17-19 on the model of Lewis lung carcinoma in BALB/c mice (n = 5 in each group)

Group, dose (mg/kg/day)	Tumor volume (mm ³), Mean±SD		TGII	Number of metastases, Mean±SD		MII
	Day 14	Day 22		Day 22	Day 22	
Control	7840±23	14340±123	-	15±4.3		
Cyclophosphamide (18.0)	5520±53*	9760±76*	31.9	0*		100
LHT-17-19 (81.5)	5670±31*	6920±42 ^a	51.7	0*		100

Note: * p < 0.05 when compared with control; ^a p < 0.05 when compared with [Cyclophosphamide](#) (ANOVA, Tukay's criterion).

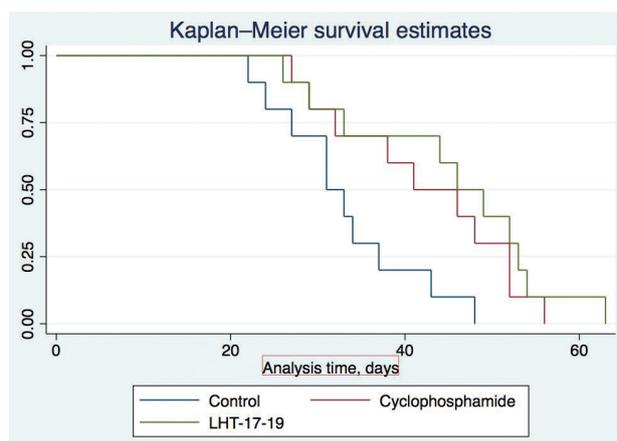


Figure 1. Experimental mice survival curves.

Antitumor and antimetastatic effect of LHT-17-19 on the heterotopic tumor model of non-small cell lung cancer

Intraperitoneal administration of both doses of LHT-17-19 – 81.5 and 40.75 mg/kg/day – for 5 days from day 7 after transplantation of tumor tissue was accompanied by statistically significant differences in the tumor node volume when compared with the control group and the group of animals receiving 18.0 mg/kg/day of Cyclophosphamide. Effect was registered from day 10 of follow-up period (Fig. 2). Development of the antimetastatic effect was accompanied by a decrease in the number of visible surface metastases, both in comparison with the control group, and with the group of animals receiving the reference drug. A proportional increase in the metastatic inhibition index was observed.

Discussion

Lung cancer remains to be one of the leading causes of death worldwide (Bray et al. 2018; Herbst et al. 2018).

Identification of intracellular signaling pathways involved in the disease onset and progression along with developing novel therapeutic approaches drastically changed the current landscape of treatment options (Herbst et al. 2018). Nevertheless, molecular and genetic variability and extreme mutation potency help the tumor escape from strict pharmacological control (Peters et al. 2018). All these provide rationale for multitude of experimental and clinical research studies aiming to cope with the issue of cancer chemoresistance.

It turned out that one of the possible sources of novel antitumor medication was pyridine derivatives. Chemical modification of the nucleotide helps it not only obtain anti-nucleotide property, but also gain other types of pharmacological actions. Among them, it is worth mentioning membrane-associated and cytoplasmic macromolecule inhibitory effects. These findings were exploited by researchers from All-Union Research Center for Safety of Biological Active Substances to develop a line of novel molecules, one of the most promising of which was extensively studied within the present research.

As many other dihydroacridine compounds, LHT-17-19 is of moderate toxicity when administered intraperitoneally to mice, which was predicted earlier by using special PASSonline software for qualitative “structure – toxicity” analysis (Shimanovsky et al. 2021). Antitumor efficacy of LHT-17-19 on both syngeneic and patient-derived lung cancer xenograft murine models indicates possibility for targeted mode of the compound action, which is to be rigorously evaluated in our further studies.

Conclusion

According to the obtained data, the following conclusions were made:

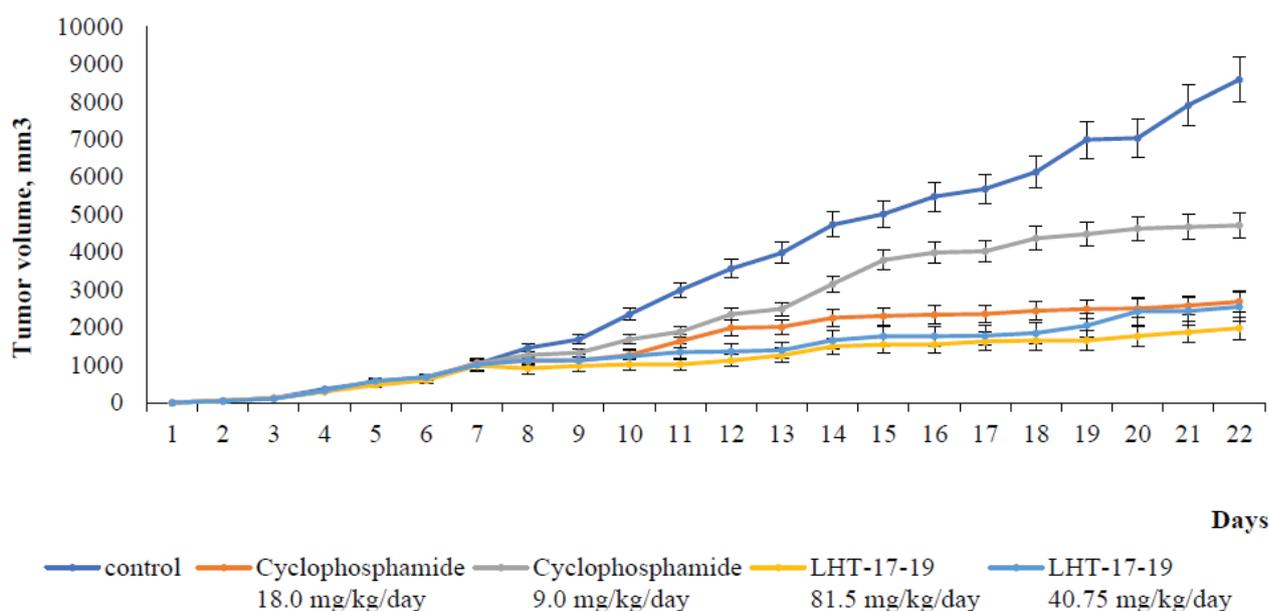


Figure 2. Growth of primary heterotopic node of patient-derived lung carcinoma in research groups.

LHT-17-19 belongs to class 3 of the toxicity classification of chemicals in accordance with GOST 12.1.007–76.

LHT-17-19 exerts antitumor and anti-metastatic effects on both syngeneic and patient-derived lung cancer xenograft murine models.

Conflicts of interest

The authors have no conflict of interests to declare.

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Author contributions

- **Ekaterina V. Blinova**, Doctor Habil. of Medical Sciences, Professor of the Department of Operative Surgery and Clinical Anatomy, Sechenov University, 8/2 Trubetskaya St., Moscow 119991 Russia; e-mail: bev-sechenov@mail.ru **ORCID ID:** <http://orcid.org/0000-0003-0050-0251>. The author organized the project, analyzed the collected data, wrote and revised the manuscript.
- **Anna V. Epishkina**, postgraduate student, Department of the Operative Surgery, Sechenov University, 8/2 Trubetskaya St., Moscow, Russia 119991; e-mail: afina-nn@mail.ru **ORCID ID** <https://orcid.org/0000-0001-7681-0382>. The author conducted the experiments on LLC and prepared the manuscript for publishing.

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- **Oksana M. Tumutolova**, Candidate of Medical Sciences, Associate Professor, Department of Oncology, Ogarev Mordovia State University, 68 Bolshevistskaya St., Saransk 430005 Russia; e-mail: tumutolov@rambler.ru **ORCID ID** <https://orcid.org/0000-0002-5657-0868>. The author conducted the statistic processing of the obtained data.
- **Olga N. Deryabina**, Candidate of Medical Sciences, Associate Professor, Department of oncology, Ogarev Mordovia State University, 68 Bolshevistskaya St., Saransk 430005 Russia; e-mail: dr.deryabina@gmail.com **ORCID ID** <https://orcid.org/0000-0002-8814-3369>. The author conducted the statistic processing of the obtained data.
- **Sofia Ya. Skachilova**, Doctor Habil. of Chemical Sciences, Professor, Head of Pharmacology Department, All-Union Research Center for Safety of Biological Active Substances, 23 Kirov St., Staraya Kupavna, Moscow region 125450 Russia; e-mail: skachilova@mail.ru **ORCID ID** <https://orcid.org/0000-0003-4486-8883>. The author supervised the synthesis of new pyridine derivatives.
- **Mihail Yu. Kudriavtsev**, postgraduate student, Department of Oncology, Ogarev Mordovia State University, 68 Bolshevistskaya St., Saransk 430005 Russia; e-mail: kudrmih@mail.ru **ORCID ID** <https://orcid.org/0000-0003-0691-4228>. The author conducted the experiments and the statistic processing of the obtained data.
- **Evgenia V. Shikh**, Doctor Habil. of Medical Sciences, Head of Clinical Pharmacology and Internal Disease Department, Sechenov University, 8/2 Trubetzkaya St., Moscow 119991 Russia; e-mail: chih@mail.ru **ORCID ID** <https://orcid.org/0000-0001-6589-7654>. The author analyzed the obtained data and supervised the project.
- **Olga S. Vavilova**, undergraduate student, Sechenov University, 8/2 Trubetzkaya St., Moscow 119991 Russia; e-mail: olga.vavilova18@yandex.ru **ORCID ID** <https://orcid.org/0000-0002-1096-9996>. The author performed the experiments on the heterotopic tumor model of non-small cell lung cancer.
- **Yulia S. Gilevskaya**, undergraduate student, Sechenov University, 8/2 Trubetzkaya St., Moscow 119991 Russia; e-mail: gilevskayaaa@inbox.ru **ORCID ID** <https://orcid.org/0000-0002-8464-4916>. The author performed the experiments on the heterotopic tumor model of non-small cell lung cancer.
- **Gordey V. Brykin**, undergraduate student, Sechenov University, 8/2 Trubetzkaya St., Moscow 119991 Russia; e-mail: sechengord@mail.ru **ORCID ID** <https://orcid.org/0000-0001-9985-8880>. The author performed the experiments on LLC tumor model.
- **Anna A. Makhrova**, Junior Researcher, Department of Molecular and Clinical Pharmacology, Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology; 1 Samory Mashela, Moscow 117998 Russia; e-mail: anna.mahrova@fcho-moscow.ru **ORCID ID** <https://orcid.org/0000-0003-3572-0625>. The author performed the experiments on LLC tumor model.
- **Dmirty S. Blinov**, Doctor Habil. of Medical Sciences, Principal Researcher, Pharmacology Laboratory, All-Union Research Center for Safety of Biological Active Substances, 23 Kirov St., Staraya Kupavna, Moscow region 125450 Russia; Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, 1 Samory Mashela, Moscow 117998 Russia; e-mail: blinov-pharm@yandex.ru **ORCID ID** <http://orcid.org/0000-0002-8385-4356>. The author designed the project, provided consulting support, and, finally, revised the manuscript.