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Research Article

Method for obtaining supramolecular complex based on 3-ohypyridine derivatives and its application for correction of osteoporosis

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Abstract

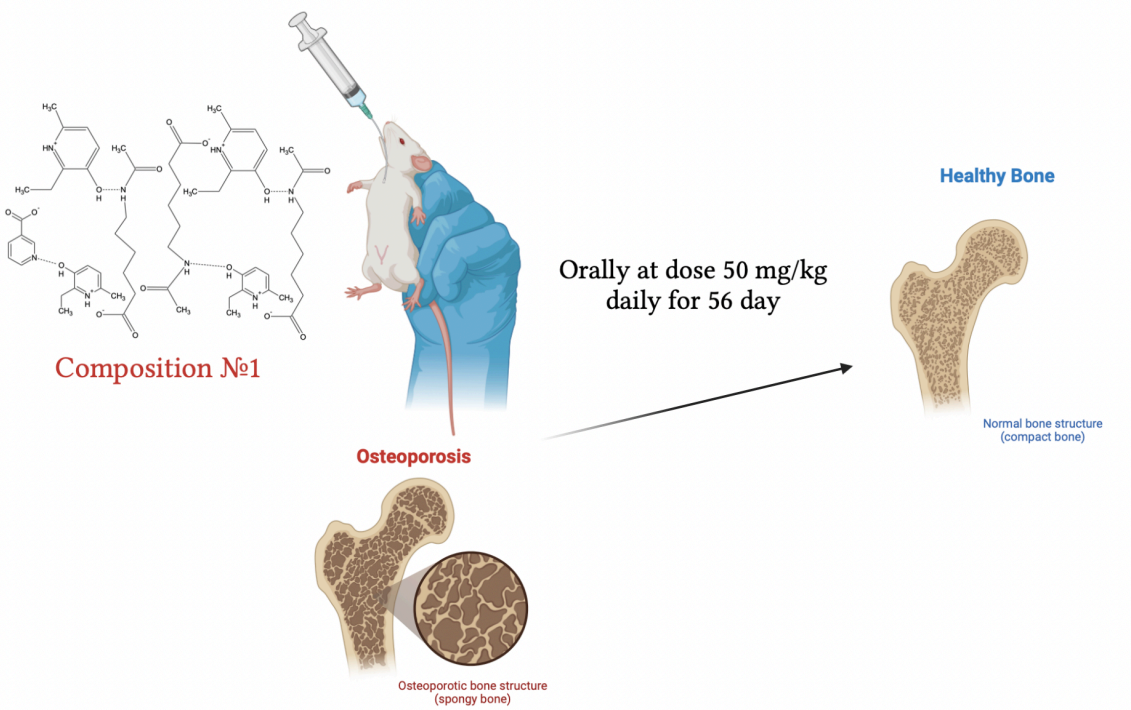
Introduction: Currently, there are no safe and ideal medicines for the prevention and treatment of oophorectomy-induced osteoporosis. The development of an effective pharmacotherapy for hypoestrogen-induced osteoporosis is an important task of pharmacology.

Materials and Methods: A new supramolecular complex (composition №1) was obtained on the basis of 3-hydroxypyridine derivatives: 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate and 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate in a ratio of 1:3 by topochemical synthesis. The study of the osteoprotective activity of the supramolecular complex at a dose of 50 mg/kg was performed on 60 white female rats of the Wistar line on a model of hypoestrogen-induced osteoporosis. The effectiveness of the osteoprotective activity of the complex was evaluated on the 57th day of the experiment.

Results and Discussion: The new supramolecular complex (composition №1) has osteoprotective activity, which is expressed in improving the indicators of X-ray and histomorphological samples. Oral administration of composition №1 at a dose of 50 mg/kg led to an increase in bone density to values of 2.55±0.02 g/cm^3, which is 1.3 times higher than in the control group 1.92±0.01 g/cm^3 ($p \leq 0.05$) and reduce bone resorption by improving cortical and trabecular bone structures.

Conclusion: The obtained data characterize the prospects of studying composition №1 for the correction and prevention of hypoestrogen-induced osteoporosis.

Graphical abstract:



Keywords

hypoestrogen-induced osteoporosis, supramolecular complex, derivatives of 3-oxypyridine

Introduction

Although several pharmacological agents are currently available for the prevention and treatment of postmenopausal osteoporosis, including estrogens and bisphosphonates, the various side effects of these antiresorptive drugs cause many patients to stop using them (Kawai et al. 2011; Martiniakova et al. 2020; Korokin et al. 2022). Given the limitations of the existing treatment options for postmenopausal osteoporosis, there is an unmet need for drug alternatives with minimal side effects.

As one of these compounds, a supramolecular complex consisting of 3-hydroxypyridine derivatives (hereinafter referred to as composition №1) including molecule of 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate (laboratory code LHT 21-16) and three molecules of 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate (laboratory code LHT 21-18).

The LHT 21-16 compound has a wide spectrum of pharmacological activity (Trunov et al. 2020; Peresypkina et al. 2020; Kesarev et al. 2017). A comprehensive study of its biological properties allowed us to establish that it can act as a potential and effective antihypoxant, antioxidant, hepato-, photo-radioprotector.

Organic acid LHT 21-18 is a derivative of aminocaproic acid is able to accelerate the cleansing of the wound surface from necrotic masses, reduce exudative processes, activate the growth of granulation tissue, vascularization, stimulate the formation of bone marrow, accelerating the healing process, and bone fractures (Pakhomov et al. 2020).

Material and Methods

Experimental animals

The study was conducted on 60 female Wistar rats weighing 220–280 g. Ethical principles for the treatment of laboratory rats were observed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental or other Scientific Purposes (ETS N 123). The experimental studies were approved by the Bioethical Commission of Belgorod State National Research University (Minutes 12/10 of 10 June 2022). All manipulations on rats were performed after intraperitoneal administration of chloral hydrate solution at a dose of 150 mg/kg and tiletamine at a dose of 60 mg/kg. The design of the experiment is schematically shown in Figure 1.

The development of generalized osteoporosis was assessed eight weeks (on day 57) after ovariectomy (Gudyrev et al. 2019). The following groups were included in the design of the experiment: sham-operated animals (daily oral administration of saline); control group, simulating bilateral ovariectomy (OV); OV + LHT 21-16 at a dose of 50 mg/kg; OV + LHT 21-18 at a dose of 50 mg/kg; OV+ composition №1 at a dose of 50 mg/kg); OV + alendronic acid (Alendronate) at a dose of 2.5

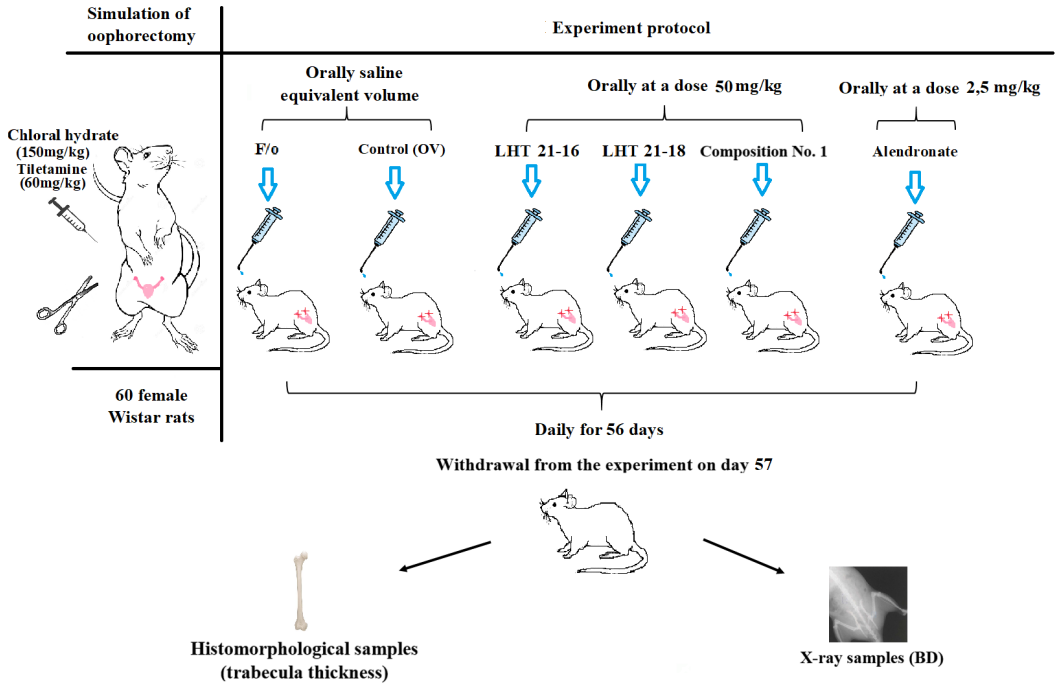


Figure 1. Experiment design

Each experimental group included 10 animals (Fig.1).

Bone density research

X-ray samples were studied using the IN-VIVO MS FX PRO multifunctional laboratory X-ray unit manufactured by Bruker (USA) with a molecular imaging system using licensed software – BoneDensitySoftware.

Morphofunctional assessment of the state of bone tissue

The tibia bones were chosen as the object of study for histological examination. For histological examination, they were isolated entirely with adjacent soft tissues and fixed in 10% formalin. The proximal segment was dissected from the fixed bones for 1 cm from the articular surface of the condyles. The material was decalcified in Surgipath Decalcifier II (Leica, Germany) according to the recommended protocol. Calcined fragments were automatically poured into paraffin according to the standard protocol, followed by staining sections 7 microns thick with hematoxylin and eosin and with Maallory.

Micropreparations were studied by scanning under a Lomo microscope with a DV1000 video camera. Using the McrAView 7.3.1.7 program (LOMO-microsystems, Russia), the thickness of the bone trabeculae and the cortical bone of the diaphysis was measured. Data registration and statistical processing were carried out using MS Excel spreadsheets according to standard formulas with a distribution type check.

Statistical analysis

The data were checked for normal distribution using the Shapiro-Wilk criterion. Normally distributed data were compared using conventional one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Non-normally distributed data were compared with the Kruskal-Wallace test and the post hoc Dunn test. Differences were determined at a significance level of 0.05. Statistical analysis was carried out using GraphPad Prism 9.2.0 software.

Results and Discussion

Chemical reagents necessary to prepare the compound were purchased from commercial suppliers who have a certificate for chemical products (Sigma-Aldrich, USA). The way of synthesis of composition №1 consisted in the following stages: 26.0 g (0.1 g/mol) of 2-ethyl-6-methyl-3-hydroxypyridinium 3-pyridinocarbonoate are loaded into the homogenizer, 93.2 g (0.3 g/mol) 2-ethyl-6-methyl-3-hydroxypyridinium N-acetyl-6-aminohexanoate. The mass was homogenized for 10-15 minutes at a stirring speed of 300-400 rpm. Next, the particle size of the resulting powder was checked, which was to be no more than 10 microns, and if necessary, additionally homogenized. The output was 119.0 g of white fine crystalline powder with Tm. = 139 - 143 °C. The resulting compound was soluble in water, forming light opalescent solution. Found, %: C 62.48; H 7.98; N 9.39 C62 H94 N8O15 m.m. 1191.46; Calculated, %: C 62.50; H 7.95; N 9.41; O 20.14. IR spectrum (v, sm-1): 3412 (OH) 3290 (NH), 2941 (CH), 2673 (N+), 1781 (C=N-), 1634 (C=C), 1561 (COO-) (the synthesis of the compounds was carried out at JSC All-Union Scientific Center for the Safety of Biologically Active Substances, Staraya Kupavna, Russia).

The mass spectrum of the protonated supramolecular complex in the positive ion scanning mode $[M+H]^+$ is, M/z: 1195.46, which corresponds to m.m. 1191.46.

The chemical formula of the compound (composition №1) is shown in Figure 2.

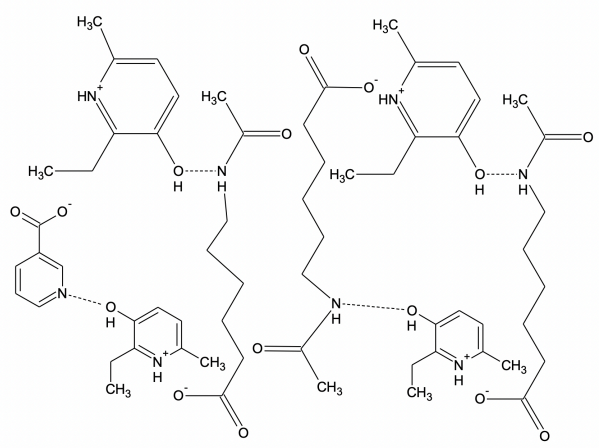


Figure 2. Chemical formula of composition No. 1

Before simulating ovariectomy, there were no significant differences in baseline bone density in any of the six experimental groups of animals. The average values of the density of the femur in the groups were 3.11±1.03 g/cm³, which confirms the absence of clinical signs of osteoporosis in experimental animals. Osteoporotic changes in the bones of the skeleton 8 weeks after ovariectomy were confirmed by the results of densitometry. Thus, in the control group on the 57th day after bilateral ovariectomy, there was a significant decrease in bone density to 1.92±0.01 g/cm³ ($p<0.05$), which is 22.7% lower compared to the group of sham-operated animals 2 .85±0.02 g/cm³ ($p<0.05$).

As a result of a histological study of the structures of the compact cortical bone and cancellous trabeculae on the ovariectomy model, a full range of changes was revealed that specifically characterize the pathomorphological picture of hypoestrogen-induced osteoporosis. Of particular note is the damage to the walls of microvessels, with detachment of intimal structures, primarily the endothelium, which was accompanied by hemorheological disorders in the form of erythrostasis and sludge. Microscopy also revealed pathological changes in the spongy bone tissue of the thigh, the thinning of the lattice network of bone trabeculae, as well as the thinning and perforation of the bone plates.

The greatest corrective effect on bone tissue was noted with the introduction of composition №1. At the same time, both qualitative and morphometric parameters of spongy substance trabeculae turned out to be informative. The general architectonics of the cortical bone and spongy substance corresponded to the intact one. In addition to noticeable cellular manifestations of osteoplastic activity, the result of neo osteogenesis in the form of lamellar bone structures on the surface of the beams was also obvious (Fig. 3B). Micrographs of the histological picture of the bone tissue of animals in the experimental group with composition №1 are shown in Figure 3 (A, B, C, D).

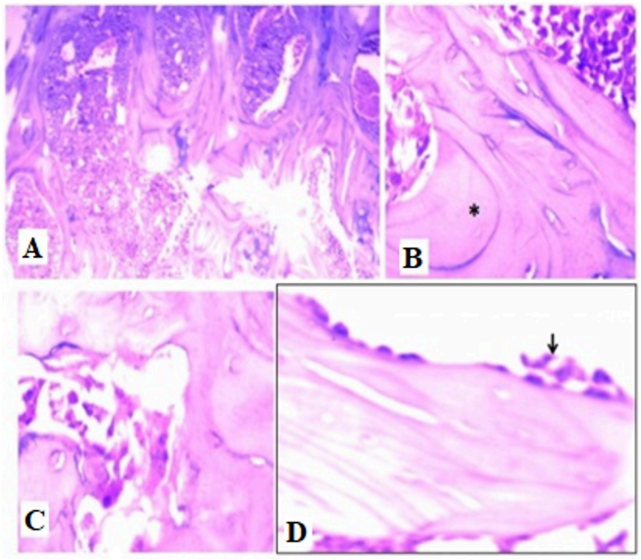


Figure 3. Micrographs of the histological picture of the bone tissue of animals in the experimental group with composition №1. ***Note*: A** – the intact microarchitectonics of the spongy substance; **B** – the osteon with a local thickening of the wall by newly formed bone plates; **C** and **D** – the accumulation of cells of the preosteoblastic type on the surface of the beams. Stained with hematoxylin and eosin. X400.

The quantitatively achieved effect reflects both the absolute and relative increase in the thickness of the bone beams, which varied from 52 to 110 microns. The average thickness is significantly ($p<0.05$) greater than after ovariectomy, and in comparison with monotherapy and reference drug Alendronate at a dose of 2.5 mg/kg. In addition, the most significant indicator is the manifestation of osteoblastic activity, judging by the histological patterns, with a high probability associated with the mobilization of the osteogenic reserve of stromal elements of the bone marrow.

Conclusion

The data obtained clearly indicate that the new supramolecular complex composition №1 based on 3-oxypyridine derivatives obtained by topochemical synthesis has protective properties on bone tissue during ovariectomy, while the degree of protective action exceeds that of compounds in monotherapy and that of the reference drug Alendronate. The obtained data characterize the prospects of studying composition №1 for the correction and prevention of hypoestrogen-induced osteoporosis.

Conflict of interests

The author declares no conflict of interests.

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