

"GEORGE EMIL PALADE" UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND
TECHNOLOGY OF TÂRGU MUREŞ

DOCTORAL SCHOOL OF MEDICINE

ABSTRACT OF PhD THESIS

INNOVATIVE METHODS TO ASSESS HEALTHY AND PATHOLOGICAL BONE TISSUE

PhD student **Emese Orban**

PhD supervisor **Conf. Dr. Pap Zsuzsanna**

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Introduction. The complexity of bone tissue biology is represented by its homeostasis and homeorhesis in normal and pathologic situations. These aspects can be influenced by various pharmacological agents. The study of the impact that lipid-lowering medication may have on bone tissue may be of clinical importance in view of its widespread use to prevent cardiovascular events which are the leading cause of mortality.

Objectives. This work aims to highlight the differences between bone tissue homeostasis in normal and pathologic conditions, as well as the evolution of the callus formation process in this context, under the influence of hypolipidemic medication represented by simvastatin and fenofibrate.

Material and Methods. An experimental study was carried out on bone tissue preparations obtained from 72 adult female Albino Wistar rats aged between 16 and 18 months (corresponding to the perimenopausal period in females, i.e. between 47 and 52 years).

The first surgery consisted of bilateral ovariectomy of 36 rats. Each group of rats, ovariectomized and non-ovariectomized, was divided into three subgroups: control (12 rats), simvastatin-treated (12 rats) and fenofibrate-treated (12 rats). Simvastatin and fenofibrate were administered orally by gavage immediately after ovariectomy daily until euthanized. The daily dose was 10 mg/kg for both drugs. The second surgery, which was performed on all 72 rats, consisted of controlled fracture of the right femur 12 weeks after the first surgery. The animals were anesthetized with ketamine and xylene (4-5 mg/kg) administered intraperitoneally. At 2, 4, 6 and 8 weeks after fracture, three animals in each subgroup were euthanized by intraperitoneal injection of a lethal dose of 8-10 mg/kg ketamine and xylene. The right femur was decalcified and paraffin-embedded for sectioning and histologic processing, including hematoxylin-eosin staining, and the left femur was preserved in 10% formaldehyde for subsequent measurements.

The study used four quantitative methods to analyze the bone tissue: visible spectroscopy, Fourier transform infrared spectroscopy, performed on biological material obtained from the paraffin-embedded femur. The formaldehyde-preserved femur was evaluated by NMR diffusometry and NMR relaxometry.

Results

Study 1: UV-VIS spectroscopy. In the non-ovariectomized rat groups, both simvastatin and fenofibrate treatment delayed the callus formation process, with the longest delay observed in the fenofibrate-treated group. In contrast, in the ovariectomized groups, fenofibrate treatment favored the callus process, whereas simvastatin treatment delayed it.

Study 2: Fourier transform infrared spectroscopy. Short-term administration of hypolipidemic drugs, i.e. 2 weeks, independent of the presence or absence of estrogens, does not affect the proportion of unsaturated fat in the callus. Evaluation of the bone callus mass in the group of ovariectomized rats showed that remodeling of the primary bone callus into a definitive callus was favored by fenofibrate treatment, with a better outcome than in the control and simvastatin-treated groups. In the group of non-ovariectomized rats, the remodeling of the primary bone callus into definitive callus is delayed in the fenofibrate-treated group compared to the simvastatin-treated group.

Study 3: NMR Diffusiometry. In the case of simvastatin administered to ovariectomized rats, its osteoprotective effect was demonstrated throughout the treatment duration, with the greatest benefit being demonstrated by long-term administration, 8 weeks. The administration of simvastatin to the non-ovariectomized group of rats induced negative osteoporotic changes in bone tissue cytoarchitectonics, but these changes were less severe than with fenofibrate.

Study 4: NMR Relaxometry. In the non-ovariectomized group, both fenofibrate and simvastatin treatment favors the development of bone fragility by showing a connectivity that favors a shift from larger to smaller spaces and a significant decrease in pores that do not show molecular exchange. In the ovariectomized group, the benefit of fenofibrate administration was demonstrated, contributing to a relatively balanced pore connectivity, but with communication predominantly from larger to smaller pore spaces, and a predominance of pores without molecular exchange between them. As for simvastatin, in the long term, the communication is both from larger to smaller spaces and vice versa realizing also a predominance of pores without molecular exchange between them.

Originality of the thesis. For the first time, the same biological material, the femur bone of Albino Wistar rats, was used in four different innovative studies - UV-VIS spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Nuclear Magnetic Resonance (NMR) diffusiometry and two-dimensional T_2 - T_2 proton magnetic resonance (Proton Magnetic Resonance) exchange maps - which allowed to investigate how the interference of pharmacological agents - simvastatin, fenofibrate - influences bone tissue biology in terms of its specific characteristics. Their effects on both the cytoarchitectonics of the proximal end of the femur and on the callus process of the femur of non-ovariectomized and ovariectomized rats were highlighted.