ABSTRACT

The term "vitamin D" refers to a group of steroid-like compounds, which include, among others, vitamin D_2, vitamin D_3 and 1α,25-dihydroxyvitamin D_3 (1,25D; calcitriol). The latter compound is formed in the body as a result of vitamin D_3 transformations, mainly in the liver and kidneys. The biologically active form is 1,25D, a hormone that acts via a specific nuclear vitamin D receptor (VDR) to which 1,25D binds inside its target cells.

1,25D affects mainly the calcium and phosphate metabolism and related tissues - intestines, kidneys and bones. 1,25D also modulates the functioning of the immune system and regulates the processes of proliferation and differentiation.

Due to the wide range of action of 1,25D and the successes in the design and marketing of drugs targeting other nuclear receptors, there was a desire to use 1,25D in the treatment of diseases such as rickets, osteoporosis, autoimmune diseases and cancer. Unfortunately, 1,25D has limited use in therapy due to its hypercalcemic toxicity at therapeutic doses. Thus, 1,25D analogs were sought that would have less side effects but retained the other beneficial properties. Thousands of compounds have been synthesized, but only a few have reached the market as drugs. These include calcipotriol used in the treatment of psoriasis or paricalcitol used in the secondary therapy of hyperparathyroidism due to chronic renal failure.

So far, the syntheses of 1,25D analogs have been carried out by trial and error, and the obtained compounds have various biological effects. One of the reasons for this fact is that the basics for the observed different effects of 1,25D on cells and tissues are unclear.

Thanks to cooperation with three research centers, five groups of newly synthesized compounds were obtained, representing a cross-section of VDR receptor ligands with different structures. These included analogues with 19-*nor*, side chain and the A ring modifications (PRI, AF and PB series of analogues), analogues with the seco-B steroid structure (UNNAT and NAT) and derivatives of lithocholic acid (SUNIL compounds), a secondary bile acid, which binds to the VDR receptor with very low affinity. Based on the existing literature, it could be assumed that all these synthesized compounds would have a better biological effect.

In the first stage of the research presented in this doctoral thesis, the obtained compounds were characterized. Research began with the determination of their binding affinity to the recombinant VDR receptor in a competition test based on the measurement of fluorescence polarization. For some compounds of the AF and PB series, the research was completed at this stage due to relatively weak affinity. Then, the study of biological activity was carried out. For this purpose, cell lines were used as models of tissues involved in calcium-phosphate homeostasis (colorectal adenocarcinoma HT-29, HEK293-FRT from embryonic kidney cells, osteosarcoma U-2-OS). Additionally, the HL-60 acute myeloid leukemia cell line was selected.

In the study of the potential for differentiation of HL-60 cells towards monocytes/macrophages, determined by flow cytometry, the strongest effect was found for the compounds of the PRI and SUNIL series. At this stage, again selection was made and research on analogues of the AF and PB series as well as UNNAT and NAT was discontinued.

The PRI series analogues and SUNIL compounds were analyzed for their biological activity by examining the ability to accumulate the VDR receptor in the nuclear fraction and to activate the ERK1/ERK2 kinases in the Western blot technique. Next, the ability to induce expression of genes related to 1,25D catabolism (*CYP24A1*), calcium uptake (*TRPV6*) and differentiation (*CD14*) in tissue model cells was analyzed using Real-time polymerase chain reaction. Comparing the above

compounds, it was found that different in vitro activity of the analogs is already achieved at the cellular level. In the next stage of the research, it was decided to investigate whether their different activity could be caused by their different transport into the cell. For this purpose, the expression of genes in cell lines encoding proteins that may be involved in the transport of vitamin D - PDIA3 (MARRS), cubilin and LRP2 (megalin) was examined. The highest expression was shown by the *LRP2* gene encoding the membrane protein in the HEK293-FRT line. This gene was knocked out using the CRISPR/Cas9 technique. Selected clonal cell lines were again treated with PRI series analogs and SUNIL-1, and CYP24A1 expression was tested. The obtained results do not allow to conclude that the different activity of the analogs is caused by their different transport with the participation of LRP2.