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STUDIES REGARDING THE PHYSICO-CHEMICAL PROPERTIES AND BIOLOGIC EFFECTS OF STRONTIUM RANELATE – THERAPEUTIC AGENT USED IN THE TREATMENT OF OSTEOPOROSIS

PhD Thesis Summary

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As musculo-skeletal diseases, particularly osteoporosis and osteoarthritis, become emerging global burdens, mainly due to sedentary and unhealthful lifestyle; in the past few decades several extensive and comprehensive research have been focused towards the elucidation of molecular mechanisms and potential therapeutic targets for the treatment of the aforementioned pathologies. In this context, strontium ranelate is one of the active pharmaceutical ingredients which, after several controversies regarding its adverse effects, was maintained in the therapy of osteoporosis as a cost-effective and reliable alternative, when other anti-osteoporotic medications are ineffective or futile. Wnt signalling is a highly conserved cellular communication system, being involved in various cell fate mechanisms, as cell proliferation, differentiation and survival. In the last years, the involvement of Wnt signalling in the pathophysiology of osteoporosis and its pharmaceutical targeting represents one of the current limelight topics in the field of osteoimmunology.

The main objectives of the present PhD thesis followed the thorough analysis of strontium ranelate for its physico-chemical characterisation, and biological as well as pharmacological effects under pharmaceutically induced osteoporosis.

Based on the above mentioned considerations, the first study focused on the development of a high-performance liquid chromatographic method for the identification and assay of strontium ranelate. The current analytical research trends often require *in-silico* modelling of the newly developed methods, thus the present study aimed to use a *quality by design* approach for the best characterization of the chromatographic analysis. In the first phase a *full factorial* experimental design was chosen, in order to perform a screening for the most influential factors, which exert a significant impact on method performance. The optimized method was validated according to the current in-force international guidelines for linearity, accuracy, precision and repeatability. A separate experimental design was carried out for robustness testing. The method proved to fulfil the requirements of analytical validations and offers a fast, cost-effective and high throughput analysis of the active substance. Furthermore, *in-silico* offers the possibility of on-line data analysis, based on different factor settings.

The aim of the second study was to develop an alternative analytical technique for the identification and quantitative assay of strontium ranelate. For this, an UV-spectrophotometric method proved to be the most adequate approach. The developed method applies a single component solvent system – tricholoroacetic acid –, and offers a high throughput sample analysis for both bulk samples and pharmaceutical dosage form. As the analytical merits of the UV-spectrophotometric method is comparable to those obtained from the HPLC analysis, this indicates that UV-spectrophotometry might represent a viable and cost-efficient alternative for modern analytical procedures and also might ease the analytical transfer in-between analytical laboratories.

Stress stability analysis for strontium ranelate was carried out using the previously described methods in the third study. The analysis revealed that the substance is highly susceptible to acidic hydrolysis, oxidation and thermal impact, alkaline environment and UV irradiation having only a negligible effect on the degradation of strontium ranelate.

The fourth study followed the selective effects of strontium ranelate on Wnt signal molecules in primary osteoblast cell cultures. Cells were divided into nine treatment groups besides the control group, as follows: 1) dexamethasone, 2) dexamethasone + strontium ranelate 0.5 M, 3) dexamethasone + strontium ranelate 0.05 M, 4) LiCl, 5) IWR-1, 6) IWR-1 + strontium ranelate 0.5 M, 7) IWR-1 + strontium ranelate 0.05M, 8) strontium ranelate 0.5M and 9) strontium ranelate 0.05M. ARN samples were isolated on days 1, 7 and 15, transcribed into cDNA and specific gene amplification was performed using qRT-PCR analysis for two housekeeping genes (β-actin and GAPDH) and four target genes (β-catenin, Wnt5a, Wnt7b and IL-6). The obtained results indicated that strontium ranelate interferes with the main signal molecule of the Wnt signalling, β-catenin, having a positive effect on the gene expression in the presence of dexamethasone. Furthermore, it was observed that strontium ranelate exerts an osteoprotective effect not only by raising the levels of β-catenin, but also stimulates the signalling pathways by increasing the expression of both canonical and non-canonical Wnt proteins. Moreover, the active substance was capable to reduce the over-expression of the pro-inflammatory cytokine, IL-6, in the presence of dexamethasone. Similar behaviour was observed when strontium ranelate was combined with Wnt pathway inhibitor IWR-1. The data indicate that besides the well-known molecular mechanisms lying under the pharmacological activity of strontium ranelate – RANK/RANKL/OPG triad –, the active ingredient is also involved in alteration of other molecular mechanisms, namely Wnt signalling.

The current PhD thesis focuses on the active pharmaceutical ingredient strontium ranelate and offers new insights in the physical-chemical and biological characteristics of the active substance from the following aspects:

1. Novel rapid and cost-efficient analytical methods offer the possibility of high throughout analysis of strontium ranelate. In-silico method development fulfils the requirements of modern research and development trends.
2. The long-term stability testing of strontium ranelate was evaluated in a wide pH range and various storage conditions, offering information about the intrinsic stability of strontium ranelate.
3. Evidence brought new insights into the pharmacological mechanisms of the active substance, elucidating important interactions between strontium ranelate and participants in Wnt signalling.