MASTER'S THESIS

Development of an osteoclast-targeted cathespin K inhibitor for postmenopausal osteoporosis: in vitro evaluation and pharmacokinetic profile

Dai, Rongchen

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ABSTRACT

Background: Postmenopausal osteoporosis which results in a reduction of bone quality and bone density is one of the most prevalent diseases affecting people around the world. Cathepsin K (CatK) is one of the most potent proteases in lysosomal cysteine proteases family, of which main function is to mediate bone resorption. Currently, the Odanacatib (ODN) developed by Merck & Co. is the only Phase III CatK inhibitor candidate with high efficacy in treating postmenopausal osteoporosis. Unfortunately, the development of ODN was finally terminated due to the cardio-cerebrovascular adverse effects. In order to enhance the specificity of ODN to osteoclasts for suppression of bone resorption in postmenopausal osteoporosis, we have previously designed and synthesized (D-Asp_8)-ODN conjugate by linking ODN with a promising osteoclast-targeted moiety D-Asp_8. The data showed that D-Asp_8 could facilitate the conjugated ODN specifically approaching osteoclasts, with reduced distribution in non-bone tissues, to inhibit the functional CatK activity within bone tissues in healthy rats. In this thesis, we hypothesized that the *in vitro* antiresorptive effects of (D-Asp_8)-ODN conjugate were comparable with that of ODN. On the other hand, we also developed a QQQ-LC/MS method for quantitation of (D-Asp_8)-ODN conjugate in plasma, which will be a valuable tool to support further pre-clinical studies.

Aim: (1) To compare the antiresorptive effect between (D-Asp_8)-ODN conjugate and ODN *in vitro*. (2) To develop and validate a practicable method for pharmacokinetic profile of (D-Asp_8)-ODN conjugate in rats.

Materials and Methods: The cytotoxic effect of (D-Asp_8)-ODN conjugate and ODN were evaluated and compared by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The effect of (D-Asp_8)-ODN conjugate and ODN on Receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclasts formation and osteoclast function-related genes were

evaluated and compared by Tartrate-resistant acid phosphatase (TRAP) staining and quantitative real time polymerase chain reaction (qRT-PCR). The effect of (D-Asp_8)-ODN conjugate and ODN on osteoclast bone resorption activities were evaluated and compared by bone resorption pit assay. Moreover, the pharmacokinetic profile of (D-Asp_8)-ODN conjugate in rat plasma was determined by using triple quadrupole liquid chromatography–mass spectrometry (QQQ-LC/MS) system.

Result: The cytotoxicity of (D-Asp_8)-ODN conjugate was significantly lower than that of ODN on the murine macrophage RAW 264.7 cell line. (D-Asp_8)-ODN conjugate had no effect on RANKL-induced osteoclast formation, which was comparable with that of ODN. (D-Asp_8)-ODN conjugate had no effect on the mRNA level of CTSK, but it could upregulate the mRNA levels of ACP5 and OSCAR, which was comparable with that of ODN. (D-Asp_8)-ODN conjugate inhibited osteoclast bone resorption activity, which was comparable with that of ODN. The newly established QQQ-LC/MS protocol had good precision and accuracy for detecting (D-Asp_8)-ODN conjugate in rat plasma. Finally, the pharmacokinetic profile of (D-Asp_8)-ODN conjugate in rat plasma was determined. Following subcutaneous administration, the time to reach maximum concentration (T_{max}) was 1.0 h, the antibiotics area under the concentration time-curves from time zero to infinity ($AUC_{0-\infty}$) was found to be 27.78 ug·mL^{-1}·h and the terminal half-life ($t_{½}$) was 1.4 h.

Conclusion: (D-Asp_8)-ODN conjugate had no effect on RANKL-induced osteoclast formation**,** which was comparable with ODN. The antiresorptive effect of (D-Asp_8)-ODN conjugate was comparable with that of ODN. On the other hand, a new QQQ-LC/MS protocol has been established for the pharmacokinetic profile of (D-Asp_8)-ODN conjugate in rat.

Keywords: Postmenopausal osteoporosis**,** Cathepsin K, Drug Development, Conjugation, (D-Asp_8)-ODN conjugate, Osteoclast, Pharmacokinetic profile, QQQ-LC/MS

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