中文摘要

骨質疏鬆症是普遍存在停經後婦女及老年人的臨床問題。在骨質疏鬆症檢測方法中，骨質代謝生化指標是測定血液和尿液中骨質生成和耗損的代謝產物，可提供骨質疾病的變化和骨質代謝速率資料，並評估骨質流失的情況。在骨代謝指標中，第一型膠原蛋白是骨骼中數量最多的膠原蛋白類型，其交聯鍵結的 N 端胜？片段（NTx）是人體中的骨骼膠原蛋白降解後所產生的物質，所以 NTx 為具有較高的特異性及敏感性的生化指標。根據本實驗室先前研究，以 NTx 片段為藍圖所設計的一系列線狀胜？中，我們發現線狀胜？ peptide 2 (P2) 與商業套組 Osteomark®中的 Anti-NTx 抗體具有較高的親合效力，因此決定與美國 Biocheck 公司合作，以此胜？(P2)為抗原製備兔子多株抗體，以開發評估骨質流失之方法。

在本實驗中，我們將 Anti-P2 的兔子多株抗體製作成親合性管柱，以便讓病患尿液檢體通過免疫親合性管柱，藉由抗體吸附尿液中具有親合性之胜？，經過沖提與 SPE C_{18} 管柱去鹽之前處理後，將析出液收集並冷凍乾燥後，應用 MALDI-TOF MS 來偵測其質譜訊號，再根據偵測訊號中最強訊號的分子量，以質譜軟體(Bruker Daltonics BioTools 3.1)推算出符合偵測樣品訊號分子量之胺基酸序列為 PRGPPGA 之胜？(P2-7mer)後，製備此胜？標準品，以製作檢量線來定量經過分離及去鹽之前處理的病患尿液中 P2-7mer 之濃度。研究結果有助於未來研發骨質流失之檢測方法，對於骨質疏鬆症的診斷，預防及治療有所助益。

關鍵詞: MALDI-TOF MS、骨質疏鬆症、免疫親合性管柱

Abstract

Osteoporosis is a serious problem of the postmenopausal women and the aged population. Among the biomarkers of bone resorption, type I collagen crosslinked N-telopeptides (NTx) are terminal metabolites specifically derived from bone collagen degradation. Type I collagen is the major collagen product synthesized by bone cells and represents more than 90% by weight of the non-mineral component of bone. Thus, the rate of cross-linked N-telopeptides (NTx) excretion in urine is regarded as a highly specific index of bone resorption, and it is sensitively suppressed in response to anti resorptive therapies. In our previous studies, we found that the epitopes for anti-NTx antibodies lie within linear peptide 2 (P2) by using commercial ELISA (Osteomark®) and surface plasma resonance (SPR) technology. Therefore, we started the collaboration with Dr. John Chen at BioCheck, Inc. (Foster City, California, U.S.A). By using the P2 peptide-conjugate as the antigen, rabbit polyclonal anti-P2 antibodies were prepared by BioCheck, Inc. In this study, the polyclonal anti-P2 antibodies (Abs) were used to prepare the anti-P2 Abs-conjugated immunoaffinity columns. The immunoaffinity columns were used to isolated peptides in patients' urine which were bound to the Abs. After elution , The eluents of bound peptides were de-salted by using SPE C_{18} column, the eluents were collected, lyophilized, and analyzed by MALDI-TOF-MS. The most significant signal identified from the mass spectra was used to explore the amino acid sequence of a peptide with the same molecular weight (MW=650) by using the software, Bruker Daltonics BioTools 3.1. The heptapeptide (PRGPPGA, P2-7mer) was identified and synthesized as the standard peptide for establishing its standard calibration curve in order to quantify the concentration of P2-7mer in the patient's urine that were purified and desalted. Results of these studies provide useful information for the development of methods for monitoring bone loss, which are important for the diagnosis, prevention , and treatment of osteoporosis.

Keywords: MALDI-TOF MS ; Osteoporosis ; Immunoaffinity column