骨細胞與生醫材料相互關係之分子生物學研究

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一、中文摘要

本研究中，我們將探討不同焦磷酸鈣離子濃度對骨母細胞行為之影響。首先使用 MTT assay 探討對骨母細胞活性之變化，並用 alkaline phosphatase stain, von Kossa stain 研究焦磷酸鈣離子對骨母細胞分化行為之作用，並進一步測定各種 bone markers mRNA 之變化。

當焦磷酸鈣離子濃度為 10^{-8}M 時，骨母細胞之增生有明顯增加之現象；alkaline phosphatase stain, von Kossa stain 則不受焦磷酸鈣離子之影響。osteocalcin mRNA 有明顯上升，而 collagen, osteonectin and osteopontin mRNA 表現則有下降趨勢。

本研究顯示，焦磷酸鈣對於骨母細胞活性之分子機轉有明顯影響。

關鍵詞：基因表現, 焦磷酸鈣, 生物適應性, 骨母細胞

Abstract

In this study, we investigated the influence of sintered dicalcium pyrophosphate ions on in vitro osteoblasts behaviour.

The effects of various concentration of sintered dicalcium pyrophosphate on bone cell activities were evaluated by using MTT assay. For the differentiation of osteoblasts, alkaline phosphatase (AP) staining, von Kossa stain for mineralized nodules and bone markers mRNA isolation and identification were performed at 3 hours, day 1, 3, 7 and 14.

At the 10^{-8}M concentration of SDCP, the beneficial effect on the osteoblasts attained to the end of 14 days' culture. The formation of alkaline phosphatase positive staining colonies and mineralization nodules formation in the osteoblast cultures were not significantly affected by SDCP ions. When osteoblasts cultured in the presence of 10^{-8} M SDCP ions, the osteocalcin mRNA expression was up-regulated; while the collagen, osteonectin and osteopontin mRNA expression were down-regulated than that of the control.

In this study, we demonstrated that SDCP has potential effects on the molecular level at the bone cells culture. This study will contribute to a better understanding of cell/biomaterial interactions and mechanisms that SDCP affect on the bone cells.

Keywords: **Gene expression, sintered dicalcium pyrophosphate, biocompatability, osteoblastic cells.**

In our laboratories, we have demonstrated sintered dicalcium pyrophosphate (SDCP) to be quite biocompatible to bone tissue in the in-vivo animal model (Lin et al. 1995). In the in-vitro model, sintered dicalcium pyrophosphate has been proved to be more biocompatible than hydroxyapatite (Sun et al. 1997). Recent work in our institute has also demonstrated that the ingestion of either bisphosphonate (Harris et al. 1993) or sintered dicalcium pyrophosphate decreased the bony porosity and increased bone mineral contents in the long bones of ovariectomized rats (Sun et al. 2002). In the later study, we demonstrated that SDCP can induce apoptosis of osteoclasts in an ultrastructural model characterized by changes of the nucleus accompanied by degradation of cellular organelles (Sun et al. 2002, accepted). However, the molecular mechanisms that mediated these processes have yet to be identified. In this study, we investigated the influence of sintered dicalcium pyrophosphate ions on in vitro osteoblasts behaviour.

The powder of sintered β-dicalcium pyrophosphate (SDCP) was prepared as previously described (Lin et al. 1995). The obtained sintered dicalcium pyrophosphate was dissolved by HCl and then diluted into different concentration of solutions by culture medium used in the bone cell culture. In the first part of this study, the effects of various concentration of sintered dicalcium pyrophosphate on bone cell activities were evaluated by using MTT assay. For the differentiation of osteoblasts, alkaline phosphatase (AP) staining, von Kossa stain for mineralized nodules and bone markers mRNA isolation and identification were performed at 3 hours, day 1, 3, 7 and 14.

When osteoblast cells cultured with 10^{-2}M or

10^{-3}M SDCP for one day, there was significant decrease in the formation of formazan; while in the 10^{-4}M or 10^{-10}M concentration of SDCP, the formation of formazan was significantly increased in the first day's culture). At the 10^{-8}M concentration of SDCP, the beneficial effect on the osteoblasts attained to the end of 14 days' culture, although it did not attain the significant level at the 3rd and 7th days' culture. We selected the 10^{-8}M concentration of SDCP ions for the further biochemical study. At 3 hours after differentaitaion medium, there little alkaline phosphatase positive staining colony was found in the culture. The alkaline phosphatase positive staining colonies first appeared at the 1st day's culture, and then progressively increased as the culture period passed, and attained a significant degree at the 14th day's culture. Similar result was observed in the group cultured with 10^{-8}M concentration of SDCP ions. Similar results were observed on the von-Kossa staining. The formation of alkaline phosphatase positive staining colonies and mineralization nodules formation in the osteoblast cultures were not significantly affected by SDCP ions.

In this model, osteoblasts have been known to express collagen type I, osteonectin and osteopontin mRNA at the 3 hours' culture, then attained their maximal expression at the first day's culture. The osteocalcin mRNA expression was quite low in the first 3 days' culture and then appeared at the 7th day's culture. In the presence of on 10^{-8} M SDCP ions, collagen type I, osteonectin and osteopontin mRNA expression were down-regulated at the 3 hour culture and then returned to that of control at the day 1. The osteocalcin mRNA expression was up-regulated in the first 3 days' culture and returned to that of control at the day 7 (Fig. 4). When osteoblasts cultured in the presence of 10^{-8} M SDCP ions, the osteocalcin mRNA expression was up-regulated; while the collagen, osteonectin and osteopontin mRNA expression were down-regulated than that of the control.

Despite encouraging preliminary in vivo reports about improvement with the use of SDCP in treatment of osteoporosis after ovariectomy, basic-science and controlled study reported to date have not established. In this study, we demonstrated that SDCP has potential effects on the molecular level at the bone cells culture. This study will contribute to a better understanding of cell/biomaterial interactions and mechanisms that SDCP affect on the bone cells. Although our study had limitations and our findings are preliminary, continued and advanced study on the alterations in gene expression of bone cells by SDCP will provide a basis for understanding the observed the bone cells responses to various pharmacological interventions.

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