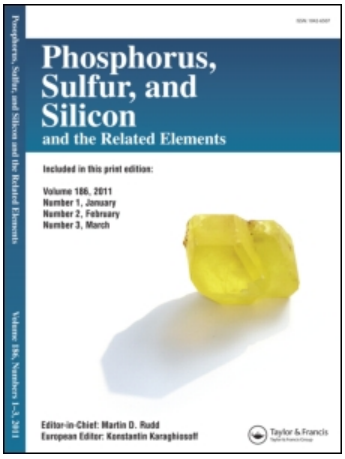

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Effects of Bisphosphonates on the Inflammatory Processes of Activated Macrophages

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All bisphosphonates are similar in terms of their inhibitory effects on bone resorption, but seems to have different effects on inflammatory processes. For example, clodronate and tiludronate have anti-inflammatory and antiarthritic activity in animals, while aminobisphosphonates exacerbate experimental arthritis in mice. Further, aminobisphosphonate induce acute phase response in patients, while non-aminobisphosphonates do not. During the last few years, we have explored the effects of various bisphosphonates on the inflammatory processes in activated macrophages in vitro.

Clodronate and tiludronate can inhibit proinflammatory cytokine and nitric oxide (NO) secretion from macrophages. In contrast, alendronate and ibandronate enhances the secretion of IL- 1β and IL-6. The intracellular delivery, and, consequently, the effects of bisphosphonates on inflammatory responses in macrophages are considerably increased by the encapsulation of the drugs in liposomes.

Clodronate and tiludronate are metabolised to an ATP-analogue by mammalian cells, while aminobisphosphonates (alendronate, ibandronate) are not. The liposome-encapsulated ATP-analogue of clodronate ($AppCCl_2p$) exerts similar effects as clodronate itself on proinflammatory cytokine and NO secretion from macrophages. In macrophages, the production of cytokines and NO is regulated by transcription factors, such as nuclear factor κB (NF-κB). In accordance with the effects on cytokine and NO secretion, clodronate and $AppCCl_2p$ inhibit the nuclear localization of NF-κB in activated macrophages, while alendronate enhances it.

The data thus strongly suggest that bisphosphonates can be grouped into those that are metabolised by macrophages and that are capable of inhibiting inflammatory responses in macrophages, thus having potential anti-inflammatory action, and those that are not metabolised and are not anti-inflammatory.

Keywords: bisphosphonates; macrophages; inflammation

INTRODUCTION

Bisphosphonates are clinically used to inhibit bone resorption and hypercalcaemia in diseases such as tumor-induced osteolytic bone disease, Paget's disease of bone and osteoporosis[1]. In addition to their ability to inhibit bone resorption, bisphosphonates, and particularly a halogenated bisphosphonate clodronate (dichloromethylene bisphosphonate), have been shown to have anti-inflammatory effects in animal models of rheumatoid arthritis (RA) as well as in human arthritis. In adjuvant- and antigen-induced arthritis in rats, clodronate suppresses the inflammatory articular lesions in the inflammed joints[2,3], whilst in human RA, clodronate decreases the levels of IL-1, TNFα, and $ß_2$-microglobulin in the circulation[4].

By contrast with clodronate, the amino-containing bisphosphonates, such as alendronate (4-amino-1-hydroxybutylidene-bisphosphonate), can cause an acute phase response in patients when administered for the first time, probably due to the increased production of IL-6 and TNFα[5,6]. In addition, aminobisphosphonates have reported to exacerbate experimental arthritis in mice[7].

Since macrophages and macrophage derived proinflammatory mediators are involved in inflammatory processes, we have studied the effects of various bisphosphonates on activated macrophages in vitro. Liposomes were used to mediate the cellular uptake of these otherwise poorly cell permeable compounds.

METHODS

Bisphosphonates and the metabolite of clodronate, adenosine5'-(β,γ-dichloromethylene triphosphate) (AppCCl2p), were encapsulated in liposomes by reverse phase evaporation method as described in details elsewhere[8]. RAW 264 macrophages were treated with liposome-encapsulated or free compounds, and the growth of the cells was evaluated in order to study the delivery of the various formulations in the cells[8,9]. The modulation of proinflammatory cytokine (IL-1β, IL-6, TNFα) or nitric oxide (NO) secretion and inflammatory signal transduction by bisphosphonates was assessed from bacterial lipopolysaccharide (LPS)

activated RAW 264 cells[9-11]. The metabolism of various bisphosphonates in the cells was studied by hplc-mass spectrometry[12].

RESULTS AND DISCUSSION

The growth inhibition studies with RAW 264 macrophages revealed that the delivery of bisphosphonates in the cells can considerably be enhanced by encapsulation in negatively charged liposomes. Liposome-encapsulated bisphosphonates were 20-200 times more potent in inhibiting the growth of RAW 264 cells than their free counterparts[8,9]. Liposomes are phospholipid vesicles, which are avidly taken up in cells by adsorptive endocytosis. Thus, they effectively deliver encapsulated material in highly endocytotic cells and provide delivery mechanism for compounds, which otherwise do not gain access in cells.

Clodronate and tiludronate were able to dose-dependently inhibit LPS-induced secretion of pro-inflammatory cytokines and NO from macrophages, and again liposome-encapsulated drugs were 10-20 times more potent than the free drugs[9-11]. Aminobisphosphonates (alendronate, ibandronate) in turn strongly augmented LPS-induced secretion of IL-1β, slightly IL-6, and had no effects of TNFα and NO secretion[10,11].

It has been generally believed thatbisphosphonates are metabolically inert compounds. However, clodronate and tiludronate can be metabolised into ATP-analogues in Dictyostelium discoideum amoebae[13]. In the present studies we showed that clodronate and tiludronate are metabolised into similar ATP-analogues also by mammalian macrophages, while aminobisphosphonates are not[12,14]. The ATP-analogue of clodronate ($AppCCl_2p$) had similar effects on the cytokine and NO secretion from activated macrophages as clodronate itself, strongly suggesting that the metabolites of clodronate and tiludronate may underlie the anti-inflammatory effects of these bisphosphonates.

In the studies on the effects of various bisphosphonates on signal transduction in inflammatory macrophages, clodronate and its metabolite were found to inhibit the nuclear localization of transcription factors NFκB and AP-1, while alendronate enhanced it. Since these transcription factors are known to play a central role in the expression of genes regulating the production of e.g. cytokines and NO, these results further confirm the

contrasting effects of metabolizable clodronate and aminobisphosphonates on inflammatory macrophages.

In conclusion, the results strongly suggest that bisphosphonates can be divided into two distinct categories in terms of their effects on inflammatory macrophages: 1) bisphosphonates that can metabolised and which inhibit the inflammatory response of macrophages, thus possessing potential anti-arthritic properties, and 2) aminobisphosphonates, which sensitize macrophages to an inflammatory stimulus and may consequently induce an acute phase response.

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References

[1] H. Fleisch, *Drugs* **42**, 919 (1991).
[2] L. Flora, *Arthr. Rheum.* **22**, 340 (1979).
[3] T. Österman, K. Kippo, L. Laurén, R. Hannuniemi and R. Sellman, Rheumatol. Int. **14** 139 (1994).
[4] F.P. Cantatore, A.M. Introsso and M. Carrozzo, *J. Rheumatol.* **23** 1117 (1996).
[5] S. Adami, A.K. Bhalla, R. Dorizzi, F. Montesanti, S. Rosini, G. Salvagno and V. Lo Cascio, *Calcif Tissue Int.* **41** 326 (1987).
[6] A. Sauty, M. Pecherstorfer, I. Zimmer-Roth, P. Fioroni, L. Juillerat, M. Markert, H. Ludwig, P. Leuenberger, P. Burckhardt and D. Thiébaud, *Bone* **18** 133 (1996).
[7] M. Nakamura, T. Ando, M. Abe, K. Kumagai and Y. Endo, *Br. J. Pharmacol.* **119** 205 (1996).
[8] J. Mönkkönen, M. Taskinen, S.O.K. Auriola and A. Urtti, *J. Drug Target.* **2** 299 (1994).
[9] J. Mönkkönen, J. Similä and M.J. Rogers, *Life Sci.* **62** P195 (1998).
[10] N. Pennanen, S. Lapinjoki, A. Urtti and J. Mönkkönen, *Pharm. Res.* **12** 916 (1995).
[11] N. Makkonen, M-R. Hirvonen, T. teräväinen, K. Savolainen and J. Mönkkönen, *J. Pharmacol. Exp. Ther.* **277** 1097 (1996).
[12] S. Auriola, J.C. Frith, M.J. Rogers, A. Koivuniemi and J. Mönkkönen, *J. Chromatogr. B* **704** 187 (1997).
[13] M.J. Rogers, X. Ji, R.G.G. Russell, G.M. Blackburn, M.P. Williamson, A.V. Bayless, F.H. Ebetino and D.J. Watts, *Biochem. J.* **303** 303 (1994).
[14] J.C. Frith, J. Mönkkönen, R.G.G. Russell, G.M. Blackburn, D.J. Watts and M.J. Rogers, *J. Bone Miner. Res.* **12** 1358 (1997).