

LIPOSOMES - AN INVALUABLE TOOL IN BISPHOSPHONATE RESEARCH

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Bisphosphonates are currently the most important class of antiresorptive drugs used for the treatment of diseases with excess bone resorption. Their pharmacokinetic properties are characterized by low oral absorption, very rapid and effective deposition in bone mineral, and the non bone-bound fraction is excreted unchanged into urine. Thus, bisphosphonates are naturally targeted to their site of action, while other tissues are not exposed to these drugs in conventional oral or intravenous administration. Due to their very hydrophilic nature, bisphosphonates are not able to penetrate through by passive diffusion, and are taken up into cells by pinocytosis. Pinocytosis is very ineffective process and very high extracellular bisphosphonate concentrations are required in order to reach effective intracellular drug concentrations. Liposomes have proven to be suitable and convenient vehicle to enhance intracellular delivery of bisphosphonates into osteoclasts and macrophages. Despite their wide clinical use, the molecular mechanism of action of bisphosphonates has been unknown until recently. By using liposomes, we have been able to study the intracellular mechanisms of action of bisphosphonates *in vitro*, and also evaluate the possible new indications for them both *in vitro* and *in vivo*.

Recent studies have shown that bisphosphonates can be divided into two groups with distinct molecular mechanism of action depending on the nature of the R² side chain. Bisphosphonates containing a nitrogen atom (ie. nitrogen-containing bisphosphonates, N-BPs) in their side chain inhibit osteoclast function and cause secondary apoptosis by preventing post-translational modification of GTP-binding proteins with isoprenoid lipids. Bisphosphonates that lack a nitrogen (ie. non nitrogen-containing bisphosphonates, non-N-BPs), such as clodronate, do not inhibit protein isoprenylation, but are metabolised intracellularly to an AppCp-type analog of ATP. This ATP-analog, AppCCl₂p, seems to be the active form of clodronate causing osteoclast apoptosis directly by inhibiting mitochondrial ATP/ADP translocase.

The differences in the molecular mechanism of action N-BPs and non-N-BPs leads to an interesting difference in their effects on the inflammatory processes in macrophages. N-BPs are not anti-inflammatory, but in fact seem to promote inflammatory reactions, seen eg. as acute phase reaction in the patients. In contrast, clodronate, or actually AppCCl₂p, inhibit the release of inflammatory mediators from macrophages, and have anti-rheumatic activity. The clinical relevance of this application is dependent on the development of appropriate drug delivery vehicles (ie. liposomes) to target clodronate into macrophages.

The possible direct effects of bisphosphonates on the tumor cells in bone metastasis and other tumor tissues is currently under active discussion. By virtue of their ability to cause apoptosis and inhibit cell proliferation, both N-BPs and non-N-BPs could have anti-tumor activity, if the the tumor cells are exposed to high concentrations of these drugs.