

Nanotechnologies for the delivery of zoledronic acid in tumors

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INTRODUCTION

Zoledronic acid (ZOL), a third-generation amino-bisphosphonate, has been approved in the US, the EU and many other countries worldwide for the prevention of skeletal-related events in patients with bone metastases of malignancy. Recently, evidence suggested that ZOL is also potent inducers of apoptosis in several cancer cell types such as myeloma and breast, prostate and pancreatic cancer as well as in macrophage and intestinal epithelial cell lines. These data indicate that the beneficial effect of ZOL on metastatic bone disease may result also from direct anti-cancer activity that may affect a broad range of tumours¹. However, both the rapid elimination from plasma and the rapid uptake and accumulation within bone hamper the direct anti-cancer activity of ZOL *in vivo*.

Liposomes have been extensively used to change drug biodistribution. In particular, liposomes with stealth properties allow to address the encapsulated drug toward tissues characterised by vessel with an enhanced permeability of the endothelium, such as tumors².

In this study, a strategy to change ZOL biodistribution upon i.v. administration is proposed. In particular, we used stealth liposomes encapsulating ZOL to avoid the accumulation of the drug into the bone and increase its concentration in non-calcified tissues, e.g. in tumour tissues. ZOL-containing liposomes were prepared at different lipid composition. The *in vitro* activity and the cytotoxicity of ZOL-containing liposomes were investigated on different cell lines. Moreover, the selected formulation was tested in two *in vivo* model of multiple myeloma and prostate cancer, respectively.

EXPERIMENTAL METHODS

Liposome preparation

The stealth liposomes encapsulating ZOL (lipoZ) were prepared by a modified reverse-phase evaporation technique². Briefly, a lipid mixture composed of EPC/Chol/DSPE-PEG 2000 (1:0.32:0.03 weight ratio, formulation A) or EPC/Chol/DSPE-PEG 2000 (1:0.32:0.30 weight ratio, formulation B) were dissolved in a mixture chloroform/methanol (2:1 v/v) and a thin lipid film was obtained by solvent evaporation. The film was then dissolved in diethyl ether and the solution was emulsified with a ZOL

aqueous solution. The organic solvent was removed until formation of a viscous gel, which was then vortexed until a liposome suspension was obtained. Liposomes were then extruded using a thermobarrel extruder. The unencapsulated ZOL was removed by purification with Sephadex. The resulting liposomes were freeze-dried, in presence of lactose as cryoprotectant, and stored at -20°C. Liposomes were characterised in terms of mean diameter and polydispersity index by photon correlation spectroscopy, amount of ZOL loaded into liposomes by HPLC, and phospholipid content by Stewart assay.

In vitro activity

The *in vitro* activity of ZOL was investigated on the following different tumour cell lines: prostate (PC3, DU145, LNCaP), breast (MCF7, MDA-MB468, CG-5), kidney (caki2, 769P), melanoma (M14, M14+), head/neck (KB), lung (H1355), pancreas (BXPC3), myeloma (RPMI, KMS, DOX, OMP) cells. Cell proliferation studies in presence of ZOL was investigated by MTT assay.

In vivo studies

For the experiments on the multiple myeloma model, CB-17 SCID mice were subcutaneously inoculated with multiple myeloma cells. After that measurable tumours were growth, free ZOL or ZOL containing liposomes were weekly injected. Tumor weight measured each 3 days. Survival was evaluated from the first day of treatment until the death. In the experiments on the prostate cancer model, SCID mice were intramuscularly inoculated with PC3 cells. After that measurable tumours were growth, free ZOL or ZOL containing liposomes were injected three times/week at two different dose. Tumor weight measured each 3 days and survival evaluated from the first day of treatment until the death.

RESULTS AND DISCUSSION

We prepared lipoZ at different lipid composition. Liposome characteristics are summarized in table 1. Liposomes had a mean size ranging between 203 and 241 nm. The different formulations were characterised by an actual loading from about 77 to 101 µg ZOL/mg lipids. Liposomes were fully characterised before and after freeze-drying. In particular, in the case of the formulation A, freeze drying led to a significant reduction of ZOL encapsulated into liposomes. On the

contrary, the formulation B showed the higher (more than 70%) retention of ZOL into liposomes after freeze-drying.

Liposome formulation	Lipid composition (EPC/Chol/DSPE-PEG 2000 weight ratio)	Zeta potential (mV \pm S.D.)	Mean diameter (nm \pm S.D.)	ZOL actual loading (mg ZOL/mg lipids)	Encapsulation efficiency* (%)
A	1:0.32:0.03	-7.2 \pm 2.4	203 \pm 26	76.9 \pm 61.8	4.2 \pm 2.6
B	1:0.32:0.30	-1.9 \pm 0.9	241 \pm 36	101.4 \pm 38.4	5.2 \pm 0.1

*Calculated as the ratio between the ZOL actual and theoretical loading X 100

Table 1. Liposome characteristics

Then, we studied the effect of increasing concentrations of ZOL, as free or encapsulated into liposomes, on the growth inhibition of different human cancer cell lines. In all cell lines, ZOL delivery by lipoZ induced a higher growth inhibition, compared with free ZOL. In figure 1 the results obtained on PC3 cell line are shown.

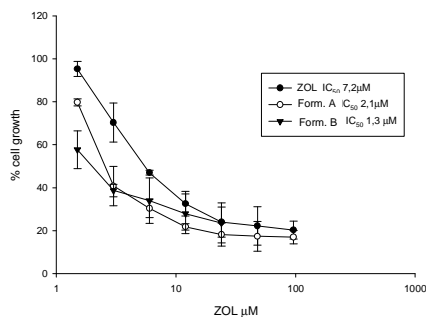


Figure 1. In vitro activity studies of lipoZ on prostate cancer PC3 cell line.

The technological characterization and the *in vitro* studies allowed to select the formulation B for the *in vivo* studies. The antitumor activity of ZOL, as free or encapsulated into liposomes, was investigated in two mice models of prostate cancer and multiple myeloma, respectively. In both cases, a resistance of the tumor to the treatment with free ZOL was found. On the other hand, at the same doses, lipoZ caused a significant tumour growth inhibition with a significant increase of mice overall survival. In Figure 2, the effect of lipoZ on the prostate cancer is shown. The treatment with all the agents used in the experiments was not toxic, not inducing changes in mice body weight and/or not producing toxic adverse events and deaths.

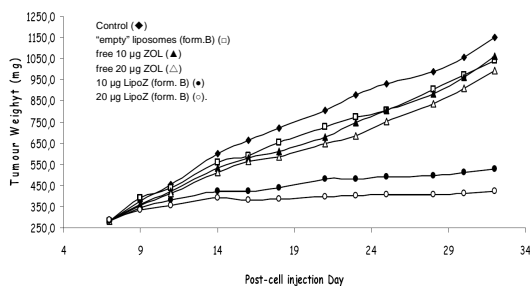


Figure 2. In vivo experiments lipoZ on mice model of prostate cancer.

CONCLUSIONS

In this study, both anti-cancer activity and tolerability of ZOL-containing liposomes were demonstrated in preclinical animal models. When encapsulated into liposome, ZOL showed a powerful anticancer effect, while the tumors were resistant to free ZOL used at the same doses.

REFERENCES

1. M. Caraglia et al. Endocrine Rel Cancer 2005
2. F. Szoka et al. PNAS 1978