Modulation of macrophage activation status by bisphosphonates and breast cancer cells.

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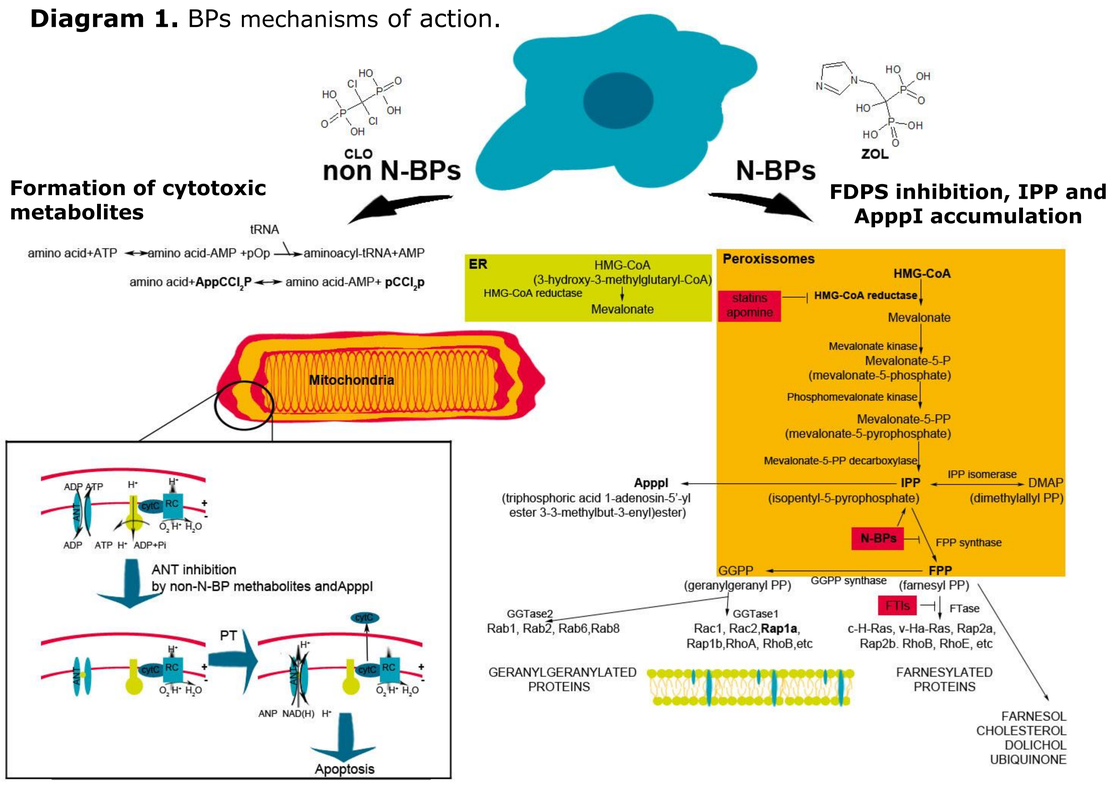
Introduction

Tumour stromal macrophages differentiate into tumour associated macrophages (TAMs), with characteristics resembling the immunosuppressive M2 polarization instead of the pro-inflammatory M1. TAMs have a central role in promoting tumour vascularization, cancer cell dissemination and suppression of anti-cancer immune response. Cancer cell dissemination leads to metastasis formation which e.g. in breast cancer often happens in bone marrow. We have studied the *in vitro* modulation of that macrophage polarization by bisphosphonates (BPs) and breast cancer cell conditioned medium (CM).

Diagram 1. BPs mechanisms of action.



Methods

The murine macrophage cell line J774 was treated for 48h with semi-logarithmic concentrations of free and liposome encapsulated clodronate (CLO) and zoledronate (ZOL) and cell viability was assessed.

J774 cells were cultured for 72h in the presence of 50%CM either from the murine breast cancer cell line 4T1 or murine fibroblast cell line 3T3. After conditioning cells were further stimulated with 10ng/mL LPS for 24h.

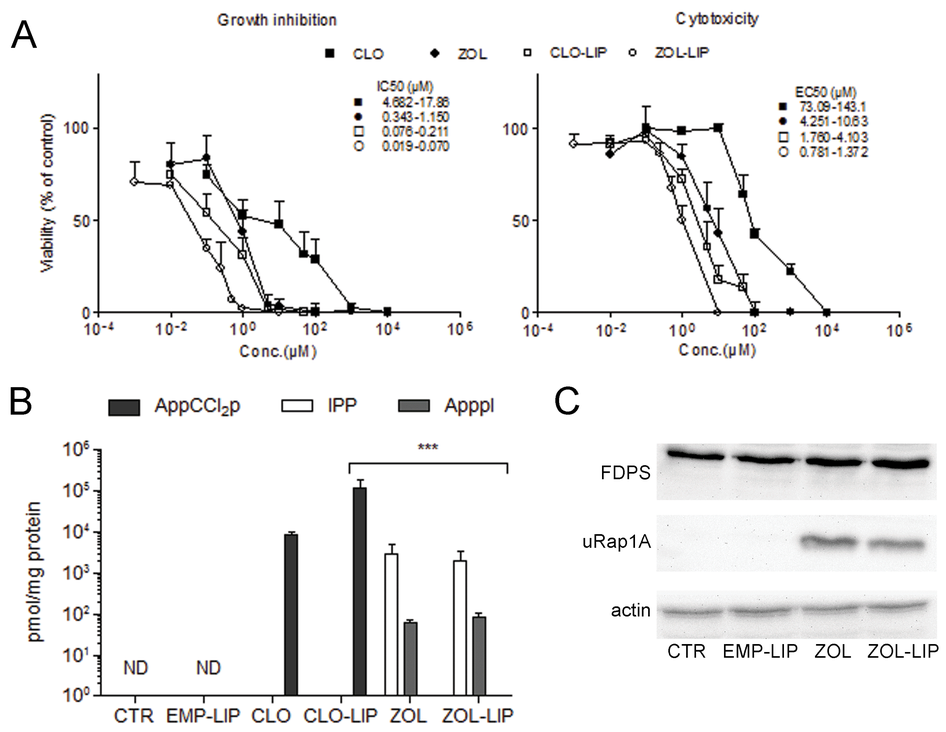
The same macrophage cell line was conditioned for 72h with 50% 4T1CM, treated for 24h with free or liposome encapsulated CLO (CLO-LIP) and ZOL (ZOL-LIP) and further stimulated with 10ng/mL LPS for 24h.

Supernatants were colected for multiplex ELISA, Griess assay and zymography.

IPP, ApppI and AppCCl$_2$p were determined by HPLC-MS in acetonitrile/water extracts from BP treated J774 cells. For western blot analysis total cell lysates were used.

Results

Fig. 1 A-Growth inhibition and cytotoxicity effects of free and liposome encapsulated BPs. B-IPP, ApppI and AppCCl$_2$p accumulation. C-Protein prenylation inhibition by ZOL.



All data presented is unpublished.

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Fig. 2 Breast cancer soluble factors impact on macrophage activation.

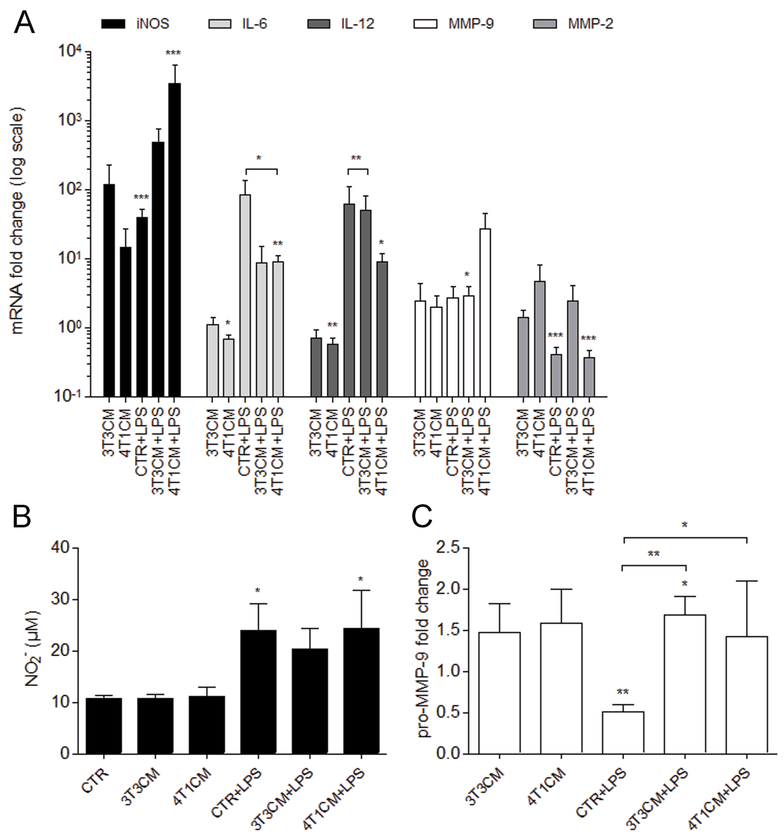
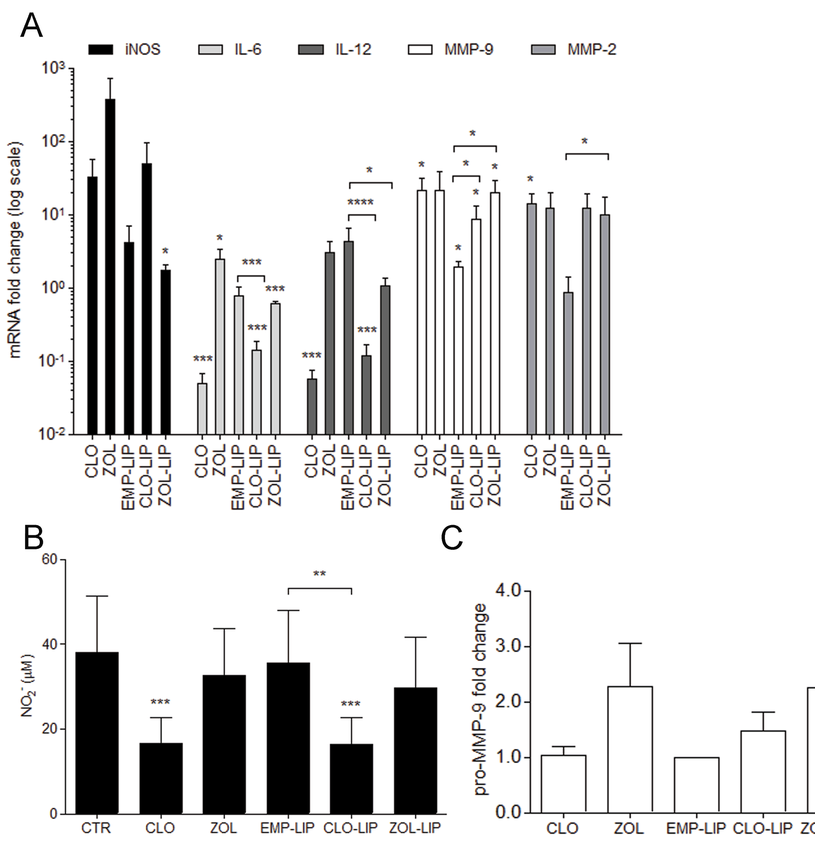


Fig. 3 Free and liposome encapsulated BPs impact on macrophage activation.



Conclusions

- Breast cancer cells secreted factors modulate macrophage activation, increasing IL-6, IL-12 and MMP-9 production upon LPS stimulus. However macrophages seem less responsive to LPS when conditioned by breast cancer factors.
- Liposome encapsulation increases BPs potency, and *in vivo* may be used to overcome the bone seeking properties of these drugs.
- CLO decreases NO, IL-6 and IL-12 production by macrophages and ZOL increases MMP-9.
- Further studies are needed to clarify the potential clinical relevance of these distinct macrophage modulation by N-BP and non-N-BP. The choice of which BP to use should be context and aim dependent. If the objective is to reduce inflammation a non-N-BP should be used. However if aiming to increase macrophage aggressiveness without decreasing inflammation, a N-BP might be beneficial.

MARIE CURIE ACTIONS BONE-NET

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