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The effects of combined treatment using paclitaxel and zoledronic acid on tumour cells and endothelial cells *in vitro*

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Effects in tumour cells

There is increasing use of combined therapies in oncology, but the optimal drug combinations and sequences have in many cases not been determined. The use of bisphosphonates (BPs) together with cytotoxic drugs may be a favourable combination, as BPs like Zometa are usually well tolerated causing few side effects in the patients.[1] In this study we have investigating the effects of zoledronic acid (Zometa) in combination with the commonly used cytotoxic drug paclitaxel, in order to determine whether this combination does induce increased levels of apoptotic cell death in breast cancer cells *in vitro*.[2]

Initially, MDA-MB436 cells were treated with 25 μM zoledronic acid for between 1 and 72 hours, and the effects on apoptosis determined by evaluation of nuclear morphology following staining with Hoechst and PI. Separate samples were treated with paclitaxel (2 nM), and cells were also treated with paclitaxel and zoledronic acid in sequence or in combination, in order to determine if the order of drug exposure influenced the levels of cell death. We found that the highest level of apoptotic cell death was induced in the cells that had been treated with paclitaxel prior to zoledronic acid, as opposed to the reverse sequence (zoledronic acid then paclitaxel) or the drugs given together.

The synergistic effect could be reversed by inclusion of GGOH, an intermediary compound in the mevalonate pathway, suggesting that inhibition of this pathway by zoledronic acid leads to induction of apoptosis in breast cancer cells.

The sequence-dependent effect of the combined treatments was confirmed by cell cycle studies, measuring the accumulation of cells in subG1 phase (corresponding to the apoptotic population). In agreement with the results shown in Figure 1, the highest number of MDA-MB-436 cells was found in subG1 in the treatment group where paclitaxel had been given prior to zoledronic acid.

One of the intracellular targets affected by inhibition of prenylation by zoledronic acid is the GTPase Rap1a. We therefore investigated how the different drug sequences caused inhibition of prenylation of Rap1a, using an antibody that specifically picks up the unprenylated form of this molecule. In MDA-MB-436 cells treated with zoledronic acid alone there was clearly detectable levels of unprenylated Rap1a at 24h but this was not affected by combined treatment with paclitaxel.

Taken together our data show that the synergistic effect on levels of apoptosis following combined treatments with paclitaxel and zoledronic acid in breast cancer cells depends on the order in which the compounds are given. The highest levels of apoptosis are obtained when cells are exposed to paclitaxel followed by zoledronic acid. The molecular mechanism underlying this sequence-dependent effect remains to be identified, but does involve the inhibition of the mevalonate pathway. Prenylation of Rap1a is not differentially affected by the drug sequencing, suggesting that this is not a mediator of apoptosis in these cells following combined treatments.

Effects in endothelial cells

Having established that paclitaxel and zoledronic acid induce a synergistic increase in apoptotic cell death in tumour cells, we next went on to determine whether the treatments could also affect endothelial cells *in vitro*. Combined treatments may cause potential side effects by targeting the normal vasculature, or may have additional anti-tumour effects by affecting tumour angiogenesis. We found that both microvascular endothelial cells (HuDMEC) and macrovascular endothelial cells (HUVEC) were relatively insensitive

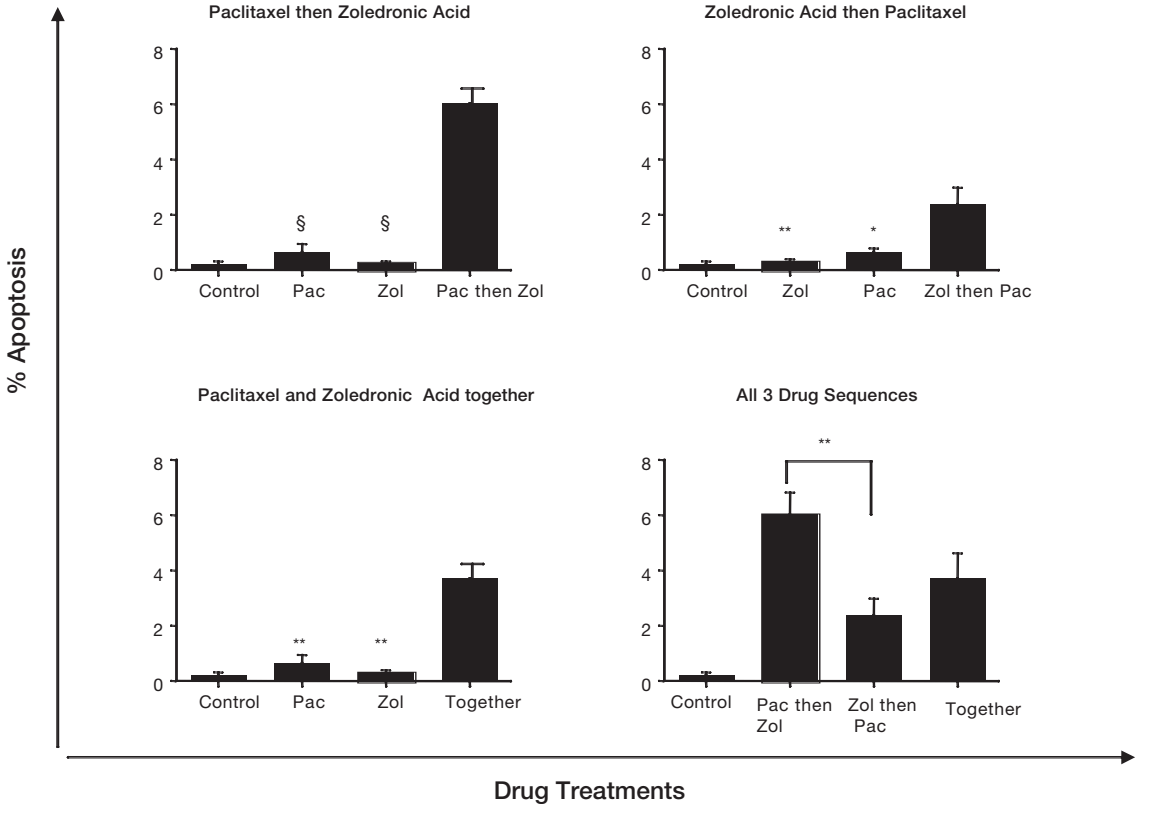

Figure 1. MDA-MB-436 cells were treated with 2 nM paclitaxel for 4h and 25 uM zoledronic acid for 1 hour in the sequences given. The levels of apoptotic cell death were determined at 72h.

to zoledronic acid *in vitro*, with growth affected only at doses above 25 μM for at least 48 hours treatment. In order to determine if the endothelial cells were able to take up zoledronic acid, the effect on prenylation of Rap1A was measured. Unprenylated Rap1A was clearly detectable in both HuDMEC and HUVEC after 48h of treatment. As for tumour cells, zoledronic acid (25 μM/48h) did cause an increase in the number of endothelial cells in S phase, suggesting that the cells are affected by the drug. However, only low levels of apoptosis could be detected in HUVEC and HuDMEC cultures treated with zoledronic acid alone, even using high doses and repeated treatments. Paclitaxel did cause increased levels of apoptosis in HuDMEC after 48h, but this was only significant at doses above 4nM. When treating HuDMEC with a combination of paclitaxel and zoledronic acid, the levels of apoptosis were substantially increased compared to the levels caused by the drugs alone. In contrast to our data using tumour cells, in endothelial cells were most sensitive to the effects of paclitaxel and zoledronic acid given together, rather than in sequence.

Taken together our data demonstrate that in terms of anti-tumour effects there may be a potential benefit in using zoledronic acid in combination with paclitaxel, although these data remain to be confirmed by studies using *in vivo* model systems.

References

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