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LETTER TO THE EDITOR

Additive/synergistic anti-tumoral effects of the combination of docetaxel and zoledronic acid on prostate cancer cells: Possible mechanisms?

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To the Editor

We read the interesting article by Ullen et al. [1] on the additive/synergistic effects of zoledronic acid (ZOL) combined with docetaxel (DOC) on prostate cancer cells, published in the September issue of *Acta Oncologica*. We especially appreciate their approach to study the anti-tumoral effects of ZOL alone and in combination with DOC on prostate cancer cells. In addition to showing that ZOL possesses anti-tumoral effects in terms of proliferation inhibition and apoptosis induction, they also reported the potential of ZOL and DOC to exert super-additive anti-tumoral effects on two hormone-refractory prostate cancer cell lines. They found an additive effect for PC3 cells when combining DOC and ZOL at concentrations of 1 ng/ml and 25 μM, respectively, whereas a synergistic anti-tumoral effect on DU145 cells was observed with DOC and ZOL at concentrations of 0.01 ng/ml and 50 μM, respectively. However, there was no mention of novel chemosensitizing effects of bisphosphonates (BPs) or possible mechanisms of action. This study supports the findings of other studies which showed that BPs act synergistically with other chemotherapeutic agents [2,3], a notion that further supports the combined use of BPs as chemosensitizing agents.

As is well known, BPs exhibit a high affinity for calcified matrices, such as hydroxyapatite in bone, and are used successfully as powerful inhibitors of increased bone resorption in several bone diseases including Paget's disease of bone, osteoporosis, and tumor-associated bone diseases. [4]. Therefore, BPs are used to decrease skeletal-related complications for a number of tumors including breast, prostate and multiple myeloma, leading to improved quality of life [5,6]. Although ZOL clearly has cytotoxic effects on prostate cancer cells in vitro, these may be mediated by the compound's ability to chelate calcium. Our previous study with EGTA, which also chelates calcium, demonstrated only a small effect on the reduction on cell number which remained significantly different from the greater effect observed following ZOL treatment [3]. Addition of EGTA to ZOL-containing cultures showed a significant decrease in cytotoxicity rather than an enhanced cytotoxic effect, which one would have expected if the mechanism was via calcium chelation. These data suggest that a decrease in extracellular calcium can actually protect cells from these drugs. The BP alendronate induces calcium leakiness in osteoclasts that leads to a rise in free intracellular calcium. Such a rise in calcium has been previously suggested to be an inhibitory signal for cells [7]. Furthermore, increases in calcium have been implicated as second messengers during the induction of apoptosis [8].

In our study, we demonstrated that ZOL induced antiproliferative and apoptotic effects on MM cell lines *in vitro* by activating protein kinase C, and these effects were augmented when dexamethasone and thalidomide were combined with ZOL [3]. In

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this study we showed by flow cytometric analysis that ZOL treatment of multiple myeloma cells increased the proportion of cells in the S-phase, possibly due to slowing the progression through S-phase or to a block between S and G_2M in the cell cycle. Another article by Matsumoto *et al*. on small cell lung cancer cell lines reported that cell growth inhibition by ZOL alone, or combined with other anti-cancer agents, may involve not only induction of apoptosis but also prolongation of cell cycle progression [9]. This ability of ZOL to arrest cells in G_2 and M or prolong the cell cycle progression raises the possibility of ZOL as a potential cell cycle chemosensitizer because G_2 and M cells are more sensitive than cells within other phases of the cell cycle [10].

Human cancers are often characterized by Ras mutations that lead to the constitutive activation of the Ras signalling pathway. Effective Ras signalling requires the attachment of Ras proteins to the plasma membrane, a process initiated by the enzyme farnesyl protein transferase. Therefore, blockage of Ras binding to the plasma membrane may be a good therapeutic target for the treatment of malignancies. Third-generation BPs deplete the cellular pool of both geranylgeranyl pyrophosphate and farnesyl pyrophosphate, and thereby inhibit both geranylgeranylation and farnesylation [11]. Matsumoto et al. showed that ZOL blocked the prenylation of Ras in squamous cell lung cancer cell lines in a dose- and time-dependent manner and induced apoptosis [9]. Salomo et al. reported that BP-resistant cells had increased farnesyl pyrophosphate synthase activity, although not due to upregulation of its gene transcription. Therefore, sensitivity differences to BPs may result, at least in part, from increased activity of farnesyl pyrophosphate synthase [12]. Thus, the chemosensitizing effect of BPs could be attributable to the Ras signalling blockade by depletion of the cellular pools of both geranylgeranyl pyrophosphate and farnesyl pyrophosphate.

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