The farnesyl-transferase r115777 and the aminobisphosphonate zoledronic acid synergize on the apoptosis and on the growth inhibition of human cancer cells

Panidronate (PAM) and Zoledronic Acid (ZOL) are aminobisphosphonate (BPs) able to affect the isoprenylation of intracellular small G-proteins. We have investigated the antitumor activity of BPs and R115777 farnesyltransferase inhibitor (FTI) against epidermoid cancer cells. In human epidermoid head and neck KB and lung H1355 cancer cells 48 h exposure to PAM and ZOL induced growth inhibition (IC50 25 µM and 10 µM, respectively) and apoptosis and abolished the proliferative and anti-apoptotic stimuli induced by epidermal growth factor (EGF). In these experimental conditions ZOL induced apoptosis through the activation of caspase 3 and a clear fragmentation of PARP was also demonstrated. A strong decrease of basal ras activity and an antagonism on its stimulation by EGF was recorded in the tumour cells exposed to BPs. These effects were paralleled by impaired activation of the survival enzymes extracellular signal regulated kinase 1 and 2 (Erk-1/2) and Akt that were not restored by EGF. Conversely, farnesol induced a recovery of ras activity and antagonized the pro-apoptotic effects induced by BPs. The combined treatment with BPs and R115777 resulted in a strong synergism both in growth inhibition and apoptosis in KB and H1355 cells. The synergistic activity between the drugs allowed BPs to produce tumor cell growth inhibition and apoptosis at in vivo achievable concentrations (0.1 µM for both drugs). Interestingly, the synergistic activity of the two drugs on apoptosis was completely antagonized by the addition of farnesol to the cells (Figure 1). Moreover, the combination was highly effective in the inhibition of ras, Erk and Akt activity, while farnesol again antagonized these effects. However, BPs are specifically accumulated in bone tissue and the extra-bone distribution is very limited. Therefore, we have chosen prostate adenocarcinoma as experimental model since it is characterized in its hormone-independent phase by development of bone metastases. We have found that ZOL and R115777 synergize on growth inhibition and apoptosis in two human prostatectic cell lines: androgen-independent PC3 adenocarcinoma cell lines and the androgen-dependent LNCaP cells. The synergistic activity between the drugs allowed BPs to produce tumor cell growth inhibition and apoptosis at in vivo achievable concentrations (0.75 µM for Zol and 1.5 µM for FTI) (Figure 2). In conclusions, the combination of BPs and FTI leads to enhanced antitumour activity at clinically achievable drug concentrations which resides in the inhibition of farnesylation-dependent survival pathways and warrants further studies for clinical translation.

We have selected a combination of R115777 and PAM that was highly synergistic at CalcuSyn elaboration and gave an about 40% growth inhibition. We have evaluated the apoptotic effects of this combination and the antagonistic activity of farnesol on KB (A–E) cells at fluorescence microscopy after PI and anti-annexin V antibody labelling (insets). A) Control cells; B) 48 h 0.07 µM R115777; C) 48 h PAM 0.07 µM; D) 48 h 0.07 µM R115777 and PAM 0.07 µM; E) 48 h 0.07 µM PAM and 0.07 µM R115777 and 1 µM farnesol. Red and green fluorescent cells were apoptotic. The bars in the insets show the percentage of apoptotic cells. Arrows show apoptotic cells.

References
Figure 1. BPs and the farnesyltransferase inhibitor R115777 combination have a synergistic effect on apoptosis that was antagonized by farnesol supplementation in KB cells.

Figure 2. Graphs. Isobologram analysis of the effects of R115777 and ZOL combinations in PC3 and LNCaP cell lines. The combination shows strong synergistic antiproliferative effects as resulted by the analysis with the dedicated software CalcuSyn (by Chou and Talalay). CI, combination index; DRI, dose reduction index. Assessment of synergy was performed quantitating drug interaction by CalcuSyn computer program (Biosoft, Ferguson, MO). Combination index (CI) values of < 1, 1, and > 1 indicate synergy, additivity, and antagonism, respectively. Tables. CI50 was calculated for 50% cell survival (ED50) by isobologram analyses performed with CalcuSyn software. DRI50 represents the order of magnitude (fold) of dose reduction obtained for ED50 effect in combination setting as compared to each drug alone.