Dual delivery of doxorubicin and zoledronic acid from an injectable calcium sulphate/hydroxyapatite carrier

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INTRODUCTION: Doxorubicin (Dox), an anthracyclin agent, is widely used as a chemotherapy agent for different kinds of malignancies. However, even when entrapped in liposomes, Dox has a terminal half-life in plasma of 69.3 h compared to 17.3 h with free doxorubicin (Rahman et al[1]). And only a small fraction (<5%) of the total administered liposomal formulation is actually delivered to the target site[2]. Alternatives to improve efficient and contained delivery of Dox locally within the tissue are needed. Zoledronic acid (ZA) is a new-generation bisphosphonate, and widely used as an adjuvant treatment for bone metastasis, to reduce tumor-related pain and skeletal-related events[3]. It could also increase bone formation and enhance repair of large bone defects[4]. Furthermore, locally delivered ZA can stay at the target site for up to 6 months due to its affinity to hydroxyapatite (HA) (manuscript in progress). Local dual delivery of Dox and ZA acts synergistically to achieve strong and long-term anti-tumor effect, as well as bone regeneration at the eradicated tumor site. Tumor recurrences and simultaneously an enhanced repair of the bone defect can be achieved. The aim of the present study was to characterize the release kinetics of Dox and ZA from an injectable FDA and CE approved calcium sulfate/hydroxyapatite (CaS/HA) carrier and evaluate the effect on human osteosarcoma cells MG-63.

METHODS: The biphasic CaS/HA material was mixed with Dox or Dox+ZA (dissolved in Iohexol and saline) and casted as a cylindrical disc (100 µl of ceramic paste). Dox pellets contained 10 µg of Dox in the CaS/HA material, and Dox+ZA pellets contained both 10 µg Dox and 10 µg ZA. Pellets (n=5) from each group were placed in 1 mL PBS at pH 7.4 or 5. The supernatants were collected on day 1, 3, 7, 14, 21 and 28. The fluorescence was detected using a spectro-fluorimeter (Doxorubicin, Excitation: 485 nm, Emission: 580 nm). In the cell culture experiment: MG-63 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, and 1% antibiotic-antimycotic.10^4 cells per well were seeded in 96 well plate and cultured for 24 h before the experiment. Bioactivity and toxicity of the released drug fractions collected on day 1, 3 and 7 were tested on the cells using MTT assay by feeding the cells with respective fractions for 7 days.

RESULTS: At pH 7, the biphasic material released 28% of the doxorubicin during the first week and additionally only <1 % during the first month. At pH 5, 36% was released during the first week (Fig. 1A), indicating that more doxorubicin could be released at the tumor site with an acidic microenvironment. A similar tendency was seen in Dox+ZA pellets with 26% Dox released at pH 7.4 and 33% at pH 5 for the first week respectively (Fig. 1B). The main difference in release between pH 7.4 and 5, occurs on day 1 in both groups (Fig. 1C,D). For ZA, our previous study[5] showed 6% of ZA originally loaded being released on day 1 increasing to nearly 10% on day 7, indicating a sustained release pattern. To further test the bioactivity of Dox or ZA released from material, fractions from different time points were added to the cells. Compared to the control, fractions collected on day 1, 3 and 7, remarkably inhibited the cell proliferation, with cell viability around 27% for day 1 or 3 group, and 32% for day 7 group (Fig. 2A). For Dox+ZA group, an even lower cell viability was observed, with around 21% for day 1 or 3 group and 28% for day 7 group (Fig. 2B). Moreover, Dox+ZA had a stronger tumor toxic effect in the day 1 and 3 group, but not in the day 7 group (Fig. 2C,D,E).

DISCUSSION: Doxorubicin binds to HA and a sustained release can be activated by osteoclastic resorption, resulting in high local Dox concentration compared to systemic injection. The bioactivity of Dox did not change after being incorporated in the CaS/HA carrier. Simultaneously loading ZA together with Dox did not affect the release of Dox significantly during the first month. An enhanced tumor toxic effect was seen by adding ZA, released from the carrier at the local site for several months as reported previously. Using the combination of Dox+ZA, high local concentrations and prolonged delivery can be reached with minimal systemic side effects. The effect of these drugs from bone regeneration perspective needs to be analyzed further. Additional long-term in vivo studies are required to elucidate the efficacy of the carrier in delivering cytostatics locally within a tumor often in combination with surgical eradication. Adding additional tumor cytostatics, with no accretion in apatite, high local release was measured during the first month along with the resorption of the cytostatic embedding sulphate[6].

SIGNIFICANCE: An injectable calcium sulphate/hydroxyapatite carrier may constitute an attractive platform for local delivery of various tumor drugs, which could have a dual effect of being anti-tumorigenic as well promote bone repair.

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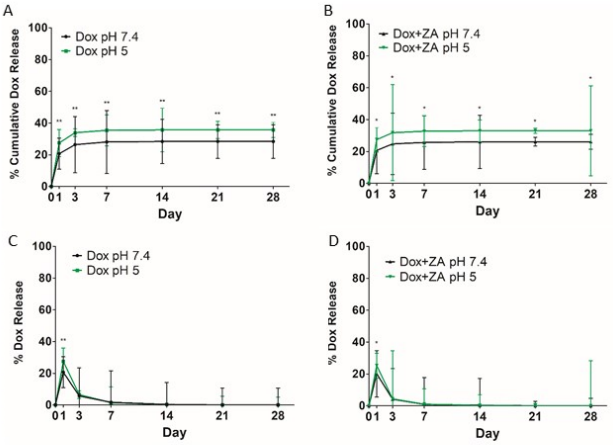

Fig.1: Dox release from a calcium sulfate/hydroxyapatite carrier at Ph 5 and 7.4. (A,B) show cumulative Dox release from the carrier with or without Zoledronic Acid at Day 1,3,7,14,21 and 28. (C,D) show the amount of Dox released at each time point with or without ZA. *p<0.05, **p<0.01.

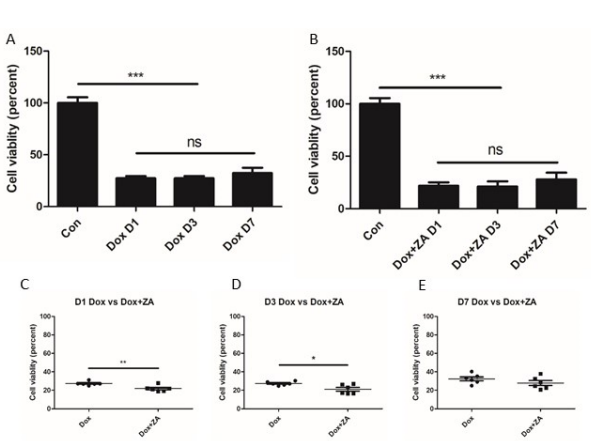

Fig.2: Bioactivity of Dox and ZA released from the carrier. (A,B) show the toxicity of fractions collected from the carriers loaded Dox or Dox+ZA at Day 1,3 and 7 on MG-63 cells. (C,D,E) compare the toxicity of Dox fractions with or without ZA collected from Day 1,3 and 7. *p<0.05, **p<0.01 and ***p<0.001.