Effects of Ionic Dissolution Products of Bioceramics on the Structure and Bioactivity of Doxorubicin

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Statement of Purpose: Studies have explored calcium-phosphates and silica based materials as drug delivery systems to treat cancer. The bioactivity reactions include an initial dissolution of the ceramic surface followed by a back-precipitation. These reactions have been shown to regulate the drug release kinetics.[1] While most of the studies have focused on the release kinetics and the bioactivity of released drug, little is known about the effects of the ionic dissolution products of the ceramic on drug toxicity. The objective of this work is to analyze the interactions between anticancer drug molecules and individual ions released from bioactive ceramics. Additionally, the effects of individual ions on Doxorubicin (Dox) toxicity for human cancer cells are evaluated.

Methods: A homogeneous solution of 1 mg/mL Dox hydrochloride in D_2O at pH 5.0 was prepared. The Dox solution was mixed with solutions containing silicon (Si), phosphorous (P), sodium (Na), or calcium (Ca) ions to form Dox/ion solutions at molar ratios of 0:1, 4:1, 1:1, 1:4 and 1:0. The concentration of Dox (500 μg/ml) and the pH value (5.0) was maintained constant in all solutions. NMR spectra were recorded on Bruker Avance 500 MHz spectrometer at 298 K. The assignments of the proton NMR chemical shifts of Dox were done using 2D COSY. The interactions of Ca and P ions with Dox were monitored by 1D proton spectra of Dox. The cytotoxicity of the Dox/ion solutions was measured on human MCF7 breast cancer cells seeded at 10^5 cells/well and grown to confluency in DMEM complete medium. The cytotoxicity of the Dox/ion solutions along with Dox alone and ion alone solutions was evaluated by measuring the propidium iodine incorporation after 72 h. The results were reported as relative fluorescent unit (RFU).

Results: NMR analyses showed that the addition of Ca ions to Dox solution resulted in chemical shifts of the NMR peaks characteristic for 1H, 2H and 3H protons of ring A and the 5H proton of the O-CH_3 group of the same ring (Fig 1). Moreover, the shift of these peaks increased as the Ca concentration increased. On the other hand, no significant shift was observed when P was added to Dox solution. Cell culture studies showed that control medium, without Dox, modified with Ca, Na, P or Si ions did not have a toxic effect on MCF7 cell (Fig 2). As expected, addition of Dox to the culture medium promoted MCF7 cell death. On the other hand, variations in MCF7 cell death in response to Dox were observed after the addition of Ca, Na or P ions (Fig. 2). Addition of various concentrations of Si ions appeared to have minimal effect on the cytotoxicity of Dox.

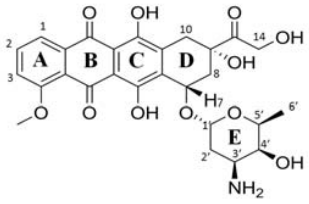

Figure 1. Chemical structure of Doxorubicin.

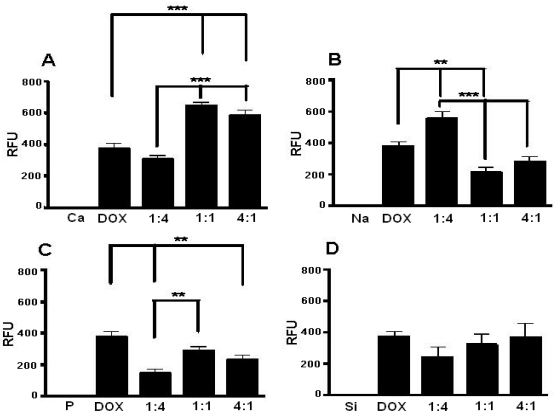

Figure 2. MCF7 breast cancer cell Dox cytotoxicity is modulated by the content of Ca (A), Na (B), P (C) and Si (D) ions at different molar ratios. **P<001 and ***P<0.001.

Conclusions: Results indicate that Si, Ca, P, and Na ions commonly released by the dissolution of bioceramics affect the bioactivity of the widely used anticancer drug Doxorubicin. Ca ions added to Dox solution at the molar ratio of 1:1 lead to 60 % increase in the death of MCF7 breast cancer cells compared to control Dox solution without addition of Ca ions (Fig. 2 A). In contrast, Si ions had no significant effects of the toxicity of Dox regardless of the concentration tested (i.e., from 25 %-75% molar ratio). NMR chemical shift data correlate with the bioactivity data and suggest that Dox interacts with Ca, but not with P ions. Ongoing NMR experiments include the analysis of the effects of Ca, P, Na and Si ions on the Dox structure.

Reference:

1 El-Ghannam A, et. al., J Mater Sci Mater Med. 21(9):2701-10. Sep 2010.

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