SOLVENT EFFECTS ON DOXORUBICIN STABILITY IN BONE CEMENT ELUTION STUDIES

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INTRODUCTION

Research to optimize local drug delivery is being actively pursued. Because of its superior mechanical properties, polymethylmethacrylate (PMMA) bone cement is the delivery vehicle of choice at structurally significant sites. PMMA cement is the most widely used local drug delivery system for antibiotics in total joint arthroplasty and the treatment of osteomyelitis. It may also provide sustained release of cancer chemotherapeutic agents for treating metastatic and primary bone tumors while minimizing systemic drug effects. Specifically, orthopaedic oncologists have investigated the potential of adding chemotherapeutic drugs such as methotrexate, cisplatin and 5-fluorouracil to cement when treating malignant disease of bone. Our laboratory is currently characterizing the drug delivery properties of doxorubicin (DOX)–PMMA cement mixtures.

Elution studies are performed to estimate the quantity of drug that will be delivered by drug-cement mixtures. Phosphate buffered saline (PBS) is considered the solvent of choice because of its ionic composition, isotonicity and pH. However, the degradation of drugs eluted from drug-cement mixtures due to solvent contact has not been well characterized. A thorough review of the published pharmaceutical literature highlights that DOX is an extremely unstable compound in a variety of solvents, most notably PBS and culture media (Janssen et al.). In vitro studies of DOX that use an ionic solvent are therefore not accurate models of in vivo drug release. This study examines the influence of solvent selection and storage conditions on the ultimate drug concentration measured in elution studies. We investigated the following hypothesis:

“The quantity of DOX measured by HPLC in PBS elution studies significantly underestimates the quantity that actually leaches out from DOX-PMMA cements.”

MATERIALS and METHODS

The degradation kinetics of DOX in PBS, pH 7.4 at 37°C was studied. A DOX-PBS solution (200 ng/ml) was prepared and divided into 8 silanized HDPE vials. One sample was frozen immediately to reflect the concentration prior to decomposition. The other 7 samples were stored at room temperature in room light. A sample was frozen on each subsequent day for 7 days. The solutions were analyzed using reverse-phase isocratic HPLC with a fluorescence detector. Standard solutions were prepared daily using a refrigerated stock solution (DOX in DMSO).

We compared the degradation products of the DOX-PBS solution with those eluted from DOX-PMMA polymerized cements. Howmedica Simplex P bone cement (40g) was mixed with PharmItalia DOX (2.0g) powder in a powder blender for 30 min and then polymerized in a Stryker mixer. Pellets were cast in a compression test mold specified in the ASTM F451-99a guidelines. Elution studies were performed in silanized HDPE vials using PBS, pH 7.4 at 37°C for 19 consecutive days. Vials containing eluant were stored at ~20°C until the day of analysis.

RESULTS

Figure 1 is a chromatogram of a standard solution of DOX showing the single drug peak.

![Figure 1. Standard DOX Solution](image1)

Typical HPLC chromatograms of the DOX-PBS solutions and eluants from DOX-PMMA cements are shown in Figures 2 and 3, respectively. Ups to six additional peaks were observed, confirming that contact with PBS results in degradation products. The chromatograms of the cement eluants showed degradation products at the same retention times as those produced in the DOX–PBS samples. An internal standard was used to confirm the location of the parent drug in the chromatograms.

![Figure 2. DOX-PBS Solution](image2)

![Figure 3. DOX-PMMA Eluant](image3)

The disappearance of DOX in PBS displayed a first-order exponential decay. Non-linear regression analysis was performed and estimated the decay constant, τ, as 8.24 days, (T_{1/2} = 5.71 days); R^2 = 0.94.

DISCUSSION/CONCLUSION

HPLC analysis of prepared DOX-PBS solutions and of DOX-PMMA eluants identified similar degradation products of DOX. Our study determined that concentration of these products increased while DOX concentration decreased exponentially with increased contact time with PBS. These findings support our hypothesis.

Blending PMMA cement with chemotherapeutic agents is a valuable strategy for local drug delivery. In vitro elution study results are used to identify suitable clinical drug loading levels. Underestimating the quantity of drug leached from cement in these studies may result in higher levels of drug loading with the potential for delivering excess quantities that may be locally toxic. Further, the excess drug may weaken the biomechanical characteristics of the PMMA cement. It is therefore important to ensure that elution studies accurately reflect the amount of drug released from bone cement. Potential drug degradation by factors such as light, temperature, solvent pH and ionic strength are fundamental experimental concerns of elution testing protocols. Finally, an understanding of the solvent interactions will prevent misinterpretation of elution data. Our findings demonstrated that the DOX degradation products were primarily due to an interaction with PBS and not formed as a result of the PMMA polymerization reaction.

DOX is an anthracycline antibiotic that is one of the primary antitumor agents in clinical use. Tavoloni et al. showed that Ringer’s lactate and cell culture medium cause similar degradation of DOX. These findings suggest that elution profiles of similar classes of drugs may have been influenced by solvent induced drug degradation, and that the drug delivery levels were possibly underestimated.

REFERENCES

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Poster Session - Hip Arthroplasty - Hall E