EFFECTS OF ECA AND MANNITOL ON MACROMOLECULE PENETRATION AND ACCUMULATION IN RAT FIBROSCARCOMA

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ABSTRACT

Penetration of drugs into tissue depends on the structure of the tissue and the molecular properties of the drugs. To quantify the dependence, rat fibroscarcoma tissue was sectioned into 600 µm slices and incubated with 10KDa, 70KDa, and 2MDa dextran molecules for 24 hours. 10 µm cross-sections were taken and imaged to determine the normalized steady-state concentration profiles as a function of molecule size. The accumulation of molecules in the center of the tissue decreased with increasing molecule size. The tissue was then incubated in separate solutions of dextran 2M and 1M mannitol solution and dextran 2M and 1 mg/ml ethacrynic acid (ECA) solution. Mannitol is hypertonic to cells and causes them to shrink, while ECA disrupts cell microtubules causing the cells to collapse. Both treatments improved the accumulation of dextran 2M in the tissue, although the improvement in penetration and accumulation was more dramatic with mannitol treatment.

INTRODUCTION

Effective treatment of solid tumors is dependent on the ability to distribute drugs, which are typically large molecules, throughout the tumor at a clinically significant dose. Drug transport is determined by interstitial structures, cell density, and molecular properties of drugs. Krol et al. [1,2] showed that the available volume fraction (K_{AV}) is dramatically decreased by the presence of a high volume fraction of cells for molecules in the range of 40KDa to 70KDa. The reduction of K_{AV} leads to decreased penetration of molecules with a size above this range into tumor tissue. Additionally, a network numerical model predicted the sharp decrease in penetration for molecules 40KDa indicating pore connectedness also plays an important role in K_{AV} for large molecules [1]. To improve macromolecule penetration in tissues, we altered the structure of rat fibroscarcoma tissue by shrinking the cells in a hypertonic solution incubation and collapsed the cells by disrupting the cell microtubules in an effort to improve macromolecule penetration within the tissue.

METHODOLOGY

Rat fibrosacorcoma tissue sectioned into 600 ìm slices was incubated with 10KDa, 70KDa, and 2MDa fluorescently labeled dextran molecules for 24 hours. A separate set of tissue slices was incubated with dextran 2M and a hypertonic solution of 1M mannitol for 24 hours. Another set of tissue slices was incubated with dextran 2M and a solution of 1mg/ml ECA. The concentration of ECA was chosen so that it would affect the cells without destroying them completely and disintegrating the tissue.

For all cases, 10 ìm cross-sections were taken and imaged using a fluorescence microscope and ImagePro® to determine normalized steady-state concentration profiles as a function of molecule size. Averaged intensities were measured across the 600 ìm thickness of tissue. An image of tissue without fluorescent dextran was used for background subtraction for each trial. For all incubations, a total of nine images were taken for each dextran size used and averaged for a final concentration profile for each tumor tested.

RESULTS

Incubation of 10KDa, 70KDa, and 2MDa dextran molecules in rat fibroscarcoma showed that more dextran 10K accumulated in the tissue than dextran 70K or 2M. Dextran 2M showed the lowest accumulation therefore, we used only dextran 2M for our tissue manipulation experiments because an improvement in penetration and accumulation would necessarily infer improvement for dextran 10K and 70K. Both shrinking and collapsing the cells within rat fibroscarcoma tissue increased the amount of dextran 2M in the tissue. However, the amount of dextran 2M that could penetrate the tumor tissue after Mannitol treatment was greater than that after ECA treatment.

CONCLUSION

These results indicate that the volume fraction of the interstitial space can be increased within tumor tissue to improve drug

distribution by treating a tumor with a hypertonic or a microtubule disrupting agent prior to drug treatment. However, shrinking the cells is more effective at increasing the K_{AV} in tumor tissue than disrupting the microtubule structure for increasing drug delivery.

REFERENCES

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