DCE-MRI evaluation of efficacy of antiangiogenic drug in an orthotopic rat glioma model: Comparison of data processing strategies

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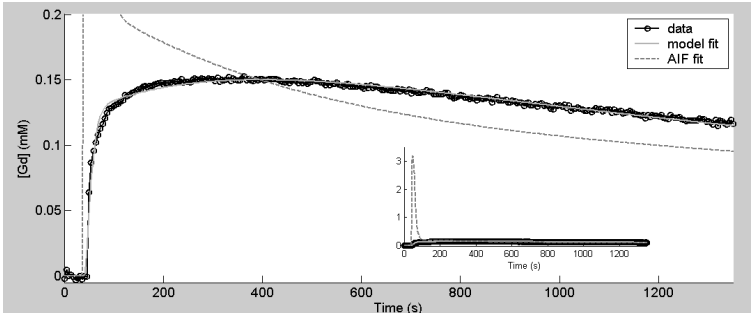
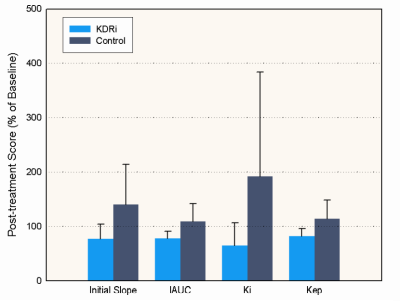
Purpose: Growth of tumors beyond a critical size is dependent on angiogenesis to maintain the supply of nutrients and oxygen for its metabolic needs. Anti-angiogenic agents, such as KDR kinase inhibitors, decreases vascular permeability and tumor vascularity, and offer a promising way of inhibiting tumor growth. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is commonly used for measurement of vascular permeability in tumors. The present study has two main goals. Firstly, to evaluate the acute effects of an experimental KDR kinase inhibitor (KDRi) in an orthotopic rat glioma model. To this end, two groups of tumor implanted rats were treated with placebo (N=10) or KDRi (N=10), and the acute responses of various DCE-MRI scores related to tumor permeability were monitored. Secondly, this work aims at evaluating the different processing strategies for obtaining the permeability scores. Four different approaches were tested: initial slope of the uptake curve, initial area under the curve (IAUC, 120 s following injection), and transfer constants from kinetic modeling approaches based on two simple two-compartment models. Comparisons of permeability scores before and after placebo treatment were considered a test-retest setting and the test-retest reliability index [1] of each approach was evaluated.

Materials and Methods: Fisher 344 rats weighing 250-300 g were anesthetized with isoflurane (induction 5%, maintenance 1% in 70% oxygen) and 10^6 9L-LacZ gliosarcoma cells stereotactically implanted in the left striatum using a Hamilton syringe through a burr hole in the skull. At 21-24 days post-cell implantation, a pre-treatment DCE-MRI evaluation was performed. On the following day, animals were treated with either methylcellulose vehicle or drug (KDRi at 100 mg/kg, p.o.) and a post-treatment MRI evaluation conducted 3-4 hours after treatment. For MRI scans, anesthesia was induced and maintained with isoflurane (5% / 2%), and the following physiological signals were monitored: expired CO_2 levels, breathing rate, and brain perfusion (arterial spin labeling MRI). MRI scans were performed on a Bruker 4.7-T/40-cm Biospec instrument with a 72 mm volume coil for excitation and an actively decoupled 2.5 x 3 cm rectangular surface coil for reception. The DCE-MRI scanning protocol consisted of 300 gradient echo images acquired every 4.55 sec (TR/TE 65.0/2.9 msec; α 60 deg; data matrix 128x70; FOV 5 cm; SLTH 2 mm; 4 contiguous slices) with a bolus of Prohance (0.3 mM/kg) injected via tail vein after the 6th image. Data were analyzed using customized Matlab (Mathworks Inc., Natik MA) software run on a Pentium 4/1.7 GHz PC and RedHat 7.2 OS. The tissue uptake curve, C_T was estimated as $C_T = (1/(R*T_{10}))*((S-S_0)/S_0)$, where R = 3.8 s^{-1}, T_{10}=1.5 s was assumed constant for all tissues, S is the MRI T1-weigthed signal and S_0 is the average signal prior to bolus injection. The initial slope of the uptake curve was estimated by fitting a straight line to the 2nd-5th data curve points, IAUC was estimated from the cumulative sum of the 2nd-28th data points following injection. The two kinetic modeling approaches assumed only passive transport phenomena were influencing the dynamics of the uptake curve. The first model assumed contrast dynamics was given by equation $(d/dt)C_T = Ki*(C_P - C_T)$, where C_P is the blood plasma concentration given by $C_P = D*[(A_0 t - A_1 - A_2)\exp(-\alpha_0) + A_1\exp(-\alpha_1 t) + A_2\exp(-\alpha_2 t)]$. Parameters A_1=2.35, A_2=1.00, α_1=15.00 x10^{-3} and α_2=1.17 x10^{-3} were taken from [2] and the first summing term in C_P is an attempt to account for the bolus "first pass" (unaccounted for in [2]). The remainder variables were estimated from non-linear (Levenberg-Marquart) curve fitting. Variable *Ki* expresses the bi-directional rate of exchange of contrast between the plasma and the tissue and is directly proportional to the tissue permeability. The second model used equation $(d/dt)C_T = K_T C_P - K_{ep} C_T$, where the contrast exchange rate into (K_T) and out off (K_{ep}) the tissue differ. In this model C_P was assumed to have the same form as in the first model, but instead of using literature value, all parameters (A_is, α_is) are treated as unknown. Non-linear fittings were used to estimate the parameters that simultaneously fit the C_T model to the data and the C_P model to experimental values measured in a separate experiment using an arterial shunt. Since in this model K_T, D and A_is are not independent, only K_{ep} was used as the permeability score. Efficacy of the KDRi was evaluated with each score by comparing (Student t-test) the post-, pre-treatment score ratios (in %) of the drug treated group (N=10) against the control group (N=10). Test-retest reliability index was estimated for each score by repeated-measures ANOVA on the pre-, post-treatment data obtained from the control group.

Results: Figure to the left show typical data set with fits from model 2. Post-, pre-treated ratios for the slope scores for treated group was 77.4±26.7 % (mean ± sd), and for the control group 139.8±74.1 % (t-test p<0.02). Ratios for the IAUC scores were 77.6±13.0 % and 108.8±33.1 % respectively for treated and control groups (p<0.007). The Ki score obtained with the first model rendered ratios 64.9±41.7 % and 192.0±191.5 % for the treated and control groups (p<0.03). Finally, model 2 Kep ratios were 82.1±13.5 % and 114±34.3 % for treated and control groups (p<0.006). Although the ratios for the control group show a trend towards increased permeability (ratio > 100%), none showed statistically significant change from baseline (ratio = 100%). Test-retest reliabilities were 0.7016, 0.8637, 0.6059 and 0.7910 for initial slope, IAUC, Ki and Kep scores, respectively. Figure to the right show summary of results





Conclusions: All scores indicate that permeability decreases acutely following treatment with a KDR kinase inhibitor, with reduction ranging from 18-25% on average. From our data, the two most reliable methods were IAUC and Kep (model 2). Ease of estimation of IAUC makes it attractive for use in the clinic. However, Kep and modeling has the potential to deliver absolute permeability figures and is a method worth pursuing. Therefore, DCE-MRI offers a reliable and quantitative methodology to rapidly evaluate pharmacodynamic responses to inhibition of vascular permeability that can be applied to preclinical models as well as in the clinical setting.

Acknowledgments: Afonso Silva, Gregory Karczman, Alan Savitz, Carol Burns, Ashleigh Bone, Angela Zeoli, Stacey Pratt, Jim Destefano

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