Preclinical Evaluation of Anti-angiogenic agent Roche1 by Dynamic Contrast Enhanced MRI at 1.5T

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Synopsis: Anti-angiogenic effects of Roche-1, a novel tyrosine kinase receptor inhibitor, have been evaluated in H460a tumor model by DCE MRI. Tumor perfusion (initial slope of the time-intensity curve) and microvessel permeability (AK_{ep}) status were analyzed. The tumor-rim slope and AKep values were lower in Roche-1 treated tumors on day 7 compared to their baseline. Controls, treated with vehicle for Roche-1, showed no significant change. On day 7 post-treatment, Roche-1 inhibited tumor growth by 50% relative to the controls. ROI Selection for the kinetics analysis influences outcome of the results.

Introduction: Tumor vascular targeting by anti-angiogenic agents is gaining importance in cancer therapy and at present various agents are under clinical and pre-clinical trials (1). Contrast kinetic parameters derived from dynamic contrast enhanced (DCE) MR imaging provides an indirect measure of tumor vascular function, which is being exploited in assessing the extent and time course of anti-angiogenic effects in tumors(2). The present study was designed to assess the anti-angiogenic potential of Roche-1, a potent inhibitor of tyrosine kinase receptors involved in angiogenesis (KDR, FGFR, and PDGFR), in H460a lung tumors by DCE MRI with 1.5T scanner and the clinically used standard contrast agent, Gd-DTPA.

Materials and Methods: The H460a (human non-small cell lung carcinoma) tumors were grown by subcutaneously inoculating 10 x 10^6 cells in the flank of female athymic/nude mice. Tumor volumes were calculated from the measures of three orthogonal diameters (d) as (d_1 x d_2 x d_3)/2 and measured on alternate days. Tumors with a volume of approximately 200 mm^3 (10-12 day after inoculation) were treated with anti-angiogenic agent Roche-1 (6.25 mg/kg twice a day p.o) daily for 14 days. A home-built small transmit-receive surface coil (1cm dia, single turn copper-foil) covering only the tumor was used for imaging on a 1.5T MRI scanner (Signa LX, GE, Milwaukee, WI,). Dynamic images were acquired before and after injection of contrast agent using fast spoiled gradient-echo (SPGR) with TR/TE/θ = 9ms/2ms/30^0, FOV=40x40mm, matrix size 256x128, 2 mm slice thickness (yielding in-plane spatial resolution 156x156µm after zero-filling). A single slice at tumor center was acquired at 7 sec temporal resolution with number of excitations (NEX) 1 and 64 time points in ~14 min. Gd-DTPA (Magnevist, Berlex Laboratories, Wayne, NJ; 0.2 mmole/kg) was injected as a bolus (0.3cc, ~7sec) through tail vein catheter after completing acquisitions of 5 baseline images. Imaging studies were conducted prior to (day0) and 7 days after start of therapy. Image analysis was performed on UNIX workstation (Ultra 20, Sun Microsystems, CA) using in-house software written in interactive data language (IDL v6.0, RS Inc., Boulder, CO). Three different regions of interest (ROIs) viz: whole tumor, tumor rim and tumor center were selected for analysis. Time-intensity curves were analyzed for each voxel. The initial uptake slope was used for characterization of the response to the contrast material bolus. The initial slope was calculated with five-point sliding linear regression applied to the first 2 minutes of the time-intensity curve (TIC). A baseline signal intensity (SI) value, SI_{pre}, was calculated as the mean intensity of three points before injection. The percentage increase per minute for each voxel was then calculated according to the following equation: % SI/min = $Slope/SI_{pre}$.100. A two-compartment pharmacokinetic model (2, 3) was applied to derive the contrast kinetic parameter Ak_{ep}, which is related to vascular permeability. The parameters A (amplitude) and K_{ep}, which describes the contrast transfer between the lesion and plasma compartments are fit independently. A Two-tailed paired t-test was employed for statistical analysis of the results.

Results and Discussion:

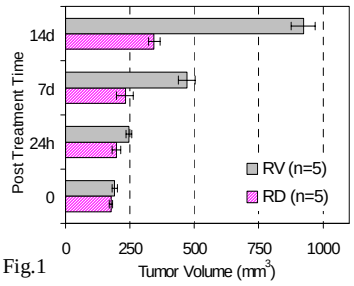

Fig.1

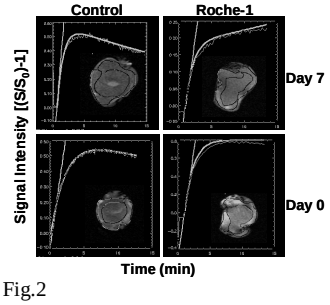

Fig.2

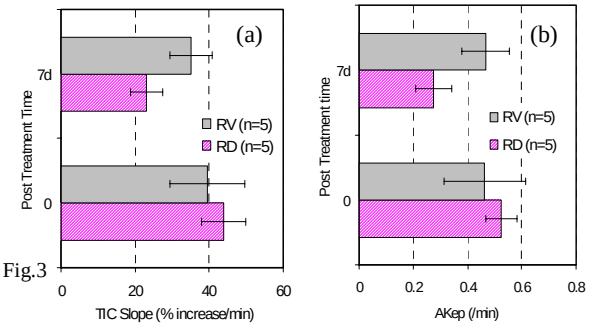

Fig.3

Fig1). H460a tumor growth inhibition by Roche-1(RV-Control, RD-Roche1). Fig 2).GdDTPA contrast kinetics at the tumor rim with corresponding model fits from representative data sets from control & Roche1 treated tumors. Fig3). The initial slope of the contrast uptake (a) and the AKep values (b) from tumor rim were reduced in Roche1 treated tumors (RD) on day 7, compared to their baseline. Controls (RV), treated with vehicle for Roche1, show no significant change.

Fig.1 shows approximately 50% tumor growth inhibition by Roche1 on day7, relative to controls. Post-treatment changes in the pattern and kinetics of contrast enhancement (fig.2) are different for controls and Roche1 treated tumors. The reduction in tumor-rim slope and AK_{ep} values observed on day 7 compared to their baseline values in Roche1 treated group (Fig 3a and 3b) approaches significance(p_{slope}<0.08, p_{AKep}<0.07). Changes observed from the tumor center and whole tumor ROIs are not significant (data not shown). The control group did not show any significant change in either slope or AK_{ep} values in all three ROIs. These preliminary results indicate that selection of ROI have great influence on the outcome of the analysis of anti-angiogenic effects. The effects of angiogenic inhibition found to be well delineated when restricting the analysis of tumor contrast enhancement kinetics to tumor rim as this region of the tumor is typically the most vascularized and least necrotic compared to tumor center. Histopathological microvascular density measurements and VEGF immunohistochemical analysis are ongoing in our laboratory to further corroborate the present results. In addition, this study demonstrates, using a custom built surface coil, the feasibility of acquiring high spatial and temporal resolution DCE-MRI data from small animals on a standard 1.5T clinical scanner.

References:

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