Characterization of Intradermal Histamine-Induced Vascular Leak in Mice

B. E. Zauscher[1], R. V. Mulkern[2], A. LaForme[3], M. W. Kieran[3]

[1]Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]Department of Radiology, Children's Hospital, Boston, MA, United States, [3]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA, United States

Introduction. Anti-angiogenic drugs offer a unique opportunity to target cancer growth via pathways independent of tumor mediated drug resistance. Inhibitors of neovascularization often require prolonged administration at biologic doses [1] targeting either the endothelial compartment or the cytokines that mediate endothelial proliferation, migration and tube formation. Vascular Endothelial Growth Factor (VEGF) is a major regulator of angiogenesis and was initially discovered as Vascular Permeability Factor (VPF) because of its ability to induce vascular leak [2]. Many anti-angiogenic drugs inhibit VEGF induced-edema, which can be used as a surrogate marker of drug activity. The administration of VEGF to humans is not feasible because this molecule could stimulate tumor progression. As such, we have investigated intradermal histamine-induced vascular leak [3] inhibition as a surrogate marker of anti-angiogenic drug activity. If a reliable correlation can be found between histamine-induced edema and tumor permeability, and this correlation changes consistently with the administration of an anti-angiogenic agent, then the ideal dosage of the agent can be determined.

Methods. Severe combined immunodeficient (SCID) mice were shaven 24 hours prior to experiments. Animals were anesthetized using a 2% isoflurane-air mixture for all procedures. An intravenous (iv) injection of Evans blue solution (1%) was given via the retro-orbital venous plexus. Mice were kept on a 37˚C pad. After 10 minutes, 50µl of saline and 50µl of 1:100 histamine solution were administered by intradermal injection. MRI scans were performed on a Bruker Biospec 4.7T magnet using a 35mm birdcage coil. A phantom with 0.1mM Gd-DTPA was used for signal intensity comparisons. A 100µl iv injection of 50mM Gd-DTPA was administered via tail-vein. Fat suppressed T1-weighted spin echo images (TE 7ms, TR 500ms, FOV 3.12cm, Matrix Size 256x256) were subsequently acquired at approximately 2-minute intervals for 25 minutes.

Results. In previous experiments, differences were observed between intradermal histamine and saline reactions evaluated after Evan's blue administration [Fig 1]. Quantitative data obtained using spectrophotometry of skin removed 10 minutes after intradermal injections demonstrated significant vascular leak in the histamine treated side. Phantom studies revealed no paramagnetic shortening of water relaxation times due to Evan's blue dye. Image series obtained with saline and histamine intradermal injections (n=5 mice) showed bright signal progress inward from the margins of the injection sites until the entire injection volume became brighter [Fig 2]. The site of the histamine reaction had a bright signal that extended further radially than did the site of the saline reaction. Volumes of fluid at injection sites were measured by summing manually selected regions of interest of the slices obtained. For the case shown in the figures, the histamine volume was 1.87 times larger than that of saline (586 pixels vs. 313 pixels). Mean signal intensities of each region of interest (ROI) were divided by the mean signal intensity of the phantom for each slice. Comparison of these intensities demonstrates that the side with the histamine injection had a slightly larger mean signal intensity at all time points measured [Fig. 3]. Results confirmed the differences observed in Evan's Blue leakage out of injection sites.

Discussion and Conclusion. These results suggest that MRI can be used to measure vascular leak due to an intradermal histamine injection. SCID mice are commonly used to grow human tumors, but evaluation of edema using Evan's blue requires sacrificing the animals, which prevents an analysis of changes over time. Accumulation of Gd-DTPA around the area of a histamine injection is similar to that of blue dye. Therefore, the ability to serially assess changes in vascular leak by MRI becomes an important methodology for evaluating novel anti-angiogenic agents and possibly tumor response.

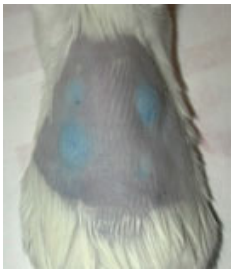

Fig 1. Evan's blue as a marker of vascular leakage. Intradermal histamine injections given on top right and bottom left. Intradermal saline injections given on top left and bottom right.

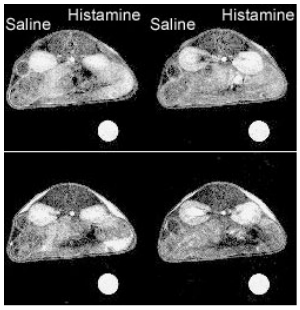

Fig 2. Axial MRI slices showing histamine and saline injections in one mouse. Images are fat suppressed and T1-weighted. Top slices are 2min post-Gd-DTPA, bottom slices are 24min post-Gd-DTPA.

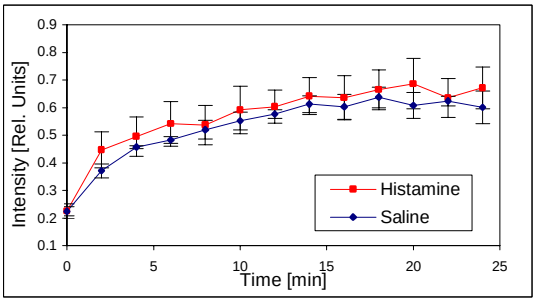

Fig 3. Total mean intensity of injection volumes vs. time for mouse shown in Fig 2. Intensity of injection ROIs was normalized to phantom intensity in each slice. Intensity at time=0min corresponds to scan prior to Gd-DTPA injection. Intensity of histamine is slightly larger than that of saline at all time points.

References

[1] O'Reilly, M. et al., *Cell,* 1997.
[2] Takahashi, Y. et al., *Cancer Res*., 1995.
[3] Majno, G. et al., *J. Bioph. Bioch. Cyt.,* 1961.