VALIDATION OF OXYGENATION AND PERFUSION SENSITIVE MRI METHODS IN HEALTHY BRAIN AND BRAIN TUMOR IN MICE BY INVASIVE MICRO PROBE MEASUREMENTS.

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TARGET AUDIENCE: Researchers developing quantitative non-invasive imaging methods and seeking for a validation of those and researchers looking for quantitative non-invasive imaging end points for clinical and preclinical cancer therapy studies.

PURPOSE: Non-invasive assessment of (patho-)physiological parameters, such as perfusion and oxygenation is of great importance for the characterization of pathologies e.g., tumors, as these parameters may be helpful to better predict treatment response and outcome. The purpose of this work was to validate MRI methods sensitive to blood oxygenation and perfusion by invasive micro probe measurements during different free breathing conditions in the brain of healthy mice and mice with brain tumors.

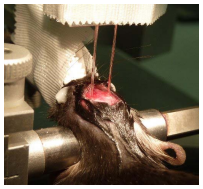

Fig. 1: Stereotactical insertion of micro probes.

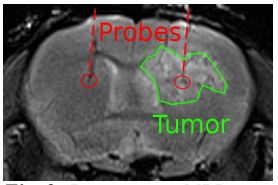

Fig. 2: Post mortem MRI to detect location of micro probe.

METHODS: Sixteen male C57BL/6J_2010 mice (age: 8 weeks, weight: 25g) were used for the validation study in healthy brain tissue and seven identical animals were used to investigate tumor tissue. The animals were anesthetized by intra-peritoneal injection of ketamine-xylazine-acepromazine (10-2-0.3mg/ml·kg). The brain tumors were established 18-21 days prior the MRI measurements by stereotactical injection of 3.5µl containing 150k GL261 tumor cells into the right basal ganglia.

MRI parameters investigated using a 7T small animal MRI were:

- irreversible (R2), reversible (R2') and effective (R2*) transverse relaxation rates assessed by multi echo gradient echo (mGRE) and multi echo turbo spin echo (mTSE) sequences
- venous blood oxygenation level (Y) assessed by quantitative blood oxygenation level dependent (qBOLD) MRI[1] and
- cerebral blood flow (CBF) assessed by arterial spin labeling (ASL) MRI[2].

All measurements were performed for three different breathing conditions: air, hypercapnia (air+10%CO2) and hyperoxia (100%O2).

The day after the MRI was performed, the micro probes were stereotactically inserted to measure tissue perfusion (normalized blood perfusion units, nBPU) by Laser-Doppler flowmetry and tissue pO2 by fluorescence quenching (Fig. 1). Micro probe measurements were successfully performed for 11 healthy and 6 tumor mice. ROI-based analysis was performed at the location of the micro probe tips. The location was confirmed by post mortem MRI (Fig. 2).

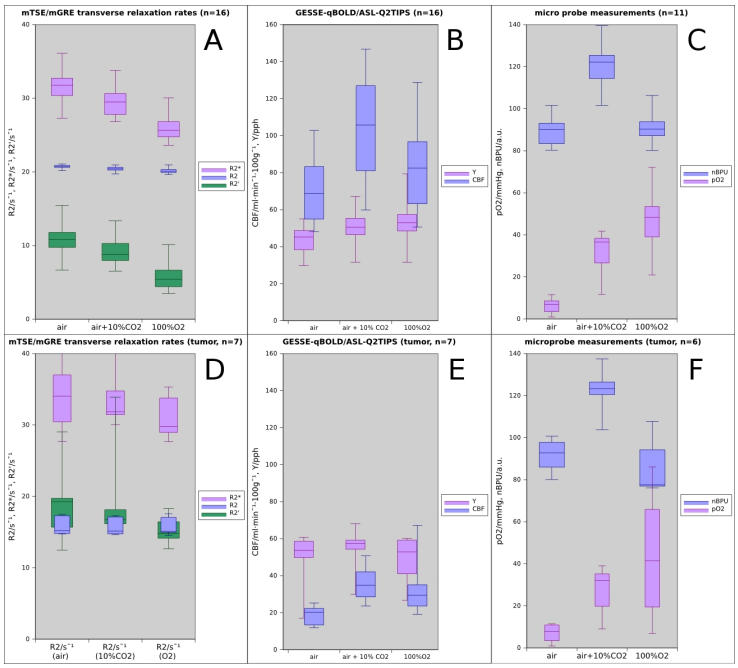

Fig. 3: Boxplots of all assessed parameters during different breathing conditions (top row: healthy mice, lower row: tumor mice).

RESULTS: Oxygenation (Y and pO2) of healthy mice increased and R2, R2*, R2' dropped simultaneously during hypercapnia (Fig. 3A-C). Oxygenation of healthy mice increased even further during hyperoxia as compared to the air breathing condition. Tumor oxygenation measured by micro probes (pO2) also increased similar to healthy controls, but showed a higher variability at 100%O2 (Fig. 3F). MRI oxygenation sensitive parameters (R2, R2*, R2' and Y) showed no clear differences comparing breathing conditions (Fig. 3D,E). Both healthy and tumor mice showed increased perfusion levels (CBF and nBPU) during hypercapnia and a subsequent decrease during hyperoxia to levels observed during air breathing (Fig. 3B,C,E,F). In contrast, absolute CBF values within the tumor were much lower compared to healthy mice (Fig. 3B,E). All parameters of healthy mice were statistically significant different between the different breathing conditions (ANOVA, $P<0.001$, Tab. 1). Tumor mice showed significant differences only for CBF, nBPU and pO2 (Tab. 1). Linear regression analysis between the parameters measured by MRI and micro probes showed good linear correlations for healthy mice which were statistically significant ($P\leq0.03$); no significant correlation was found for tumor mice (Tab. 2).

Table 1: Mean ± standard deviation of MRI and micro probe data for different breathing conditions (see Fig. 3).

Healthy mice brain	**Air**	**Air+10%CO2**	**100%O2**	***P* value**
R2/s^{-1}	20.7±0.26	20.4±0.34	20.1±0.34	<0.001
R2*/s^{-1}	31.6±2.3	29.6±2.2	25.9±1.6	<0.001
R2'/s^{-1}	10.9±2.2	9.2±2.0	5.7±1.7	<0.001
Y	0.43±0.07	0.51±0.09	0.56±0.08	<0.001
CBF/ml·min^{-1}·100g^{-1}	70.6±17.2	105.5±28.9	81.8±22.4	<0.001
pO2/mmHg	6.3±2.5	32.3±5.7	46.7±6.8	<0.001
nBPU/a.u.	89.2±9.4	120.2±11.0	90.6±9.5	<0.001
Brain tumor mice				
R2/s^{-1}	15.9±1.33	15.8±1.27	15.9±1.27	0.99
R2*/s^{-1}	34.7±6.3	35.6±7.4	31.2±3.1	0.41
R2'/s^{-1}	18.8±5.3	19.2±6.6	15.3±2.0	0.29
Y	0.49±0.15	0.55±0.12	0.49±0.13	0.67
CBF/ml·min^{-1}·100g^{-1}	18.4±5.4	35.8±10.5	30.1±17.0	0.04
pO2/mmHg	7.0±2.6	27.5±5.2	43.7±6.6	0.02
nBPU/a.u.	91.6±9.6	122.5±11.1	85.9±9.3	<0.001

Table 2: Linear regression and correlation analysis between micro probe (pO2, nBPU) and MRI data.

Healthy mice brain	**Person's R**	**Slope**	**Intercept**	***P* value**
pO2 vs. R2	-0.59	-0.011 mmHg^{-1}·s^{-1}	20.8 s^{-1}	<0.001
pO2 vs. R2*	-0.56	-0.09 mmHg^{-1}·s^{-1}	31.9 s^{-1}	<0.001
pO2 vs. R2'	-0.52	-0.078 mmHg^{-1}·s^{-1}	11.1 s^{-1}	0.002
pO2 vs. Y	0.43	0.00198 mmHg^{-1}	0.45	0.012
nBPU vs. CBF	0.38	0.63 ml·min^{-1}·100g^{-1}	24.5 ml·min^{-1}·100g^{-1}	0.03
Brain tumor mice				
pO2 vs. R2	0.01	0.0004 mmHg^{-1}·s^{-1}	16.1 s^{-1}	0.97
pO2 vs. R2*	-0.25	-0.06 mmHg^{-1}·s^{-1}	36.0 s^{-1}	0.32
pO2 vs. R2'	-0.28	-0.06 mmHg^{-1}·s^{-1}	19.9 s^{-1}	0.25
pO2 vs. Y	0.21	0.0011 mmHg^{-1}	0.49	0.41
nBPU vs. CBF	0.19	0.14 ml·min^{-1}·100g^{-1}	15.2 ml·min^{-1}·100g^{-1}	0.46

DISCUSSION: Measurements obtained by MRI and those using micro probes were in good agreement and showed a significant correlation in healthy brain tissue providing strong evidence for valid data measured by the oxygenation-sensitive and perfusion-sensitive MRI methods. However, MRI parameters of tumor tissue did not show a significant correlation with the invasive micro probe measurements. This lack of significance may be a result by the low number of tumor mice investigated thus far. Further experiments in tumor mice are underway to overcome this limitation. Furthermore, the very low absolute CBF values measured in tumor tissue may suggest that an insufficiently organized tumor vasculature significantly prolongs the blood transition time which negatively interferes with the CBF quantification model. The chaotic tumor vasculature may also interfere with the qBOLD method where randomly oriented infinitely long cylinders are assumed to model the blood vessel network.

CONCLUSION: A validation of perfusion- and oxygenation-sensitive MRI methods in healthy mice brain was performed by invasive micro probe measurements. However, the missing correlation between MRI and micro probe measurements in tumor tissue demonstrates the current limitation of these MRI methods and corresponding quantification models which were mainly developed and verified in healthy tissue. Further investigations are necessary to extend these MRI methods and quantification models for pathologies such as brain tumors.

REFERENCES: 1. He X, Yablonskiy DA. MRM 2007;57(1):115–26. 2. Luh WM, Wong EC, Bandettini PA, Hyde JS. MRM 1999;41(6):1246–54.

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