Imaging of Cerebral Blood Flow in Rodent Models with SPECT, MRI and Autoradiography

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1 BACKGROUND

Regulation of blood flow and oxygen delivery in the brain is essential for survival. In a normal physiological state the cerebral blood flow (CBF) stays very constant due to persistent adjustment of vascular resistance according to blood pressure. Imaging of CBF has clinical relevance as functional abnormalities can signal pathophysiological changes e.g. stroke and neurodegenerative diseases. In this study methods to measure CBF in rodent models are presented.

Single photon emission computed tomography (SPECT) imaging after intravenous injection of ^{99m}Tc-exametazime (^{99m}Tc-HMPAO) is a conventional method to measure CBF *in vivo*. It has been shown to correlate strongly with regional brain perfusion and is used in clinical nuclear imaging to detect stroke and other cerebrovascular diseases.

Arterial spin labeling allows capturing of brain perfusion with magnetic resonance imaging (MRI). It is a noninvasive method where protons in arterial blood are magnetically labeled and subsequently imaged in the region of interest. Therefore, no injection of contrast agent is needed to obtain information about cerebral perfusion (Figure 6). Arterial spin labeling MRI sequences are increasingly being used in clinical imaging to provide quantification of CBF.

Although ^{99m}Tc-HMPAO-SPECT and arterial spin labeling MRI techniques are translational between clinical and preclinical studies, differences arise in the required use of anesthesia. One of the major challenges to study neurological function in rodents is due to use of restriction, anesthesia or paralyzing agents. All of these methods have impact to brain perfusion e.g. altering the vasoconstriction and blood pressure. To study immediate neuronal effect, delivery of radiotracer that reaches a cerebral equilibrium in a short time frame will allow imaging with great temporal resolution. To avoid these effects, animals were cannulated in jugular vein week prior to administration of radiotracer. Tracer was injected trough cannula connected to a tether, followed by immediate dosing of euthanizing solution. Brains were collected and quantified using autoradiography. Alterations in CBF between studied brain regions were quantified.

As a summary, several translational approaches can be applied in rodent models to monitor CBF alterations associated to disease progression or drug effect.

2 AUTORADIOGRAPHY

When studying CBF with radioisotopes, delivery of radiotracer that reaches a cerebral equilibrium in a short time frame will allow imaging with great temporal resolution. ^{14}C-iodoantipyrine has shown a strict linear proportionality between tissue radioactivity and CBF when the data is captured within a brief interval after the tracer injection.

To study CBF without restrain or anesthesia in Q175KI mice, a model for Huntington's disease, the animals were cannulated in jugular vein week prior to administration of radiotracer. Tracer was injected trough cannula connected to a tether, followed by immediate dosing of euthanizing solution.

Brains were collected, cryosectioned and quantified using digital autoradiography (Figure 1). Alterations in CBF between WT and HET mice in different brain regions were quantified (Figure 2). HET mice showed lowered CBF in the cortex, striatum and thalamus regions compared to WT littermates (Table 1 and Figure 2). For more detailed description about CBF in HD mice, visit poster 226.02.

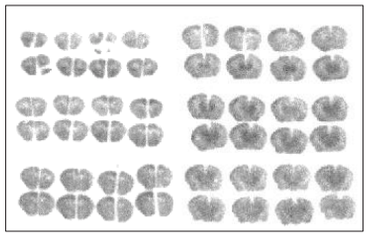

Figure 1. Example autoradiography images from coronally sectioned brains.

Table 1. Decrease in brain perfusion in various studied brain regions at 9 and 12 mo zQ175KI mice (*, p<0.05).

	9 mo	12 mo
Brain Region	**Decrease, HET vs. WT (%)**	**Decrease, HET vs. WT (%)**
Somatomotor cortex	23.2	10.8
Cingulate cortex	25.4	*15.5**
Striatum	21.1	*16.4**
Globus pallidus	3.0	6.8
Thalamus	6.1	11.1
Hippocampus	14.7	7.0
Pons	7.9	6.2
Medulla	12.2	6.8
Cerebellum	13.6	4.3

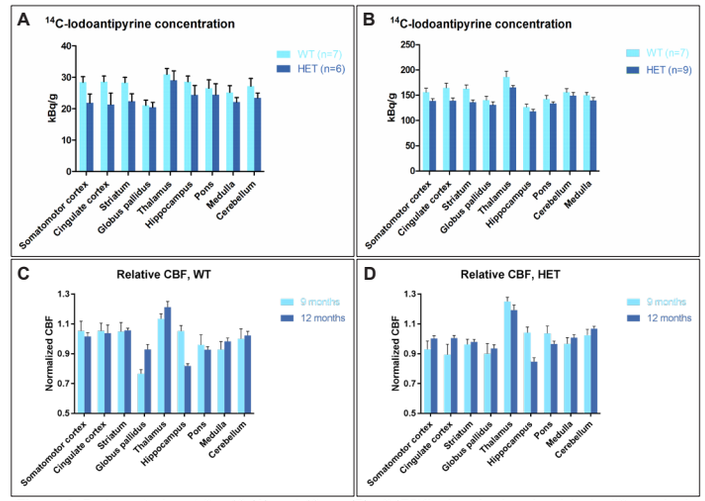

Figure 2. Brain perfusion at 9 – 10 (A) and 12 mo (B) zQ175KI mice. Analysed activity in different brain region, values normalized to external standard and shown as kBq/g. Values shown as mean +SEM.

Further, data from each region was normalized to total activity in brain to compare relative perfusion over time within WT (C) and HET (D) male mice.

3 SPECT

To study CBF of conscious C57/6J mice, the mice were surgically operated with a jugular cannula connected to a injection port in the back one week prior imaging. To confirm successful jugular vein cannulation prior SPECT, dynamic contras enhanced magnetic resonance imaging (DCE-MRI) with gadolinium contrast agent was performed.

On the day of imaging mice were connected to a tether for tracer injection. The mice were let to move freely in their home cage prior and during the injection to minimize the effect of stress (Figure 3). Further, CBF was also combined with behavioural test (forced swim test, FST). Three minutes after injecting ^{99m}Tc-HMPAO (157 – 199 MBq) the mice were anesthetized with isoflurane and imaged with SPECT/CT (NanoSPECT, Mediso) for 60 min (Figure 4A and B).

SPECT images were fused with mouse brain MRI template and the radioactivity in different brain regions was analysed with PMOD software (PMOD, v 3.7). Highest tracer uptakes were observed in inferior colliculi, superior colliculi and cerebellum (Figure 4C).

In addition, after the SPECT/CT imaging mice were terminated, brains were collected and cryosectioned for autoradiography analysis (Figure 5).

Figure 3. To administrate perfusion agent i.v. without restriction, anesthesia or analgetic agents, mice were surgically cannulated to jugular vein with skin button dosing port dorsally sutured ca. week prior perfusion experiment. On the study day, mice were connected with tether through which brain perfusion tracer was administrated within their home cage.

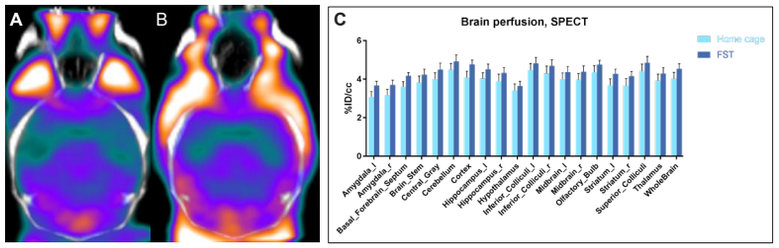

Figure 4. Average SPECT/CT images fused together representing ^{99m}Tc-HMPAO uptake in the brain. Images from home cage (A) and FST (B) in coronal direction. ^{99m}Tc-HMPAO uptake in different brain regions of mice measured with SPECT (C). Uptake expressed as percentage of injected dose/cm^3 of tissue (%ID/cc).

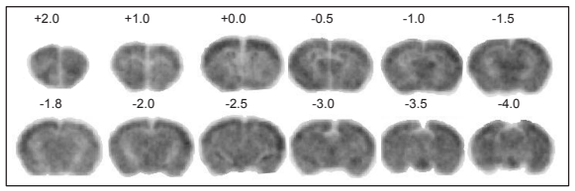

Figure 5. Autoradiography can be also performed using SPECT isotopes e.g. after *in vivo* dosing with ^{99m}Tc-HMPAO. In digital autoradiography (BetaImager) only the low energy beta particles of ^{99m}Tc will be detected and hence providing better resolution than traditional film or phosphorimager plates where gamma emitting isotopes cause the exposure. Coordinates shown as relative distance (mm) from bregma.

4 MRI – ARTERIAL SPIN LABELING

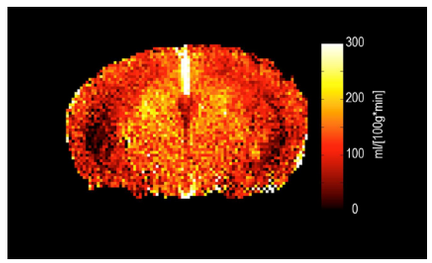

Figure 6. FAIR-ASL (RARE-sequence) was measured using selective and non-selective T1 mapping approach at 11.7 T small animal MR system from naïve mouse *in vivo*. Single slice measurement results to quantitative values of cerebral perfusion in units of ml/(100 g*min). Essentially, this approach allows longitudinal evaluation of *in vivo* changes upon pathological settings or pharmacologically induced challenges.

5 SUMMARY

CBF imaging is widely used in the clinics as changes in cerebral blood flow can tell about pathophysiological states. In preclinical imaging, the used of anaesthesia can cause severe bias in the blood flow and should be avoided when possible. SPECT, MRI, and autoradiography can be combined to study CBF in CNS rodent models combined simultaneously also with behavioural experiments.

As a summary, several translational imaging techniques and tracer approaches can be applied in rodent models to monitor CBF alterations associated to disease progression or drug effect. Furthermore, autoradiography techniques avoid the use of anesthesia and provide good temporal and spatial resolution.