Impact of contrast agent osmolarity and dose on the quantification of blood-brain barrier disruption in a DCE-MRI study

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Introduction: Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is an imaging tool that is particularly suitable for characterizing blood-brain barrier (BBB) damage in humans as well as in animal models as it allows in vivo identification of BBB compromise with high spatial resolution; the contrast agents used are typically gadolinium chelates. There are several gadolinium chelates marketed at this moment, all of which have been employed in animal studies; the administration route, dose, and dilution factor used in these studies vary widely; reasons for a particular selection of conditions are usually not given. The dose of contrast agent (CA) for clinical investigations recommended by manufacturers is 0.1mmol/kg body weight. In animals, however, this dose is usually increased several fold in order to compensate for their small size [1, 2]. Such a dose is considered sufficient for animal models of severe barrier disruption; in experiments where the disruption of BBB is uncertain, much higher doses are used [3, 4]. The purpose of this study was to identify optimal conditions for detecting low/uncertain BBB disruption and to provide an improved protocol therefor. The studied doses for our mouse experiments were chosen as follows: 1mmol/kg was chosen to mimic the clinical protocol; 4 mmol/kg Dotarem and 8 mmol/kg Prohance are the maximum doses that can be injected i.p. in mice, when diluted to plasma osmolarity; 10 mmol/kg Dotarem is the maximum dose that can be injected undiluted without triggering immediate toxicity. In order to provide a more temporally consistent and sustained enhancement than an intravenous bolus, the CA was administered as an i.p. bolus. In the present study two different gadolinium-based contrast agents have been employed – gadolinium-tetraazacyclododecanetetraacetic acid (Dotarem®), and gadoteridol (ProHance®) – in order to demonstrate the impact of the osmolarity and dose of the contrast agent on the estimation of the BBB damage.

Methods: *Mouse model:* An animal model of mild BBB disruption was chosen, using repeated induced cortical spreading depression [5] (CSD) in the brain of healthy wild type mice, which has been reported to induce BBB damage in the mouse brain [6]. In this study, seven consecutive CSDs were induced in the right hemisphere before allowing the animals to recover. The dynamics of BBB permeability were followed by MRI 24h post-surgery. *MRI scans:* The mice were divided into four groups and administered a different osmolarity and dose of contrast agent (CA) (see table 1). In vivo RARE T1-weighted DCE-MRI measurements were performed with a Bruker 9.4 T system, consisting of a pre-scan followed by 6 consecutive scans immediately after the intraperitoneally (i.p.) injected CA. Parameters were: TE/TR = 11.67/870ms, resolution = 0.078mm/pixel, 22 slices of 0.5mm thickness, acquisition time 11min. *Processing:* A voxel-by-voxel analysis was performed by Patlak plots [7] consisting of plotting the ordinate $C_t(t)/C_p(t)$ versus abscissa $\mathrm{Int}\{_0^t(C_p(\tau)d\tau/C_p(t))$; the slope of the plot is the CA blood-to-brain transfer rate (Ki). The total volume of the affected area was defined as having a threshold Ki > 0.001 ml/g min^{-1} and an R^2 of the Patlak plot > 0.7, and was composed of all pixels satisfying these conditions after smoothing the image.

Group label	Dose CA (mmol/kg)	Formulation	Osmolarity (mOsm/L)	Injected volume (µL) /25g mouse
D10	10	Dotarem	1350	500
D4	4	Dotarem	285	950
D1	1	Dotarem	285	250
P8	8	Prohance	285	900

Table 1: Dosage and osmolarity of CA used in different groups of mice.

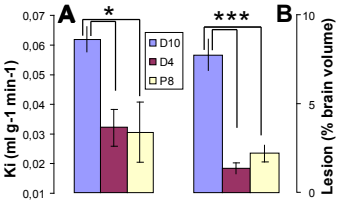

*Figure 1: (A) Average Ki and (B) lesion volume expressed as a percentage of the total brain volume, for each group, with standard deviation values. *p<0.01 ***p<0.0001*

Results and discussion: At the dose corresponding to clinical practice (D1), no BBB disruption was detectable, therefore this dose was excluded from further analysis. The D4 and P8 groups showed comparable blood-to-brain transfer rate (Ki) values and affected brain volumes (Fig. 1). Interestingly, 10 mmol/kg Dotarem (D10) yielded Ki values that were twice as high as for group D4 (Fig. 1A), as well as a BBB-compromised brain volume that was about 3 times larger (Fig. 1B). This difference could be explained at first glance as an increased sensitivity achieved by a higher dose, but close inspection of the raw MRI data revealed that 6.3±0.6% shrinkage of the total brain volume occurred after CA administration (see Fig. 2); the shrinkage represents a temporary dehydration which can be attributed to the high osmolarity of the injected CA [8]. The BBB damage in the D10 group is thus exacerbated by the high osmolarity of the injected CA.

Conclusions: A high CA dose is required to ensure the visualization and quantification of areas characterized by mild BBB disruption, but a high osmolarity of the contrast agent can aggravate BBB damage. Our optimized protocol represents a combination of maximum dosage and iso-osmolarity of CA.

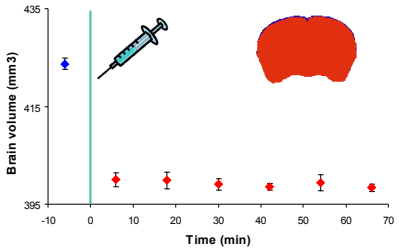

Figure 2: Decrease in brain volume after injecting 10mmol/kg (p<0.02). In the upper right corner – an example of the observed brain shrinkage in a mouse from D10 group (-3.0 mm Bregma), shown by overlapping the brain area in the last acquired image post-injection (red) over the brain area of the same slice in the pre-injection scan (blue).

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