Supplemental Digital Content (SDC)

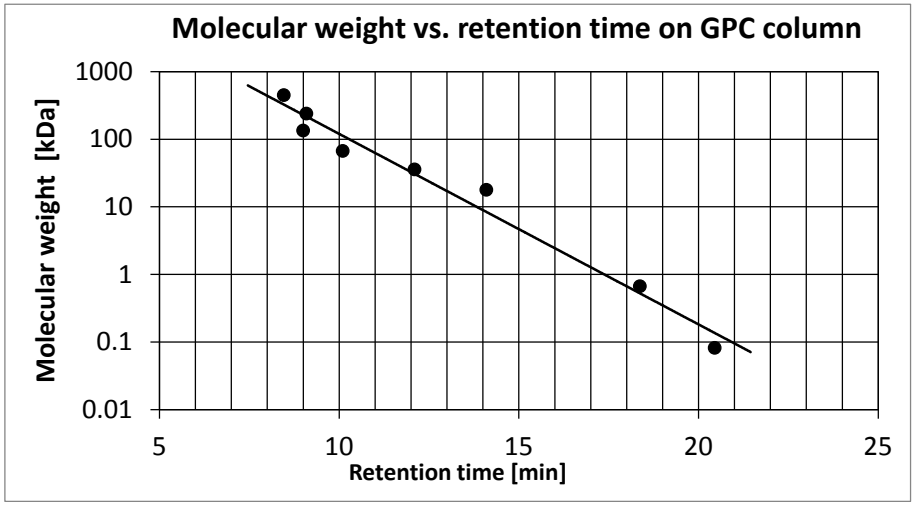
Gel Permeation Chromatography (GPC)

GPC is a tool to assess the molecular weight of macromolecules such as proteins or carbohydrates. In general, an inverse linear relationship exists between the logarithm of the molecular weight (MW) and the retention time of the compound. The used column, Sephadex 75, was calibrated using purified proteins of known molecular weight and some small molecules, to obtain this relation. The following compounds were used for calibration. The peaks were detected by UV absorption (200-300 nm):

Table SDC 1: Molecular weight standards used for the calibration of the GPC column and their retention times

MW-standard	MW [kDa]	Retention time [min]
Sodium acetate	0.08	20.4
Gadobenate	0.67	18.4
Myoglobin, monomer	17.8	14.1
Myoglobin, dimer	35.6	12.1
Bovine serum albumin, monomer	67	10.1
Bovine serum albumin, dimer	134	9.0
Catalase	240	9.1
Ferritin	450	8.5

Figure SDC 1: Retention times on GPC of standards of different molecular weight.



The used column provides separation of molecules up to a molecular weight of a few hundred kDa. Molecules larger than 250-300 kDa elute in the exclusion volume around 8.5 min.

Recovery of GBCAs and gadolinium during the extraction process

The recovery of each step during fractionation and chromatography was investigated. Cerebrum (about 650 mg each) from untreated rats was spiked with 65 µL of a 100 µM GBCA solution. This mimics a tissue concentration of about 10 nmol/g wet tissue (n=2 preparations for each GBCA and for saline). The tissues were then treated like the samples from the animals treated with GBCA. The whole processing time was about 4-5 h and the samples were kept on ice or at 4°C during this time and the individual fractions were stored at -20°C until analysis.

Overall recovery: Based on the initial concentrations in the homogenates in this recovery experiment, around 94-96% of the Gd of the added GBCA was found in the different fractions (pellet + supernatant A), indicating that only little gadolinium was lost during the sample fractionation.

Pellet - insoluble fraction: A small percentage (~ 5-7%) of the intact GBCAs, linear or macrocyclic, remained in the washed pellet, indicating that such a small amount should not be interpreted as degradation or precipitation of insoluble Gd species, but rather as incomplete washout and incomplete separation between the soluble and insoluble fraction.

Supernatant - GPC: The chromatographic system produced a small system peak in the retention time range of macromolecules when blank samples from untreated animals spiked with saline were injected. Therefore, these blank chromatograms were subtracted from spiked samples. For Gd-DOTA, gadobutrol, Gd-DTPA and gadobenate less than 0.5% of the total Gd was found in the remaining macromolecular region. No prominent difference was observed between these compounds. The samples spiked with gadodiamide, however, contained a clear peak around 250-300 kD which contained about 14% of the total Gd.

Chromatograms of the GPC of the spiked brain tissue are presented in Figure SDC 2.

The Gd concentration in each fraction and the total recovery are summarized in Figure SDC 3

The recovery of Gd from all tissue samples of the treated animals was determined as the sum of the measured amount of Gd in the soluble and insoluble fraction relative to the homogenate. The mean recovery was 87 ± 12 %. Individual results are presented in Figure SDC 4.

Figure SDC 2: Gel permeation chromatograms of the soluble fractions of cerebellum spiked with 10 nmol Gd/g GBCAs.

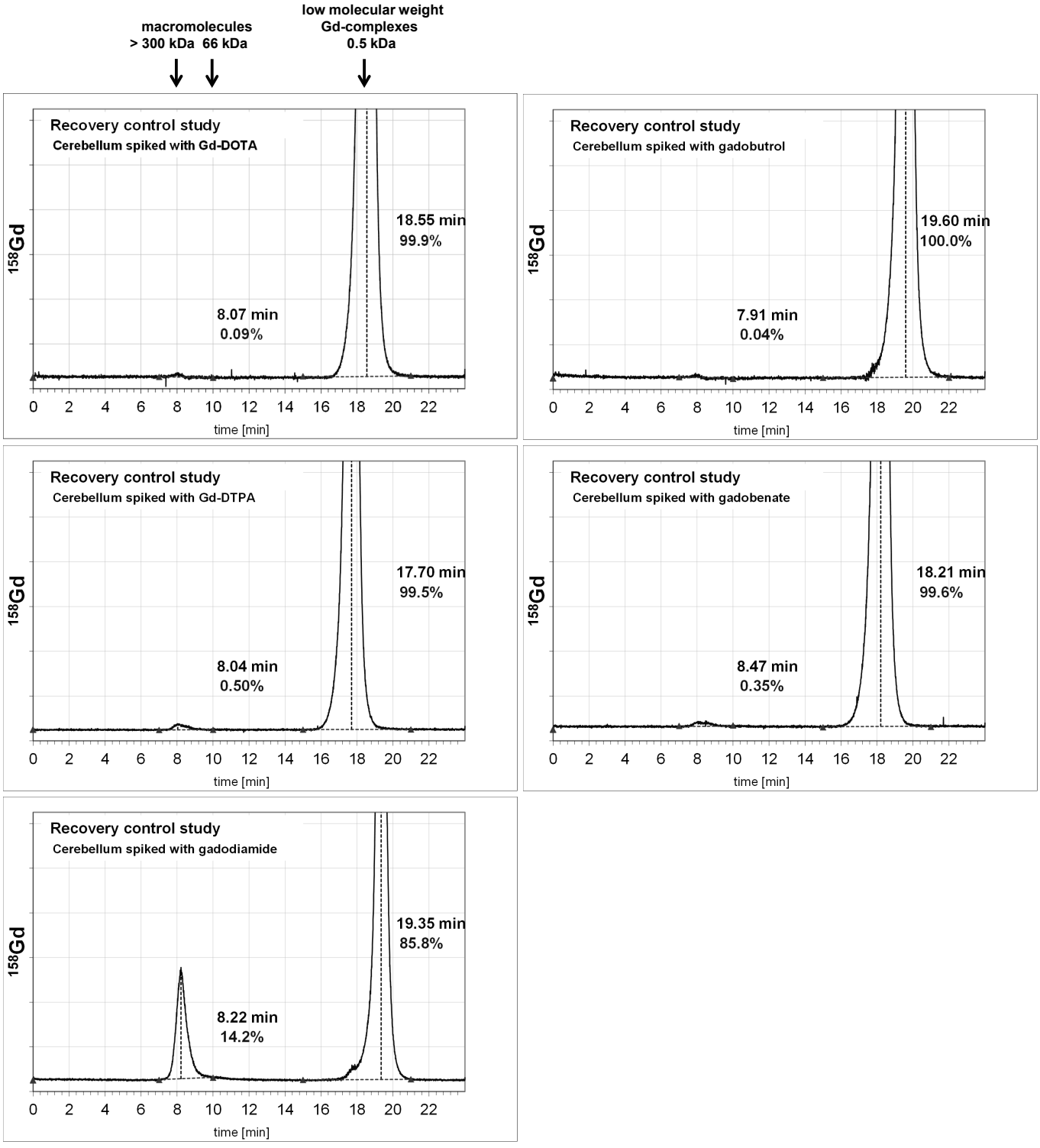
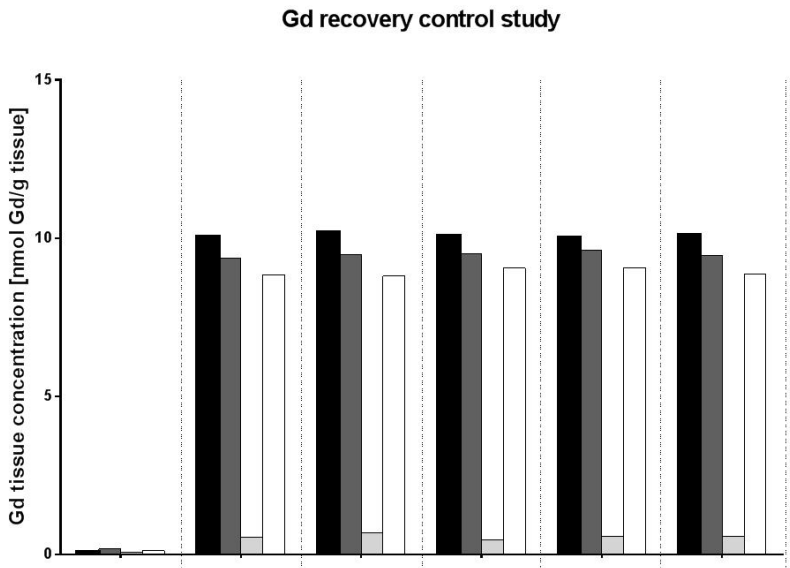
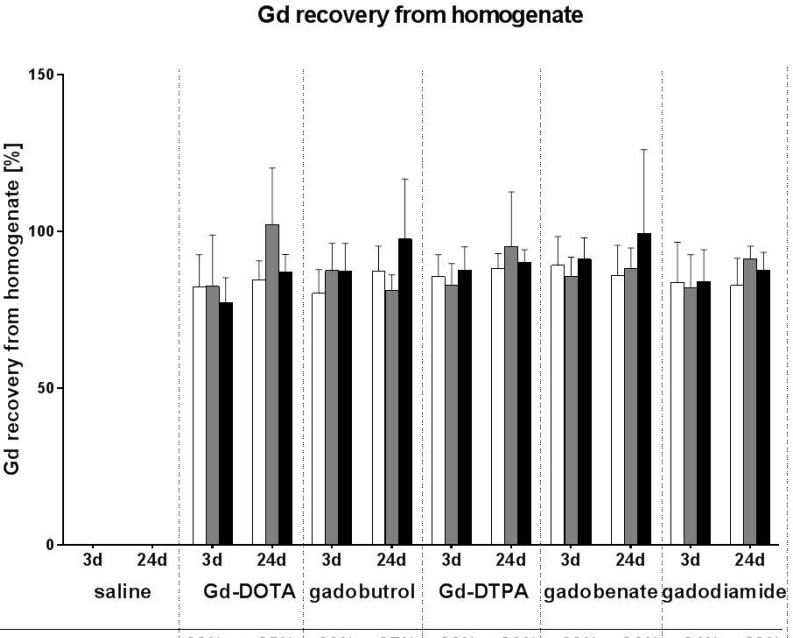


Figure SDC 3: Recovery control study: Gd concentration (mean of n=2) in each fraction of cerebellum spiked with 10 nmol Gd/g GBCAs.



	saline	Gd-DOTA	gadobutrol	Gd-DTPA	gadobenate	gadodiamide
Homogenate	0.1	10.1	10.2	10.1	10.0	10.1
Total Gd recovery	0.2	9.4	9.5	9.5	9.6	9.4
Insoluble fraction	0.1	0.5	0.7	0.5	0.6	0.6
Soluble fraction	0.1	8.8	8.8	9.1	9.1	8.9

Figure SDC 4: Recovery of Gd from the tissue fractionation of brain tissue from treated animals as the sum of the insoluble and soluble fraction relative to the tissue homogenate. The tables show the mean of 5 animals. The SD is shown in the error bars.



	saline		Gd-DOTA		gadobutrol		Gd-DTPA		gadobenate		gadodiamide	
	3d	24d	3d	24d	3d	24d	3d	24d	3d	24d	3d	24d
Cerebrum			82%	85%	80%	87%	86%	88%	89%	86%	84%	83%
Cerebellum			83%	102%	88%	81%	83%	95%	86%	88%	82%	91%
Pons			77%	87%	87%	98%	88%	90%	91%	99%	84%	88%