Regional absolute quantification in neurochemical profile of the canine brain: Investigation by proton nuclear magnetic resonance spectroscopy and tissue extraction

D-C. Woo[1], C-B. Choi[2], S-H. Lee[3], E. Bang[4], S-S. Kim[1], H-S. Rhim[1], S-Y. Kim[1], and B-Y. Choe[1]

[1]The Catholic University of Korea, Seoul, Seoul, Korea, Republic of, [2]Kyung-Hee University of Korea, [3]Konkuk university of Korea, [4]Korea Basic Science Institute

INTRODUCTION

In vitro magnetic resonance spectroscopy (MRS) method is able to analyze metabolism exactly rather than *in vivo* MRS because *in vitro* MRS used to employ more homogeneous NMR solution samples and higher magnetic field strength. Thus, the results of *in vitro* MRS could be used to support pathologic/physiologic analysis. Although the various brain diseases of canine models have been applied by proton MRS [1], the quantitative concentrations of canine brain metabolites have not been fully provided yet. Thus, the present study was designed to characterize the neuro-chemical profile in the various parts of canine brain using proton MRS, tissue extraction and the external simulated phantom concentration quantification, compared with those of human and rat which has already been reported.[2,3]

MATERIALS AND METHODS

Phantoms as external references, which were made of ten important metabolites of brain were separately made: NAA, tCr, Lac, Cho, Glu, Gln, GABA, mIns, Ala and Tau. Five pure bred 8kg (60 months) adult beagles were injected with intravenous propofol (6 mg/kg) and anesthetized by 2% isoflurane. After sacrificed, a canine skull was sectioned on the midline, and cut around the occipital, frontal, temporal, thalamus, cerebellum cortices and spinal cord (C-spine part). All tissues were frozen *in situ* by soaking liquid nitrogen directly in order to prevent decomposition. After the metabolite powders of each tissue were prepared using methanol-chloroform-water (M/C) extraction method [4], 90% heavy water solutions were made for MRS sample in 5mm MRS tubes. MRS parameters were: Proton resonance frequency = 500.384MHz (Varian spectrometer), CPMG spin echo pulse, relaxation/saturation delay time = 0.5/1.5 sec, acquisition time = 1.892 sec, data points = 15146, spectral width = 8000 Hz, number of scan = 256. The concentrations of 10 metabolites were measured as each metabolite peak was subtracted from an original signal using AMARES method, Lorenzian fitting and apodization (3Hz) of jMRUI-TM for the post-process and external phantom simulated quantification method. (Fig. 1)

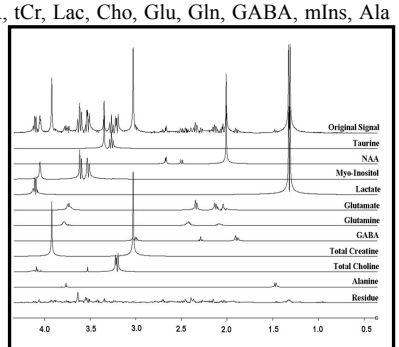

Fig.1 Each metabolite peak was analyzed and subtracted from the original signal (Canine cerebellum)

RESULTS

Table 1 shows the comparison of concentrations of brain metabolites in human, rat and canine brain tissues (mean±SD, mM). Figure 2 shows the 2D correlation spectroscopy (2D-COSY) in the canine frontal cortex and the cross peaks of Lac, Glu/Gln, mIns were identified.

DISCUSSION AND CONCLUSIONS

The concentrations of most metabolites in the cerebral cortex tended generally to be higher rather than other neuronal parts such as cerebellum, thalamus and spinal cord. Comparing with human's and rat's [2,3], the metabolic concentrations of a canine brain tissue were more close to rat's metabolic concentration than human's metabolic concentration. The present study demonstrated the absolute quantification of canine neuronal parts using *in vitro* high resolution MRS with tissue extraction as a method to accurately measure metabolite concentration. Therefore, the present findings provide metabolic information of various canine neuronal regions and can be applied in the other canine studies.

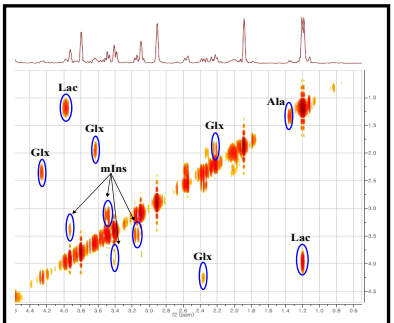

Fig. 2 2D-COSY in frontal cortex shows the 3 bond coupling connectivity of a metabolite as the cross peaks.

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REFERENCES

[1] Barker PB, Breiter SN, Soher BJ, et al. Magn Reson Med. 1994;32:157-163
[2] Pouwels PJW, Brockmann K, Kruse B, et al. Pediatr Res. 1999;46:474
[3] Florian C-L, Williams SR, Bhakoo KK, et al. Neurochem Res. 1996;21:1065-1074
[4] Le Belle JE, Harris NG, Williams SR, et al. NMR Biomed. 2002;15:37-44

Table 1. The me concentrations of brain tabolites in human, rat and canine brain tissues (mean±SD, mM)

	Human adult (ref. [2])		Rat (ref. [3])		Canine tissues (Our findings)					
	Cerebral cortex	Cerebellum	Cerebral cortex	Cerebellum	Occipital cortex	Frontal cortex	Temporal cortex	Thalamus	Cerebellum	Spinal cord
tNAA	9.1±0.7	8.0±0.9	7.11	4.17	8.72±1.58	7.16±1.11	6.45±0.31	3.68±0.42	3.51±0.82	3.63±0.70
tCr	6.5±0.5	8.3±0.9	9.35	9.20	11.08±0.62	10.27±1.84	11.31±0.86	6.64±0.55	8.77±1.54	6.83±1.53
tCho	1.2±0.1	2.1±0.3	4.79	1.62	2.44±0.90	2.73±0.44	3.5±1.99	2.15±0.59	1.43±0.43	2.03±0.51
mIns	4.4±0.6	5.2±0.7	2.52	3.14	4.78±0.84	4.89±0.66	9.78±2.87	3.21±0.71	3.50±1.24	10.30±5.19
Tau	1.1±0.4	1.8±0.7	1.57	0.60	3.16±0.53	2.86±0.72	3.89±0.24	2.17±0.84	1.89±0.45	4.59±2.48
Gln	4.1±1.3	5.1±1.2	3.58	2.71	3.8±0.85	3.61±1.02	8.17±2.16	5.22±1.02	3.76±1.24	2.73±1.96
Glu	8.8±1.1	7.6±1.3	4.61	6.10	5.95±0.89	4.02±0.95	8.41±3.01	3.24±1.72	4.69±1.07	3.39±1.51
GABA	1.4±0.7	2.2±0.7	1.71	1.63	5.00±2.04	4.91±1.28	4.7±0.69	3.81±0.88	2.24±1.10	3.16±2.32
Ala	-	-	1.52	0.74	1.51±0.20	1.11±0.21	2.52±1.30	1.12±0.52	0.87±0.25	0.77±0.15