Simultaneous measurements of ascorbic acid and NAA in the rat brain *in vivo* using a single-shot, two-echo selective multiple quantum spectroscopy

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INTRODUCTION

Ascorbic acid (Asc) also known as vitamin C is a major antioxidant in the brain. Asc treatments are suggested to bring positive clinical outcome in cancer and diabetes. Most studies rely on peripheral measurements of Asc but *in vivo* measurement of Asc is vital to evaluate the true status of Asc in tissue. To date, however, only a few non-invasive measurements of Asc were reported in the rat brain [1, 2]. The aim of this study was to develop a reliable editing method for Asc using doubly selective multiple quantum filtering method [3, 4]. We used a single shot, two-echo method [4] for simultaneous detection of MQ filtered Asc and single quantum (SQ) NAA. The NAA signal served as a reliable reference for the phase and frequency as well as an internal concentration reference to quantify the Asc signal.

METHODS

The two-echo method of Asc comprises two parts: the first echo part for the MQ filtered Asc acquisition and the second echo part for the SQ NAA acquisition. The pulse sequence is specially designed not to disturb longitudinal relaxation of Asc during the SQ NAA acquisition by the band-selective excitation. A double-band frequency selective 180^{o} pulse was applied in the MQ sequence part during the MQ preparation period for the selection of Asc C6 methylene protons (3.7 ppm) and Asc C5 proton (4.0 ppm). The SQ NAA sequence part is based on a localized PRESS sequence. *In vivo* Asc concentration was estimated using the internal reference method: the *in vivo* Asc-to-NAA concentration ratio was obtained by comparing the signal intensity of MQ filtered Asc with that of SQ NAA. All studies were performed on a 9.4 T Varian system using a ^{1}H quadrature RF coil. The volume of interest was positioned in the neocortex region of the rat brain.

RESULTS AND DISCUSSION

Both MQ Asc and SQ NAA spectra were acquired simultaneously using the single-shot two-echo method. The specially designed frequency selective excitation and refocusing scheme during the SQ acquisition part allowed no signal loss of Asc during the SQ acquisition of NAA, creatine and choline, and very robust water suppression. The observed pattern of *in vivo* Asc signal (Fig. 1B) was consistent in all rat brains and matched that of the *in vitro* Asc signal from a solution phantom (Asc 10 mM, creatine 10 mM, NAA 10 mM). The individual traces of the MQ Asc and SQ NAA spectra were acquired with an average of 32 transients. In addition, reference frequency was dynamically updated at every block of 32 transients (64 s) by measuring unsuppressed water spectrum (1.3 s). This simultaneously measured NAA singlet served not only as an internal concentration reference but also as a navigator to correct any frequency drifts in each acquisition. Figs. 1A and 1B show the averaged spectra (NT = 1536) after individual frequency drift correction based on the frequencies of each SQ NAA spectrum. The phase of MQ Asc was corrected based on that of SQ NAA since the zeroth-order phase difference between MQ Asc and SQ NAA was consistent in all subjects. Since the double-band frequency selective pulse during MQ preparation exclusively selects 3.7 ppm and 4.0 ppm, spectral selectivity of MQ Asc was excellent by suppressing overlapping signals from glutathione, glutamate and glutamine around ~3.7 ppm (data not shown). In the preliminary data analysis, the Asc-to-NAA ratio was 0.21 ± 0.05 (mean ± SD, n= 5) and the concentration of Asc in the rat brain *in vivo* was 1.86 ± 0.42 (mean ± SD, n= 5) using the internal reference method.

In conclusion, this study demonstrates single-shot, three-dimensionally localized simultaneous ^{1}H NMR spectroscopy of MQ filtered Asc and SQ NAA, creatine and choline in the rat brain *in vivo* with minimal sensitivity reduction. The proposed method should be applicable for other J-coupled metabolites and can be adapted to chemical shift imaging [5] to assess regional distribution of the metabolites in health and any focal alterations in diseases.

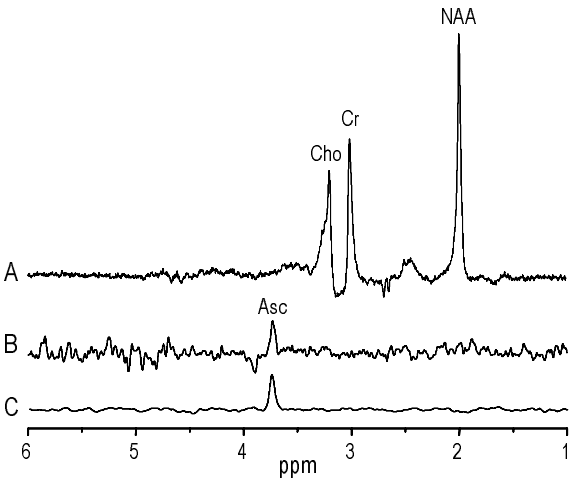

Fig. 1 MQ Asc and SQ NAA spectra in the rat brain *in vivo* using the two-echo selective MQ method (tr = 2 s, TE = 140 ms, 90 μL). (A) *In vivo* SQ NAA spectrum, (B) the corresponding *in vivo* MQ Asc spectrum, (C) *in vitro* MQ Asc spectrum from a 10 mM Asc solution phantom.

REFERENCES

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