In vivo measurements of cerebral ascorbate increases after systemic ascorbate infusion

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INTRODUCTION

Brain and neuroendocrine tissues have the highest concentrations of ascorbate (vitamin C), approximately 50-100 fold higher than plasma [1]. Under physiologic conditions, ascorbate is first accumulated into cerebrospinal fluid, with concentrations several-fold above plasma concentrations, and subsequently accumulated intracellularly in brain tissues [2]. Ascorbate is also administered parenterally, as a drug, producing pharmacologic concentrations in plasma as much as 100 fold higher than those possible with oral ingestion [3]. We hypothesized that pharmacologic plasma ascorbate concentrations might overwhelm normal brain transport mechanisms, thereby producing higher concentrations in the brain. If this occurred, pharmacologic ascorbate concentrations could have novel central nervous system actions. In this study, we investigated whether parenteral (intraperitoneal, i.p.) ascorbate administration could modulate brain ascorbate levels in living animals, using ultra-short echo time ^{1}H MRS at 9.4 T.

METHODS

Total 9 Sprague-Dawley rats were used in this ascorbate infusion study. Prior to ^{1}H MRS scans, an i.p. catheter was placed for infusion of ascorbate solution. During MR measurements, the rats were anesthetized (air:oxygen = 1:1 with 1-2% isoflurane) and their core temperatures were maintained at 37˚C. Ultra-short echo-time ^{1}H MRS (TE=3ms, TR=4s) method [4] was used to acquire spectra data from a voxel (90 µl) in the hippocampus. The voxel was localized using T_2-weighted fast spin echo images. All measurements were performed on a Varian 9.4 T MR scanner using a quadrature surface RF transceiver coil. After acquiring the baseline spectra prior to the ascorbate infusion (Pre infusion), ascorbate solution was administered for 5 min via i.p. at the doses of 0.5 - 0.75 g/kg (n = 6), 1 g/kg (n = 4), and 3 g/kg (n = 5). Some animals were scanned multiple times at different ascorbate doses with ~1 month interval. The ascorbate was quantified using LCModel [5] analysis after spectral phase/frequency correction based on a total creatine (Cr+PCr) signal at 3.03 ppm.

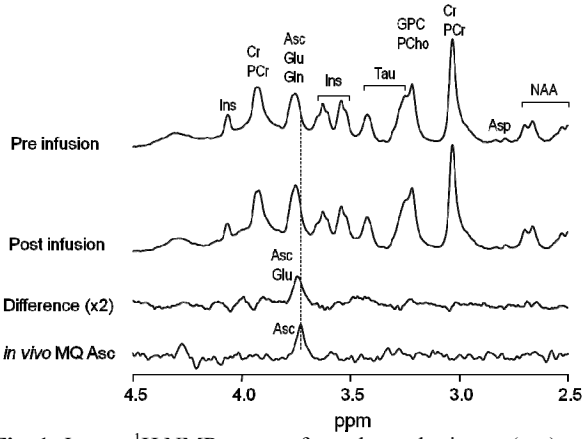

Fig. 1. *In vivo* ^{1}H NMR spectra from the rat brain pre (top) and post infusion of i.p. ascorbate (3 g/kg). A difference spectrum obtained from direct subtraction between post and pre infusion spectra. Ascorbate (Asc) detection using the MQ two-echo method (bottom) for comparison (choi, 2007).

RESULTS AND DISCUSSION

Figure 1 shows ^{1}H MRS spectra acquired before and after 3 g/kg ascorbate i.p. infusion. The difference spectrum from post and pre ascorbate infusion spectra showed a distinct peak at ~3.74 ppm, consistent with the ascorbate (Asc) resonance at 3.73 ppm as well as glutamate at 3.75 ppm. The peak at ~3.74 ppm in the difference spectrum (Fig. 1) was compared with the Asc signal detected in the rat brain using a multiple quantum filtering technique [6], suggesting that the signal attributes to both Asc and glutamate, which is consistent with the outcome of the LCModel analysis. Time courses of Asc following the Asc infusion (Fig. 2) show significant increases of Asc concentrations compared with those at the baseline by 20% and 60% ~1 h post infusion at the doses of 1 and 3 g/kg, respectively. Lower dose of Asc infusion (0.5 – 0.75 g/kg) did not yield any detectible increase of Asc levels.

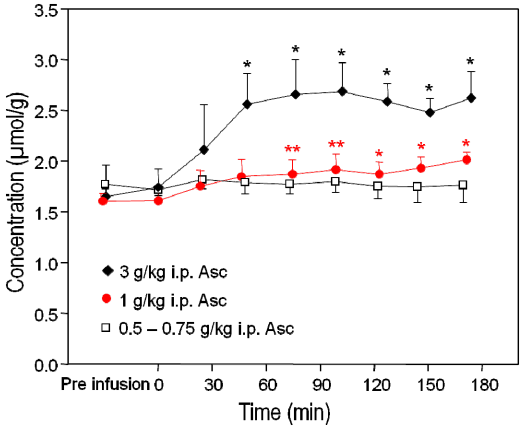

Fig. 2. The effect of i.p. Asc infusion on cerebral Asc levels in the rat brain *in vivo*. (*p<0.01, **p<0.05)

The potential influence of the substantially high plasma Asc after the infusion to Asc signals was estimated using the known blood volume in the rat brain of 3.4 ml/100 g [7] and Asc concentrations in plasma following i.p. infusion [8]. When assuming the same Asc concentrations in plasma and red blood cells, the contribution of the blood to Asc signals can result in at most 9% and 25% Asc signal increases for 1 and 3 g/kg, respectively. If the Asc concentrations are lower in red blood cells compared to plasma, its contribution will be less. Thus the observed Asc signal increases of 20 – 60% that we have observed cannot be explained by the contribution of elevated plasma Asc levels. Therefore, our data indicate that cerebral Asc accumulation can occur by the presence of sufficiently high levels of plasma Asc.

In summary, we demonstrated the modulation of Asc levels in the living brain with the i.p. administration of Asc and the increased cerebral Asc levels were sustained even during continuous decreases of plasma Asc levels.

REFERENCES: 1. Shimizu et al., *Nature* 186, 479 (1960). 2. Harrison & May, *FRBM* 26:719 (2009). 3. Chen et al., *PNAS* 105:11105 (2008). 4 Mlynarik et al., *MRM* 56:965 (2006). 5 Provencher, *MRM* 30, 672 (1993). 6. Choi & Lee, *PISMRM* 15:2357 (2007). 7. Shockley & LaManna, *Brain Res* 454:170 (1988). 8. Chen et al., *PNAS* 104:8749 (2007).

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