Cerebral Metabolic Response to Methylene Blue: A ^{31}P Magnetization Transfer Study at 11.7T

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Target Audience Researchers involved in stroke, metabolic applications of magnetic resonance and ^{31}P NMR.

Purpose Methylene blue (MB) has unique energy-enhancing and antioxidant properties.[1-3] MB's auto-oxidizing property acts as an electron cycler that allows MB to redirect electrons to the mitochondrial electron transport chain, thereby enhancing adenosine triphosphate (ATP) production and promoting cell survival. *In vitro* studies have firmly established that MB enhances mitochondrial ATP production via oxidative phosphorylation (OXPHOS).[4,5] In bypassing complex I-III to generate ATP, MB also reduces reactive oxygen species production from the mitochondrial electron transport chain, which has an antioxidant effect. MB produces positive therapeutic effects in a number of neurological disorders, including Alzheimer's and Parkinson's diseases, and traumatic and ischemic brain injury.[6,7] While MB's mechanisms of action are well-studied *in vitro*, the effects of MB on *in vivo* metabolites and metabolic rates remain unknown. In this study, we implemented ^{31}P NMR based on the Four Angle Saturation Transfer (FAST)[8] method for rapid measurements (~5min) of relative concentrations of ATP, phosphocreatine (PCr) and inorganic phosphorus (Pi) and the forward creatine kinase (CK) rate ($k_{f,CK}$, ADP+PCr→ATP+Cr) of ATP synthesis. We applied this method to study the effects of MB on these metabolites and the CK metabolic rate in rat brains.

Methods Male Sprague-Dawley rats (n=6, 225-250g) were anesthetized using isoflurane (~2%). A line for MB was placed in the tail vein and the animals were moved to the magnet (iso ~1.2%). O_2 saturation, rectal temperature and breath rate were monitored continuously and maintained at normal physiological levels. MRI was performed on an 11.7T Bruker Biospin Magnet using a dual-tuned (500/202.5 MHz) 2-cm diameter surface coil. The 1H (500MHz) element was used for positioning and shimming prior to ^{31}P NMR. ^{31}P magnetization transfer (MT) data was acquired using the FAST method, where $k_{f,CK}$ is calculated, as previously described[8], using four spectra acquired with 30° and 60° FA's with and without γ-ATP saturation (TR=1100s, NA=64, DS=6). Accurate FA's throughout the brain were set using BIRP[9] plane rotation adiabatic RF pulses. Narrowband ATP saturation with negligible bleed over was achieved using the BISTRO[10] saturation scheme with 8x50ms hyperbolic secant RF pulses. Total acquisition time for a $k_{f,CK}$ measurement was ~5min. ^{31}P data were acquired before MB, immediately after 1mg/kg MB, every 30 mins for 2 hrs and again at 24 hrs. Animals recovered from anesthesia after the 2 hrs time points.

Results Figure 1 shows a typical ^{31}P data set consisting of the four spectra used to calculate $k_{f,CK}$ in the FAST method (whole brain). **Figure 2A** shows that the ratio PCr/Pi, an indicator of phosphorylation potential, was elevated following delivery of MB and remained elevated at 24 hrs, while PCr/ATP, a measure of the balance between energy use and energy availability decreased slightly, but remained relatively stable. **Figure 2B** shows that $k_{f,CK}$ decreased significantly immediately following the MB dose, but then returned to its normal value at 1.5 hrs and was elevated at 24hrs.

Discussion The FAST ^{31}P method provides the ability to measure quickly-changing cerebral metabolite concentrations and determine CK reaction rates. Healthy brain tissue relies almost exclusively (~90%) on OXPHOS to produce the ATP needed to meet energy requirements. The CK pathway, not relying on oxygen, acts as an energy buffer, maintaining ATP levels during oxidative stress and transports ATP from the site of production (mitochondria) to the site of use (cell membranes). MB's enhancement of OXPHOS is well characterized [(1-7)]. The less well understood CK response to MB is important for understanding MB's potential role in the treatment of neurological disorders. The elevation of the ratio of PCr to Pi supports that mitochondrial ATP synthesis via OXPHOS increases after 1mg/kg MB. In normal animals, the stable ratio of PCr to ATP suggests that the dose of MB given does not significantly disturb the balance of energy use and availability. Decreased ATP synthesis via CK post-MB implies that there is a compensating mechanism preserving the energetic equilibrium ≤1hr after MB.

These data provide fresh insights into the effects of MB on cerebral metabolism in vivo and could be used to help to optimize MB treatment regimens (i.e., dosing) of neurological disorders. Future studies will use this approach to evaluate MB effects on ischemic stroke and aging.

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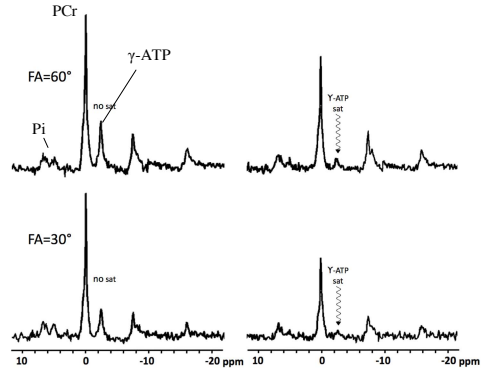

Figure 1. 31P-MT FAST spectra FA's 60° and 30° with and w/o saturation of γ-ATP.

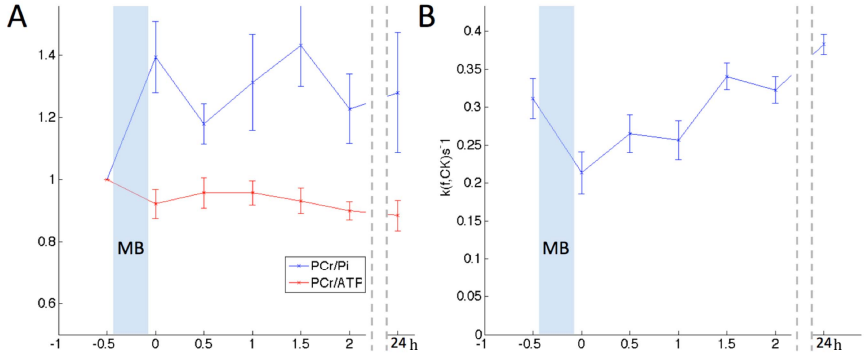

Figure 2. Time course of the (A) normalized 31P metabolite ratios, PCr/Pi and PCr/ATP and (B) forward creatine kinase rate before and after delivery of 1mg/kg MB.