Pharmacological-challenge MRI reveals effects of the antibiotic minocycline on neurovascular coupling: A combined phMRI and fMRI study

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Introduction

Minocycline is a safe, widely prescribed broad-spectrum antibiotic effective against neuro-inflammatory and oxidative cell stress. These actions may mediate the beneficial effects of minocycline on negative symptoms in schizophrenia [1]. However, recent evidence suggests that minocycline can also ameliorate cognitive and behavioral deficits induced by NMDA glutamate receptor antagonists in mice [2]. Here, we used phMRI to assess whether pre-treating with minocycline modifies the functional activated response to acute ketamine challenge in the rat brain. As the signal changes observed in phMRI are potentially susceptible to pharmacological modulation, we also assessed whether minocycline could affect neurovascular coupling as its main mode of action.

Methods

Animals: 29 male Sprague-Dawley rats [mean (±S.E.M.) weight, 290±5g] were used for the present study. All in vivo experiments were conducted in accordance with the Animals Scientific Procedures Act, UK, 1986, and approved by the University of Manchester ethical review process. Preparation/monitoring were the same for all subjects. Animals were anaesthetised with 3% isoflurane in a medical air: O2 gas mixture (0.9:0.1) l/min, then maintained with continuous (i.v.) infusion of α-chloralose 30 mg/kg/hr.

phMRI measurements: 16 rats were randomly assigned to one of four challenge arms: veh/veh, veh/ket, min/veh, and min/ket (N=4 per group). The pre-administration of 50mg/kg minocycline or vehicle i.p. was followed by 30mg/kg ketamine or vehicle s.c. 30min later. MRI data were acquired using a Varian 7T system, with a birdcage resonator and custom-made RF receive coil. T2-weighted anatomical images were acquired via FSE sequence (TR=240ms, TE=60ms, FOV=32×32mm2, 256x256 matrix, 24 contiguous 1mm slices), followed by a three-echo GE BOLD-sensitive time-series with the same spatial coverage (TR=649ms, TE=10ms, 64x64 matrix, resolution=0.5mm3, T1=30sec). Data analysis was conducted in SPM5, and individual subjects were spatially normalised to a stereotaxic rat brain MRI template set [3]. Functional data was smoothed to a FWHM 1mm (2x in-plane pixel dimensions), and multiplied by a brain parenchyma mask to remove extra-cranial and CSF contributions. Time-series analysis was performed using the “p-block” analysis method as described previously [4]. Statistical parametric maps were thresholded using a significance value of p < 0.05 FWE corrected, and volumes of interest (VOI) analysis was conducted using a 3D digital reconstruction of a rat brain atlas (Paxinos and Watson (1998)) co-registered with the MRI template [3]. fMRI measurements: 8 rats were randomly treated with either 50mg/kg minocycline or vehicle i.p., followed by electrical hindpaw stimulation (3mA, 0.3msec, 3Hz) 30min later (N=4 per group). The electrical stimulation paradigm consisted of five continuous OFF–ON cycles, where OFF & ON = 2 seconds each. MRI data were acquired using a Bruker Avance III 7T system, with a 72-mm birdcage resonator for RF transmit and a quadrature receive coil. T2-weighted anatomical images were acquired using a RARE sequence (TR=2500ms, TE=33ms, RARE factor 8, FOV=35×35mm2, 256×256 matrix, 16 contiguous 1mm slices), followed by a BOLD-sensitive time-series with matching spatial coverage (GE-EPI, TR=3000ms, TE=16ms, matrix = 64×64, 2 shot EPI, FA= 30°, resolution=0.5mm3, T1=96sec). Image preprocessing and statistical analysis were also performed in SPM. Two-sample t-Test compared the main effects of the minocycline vs vehicle for the localized S1HL BOLD response.

Local Field Potential (LFP) measurements: 5 rats had a 32-contact probe (NeuroNexusTech, USA) inserted in the right S1HL cortex by 10’ from vertical using the following coordinates from the cortex surface: AP:-2.0mm, ML:2.2mm, DV:-1.7mm (These coordinates were inferred from the peak responding voxel in the fMRI data). LFP measurements were carried out at baseline and 30min post-minocycline administration. Stimuli lasted 0.3ms at a 3Hz frequency, with 50 single-pulses of magnitude 0.1, 0.25, 0.5, 1, 3mA.

Results

Pre-treating with minocycline, strongly suppressed ketamine-induced functional activation (Figure 1). In response to electrical hindpaw stimulation, the pre-administration of minocycline completely suppressed the BOLD fMRI response (Figure 2). However, the individual components of the LFP response (i.e. amplitude, latency and slope) revealed no significant changes between pre- and post-minocycline conditions (Figure 3).

Conclusions

Our results extend previous findings of a role of minocycline in modulating aberrant glutamate NMDA function. However, through characterizing the functionally specific effects of minocycline on haemodynamic and neuronal responses to electrical hindpaw stimulation, the data also describe a prolonged, pharmacologically induced disruption of neurovascular coupling. Taken together, these data implicate the role of nitric oxide synthase (NOS) inhibition in the antipsychotic activity of minocycline [5]. This unique property of tetracyclines could make them promising candidates as safe and acceptable modulators of existing pharmacological therapies for schizophrenia.

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References