PERSPECTIVE

Pretreatment of caffeine leads to partial neuroprotection in MPTP model of Parkinson's disease

Parkinson's disease (PD) is a common neurodegenerative disorder affecting more than 1% people above 60 years of age worldwide, manifesting as the impaired motor function such as tremors, rigidity, akinesia/bradykinesia and postural inefficiency with a reduced life expectancy (Dorsey et al., 2007). PD is believed to be the end result of the progressive death of dopaminergic neurons in the substantia nigra pars compacta (SNc). This loss of dopaminergic neurons results in deficit in neurotransmitter dopamine in the basal ganglia that leads to impairment of motor control. The pathologic changes are reported to precede by a decade or more before the manifestation of clinical symptoms. Analysis in postmortem human brain tissue and various animal studies have reported activation of inflammatory microglial cells causing the oxidative stress. The reactive oxygen species (ROS) may cause severe damage to DNA, proteins and lipids in cells (Jenner, 2003).

The familial form of PD is associated with mutations in various genes such as *Parkin*, *LRRK*, *α-synuclein*, *Pink 1*, *DJ 1* and *UCH-1*. Additionally, several chemicals act as specific dopaminergic neuron neurotoxins, and are used to model PD in animals. These include treatment of rodents with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat, rotenone, and 6-hydroxy-dopamine. MPTP is a widely used neurotoxin for studying PD as it mimics the progression of the disease by selectively destroying the dopaminergic neurons in the SNc by accumulation into these cells and inhibition of the ATP synthesis. Cell death triggers microglial cell activation and glial proliferation leading to neuroinflammation, ROS generation and activation of the midbrain adenosine receptors (A2A) (Smeyne and Jackson-Lewis, 2005). Suppression of astroglial A2A receptor activity is suggested as a strategy of neuroprotection by reducing the proliferation of microglial cells.

Caffeine is a widely consumed central nervous system stimulant worldwide (Fredholm et al., 1999). A number of epidemiological data have associated higher caffeine intake to a lower risk of developing PD signifying a neuroprotective role of caffeine. Caffeine has been reported to play a protective role in various studies in the animal models including MPTP mouse model of PD (Prediger, 2010). While the exact mechanism of neuroprotection by caffeine is not clear, the most well-known hypothesis is that caffeine is an antagonist of the A2A receptor. Hence, blocking of A2A receptors may inhibit the extracellular glutamate release and attenuate excitotoxicity. Moreover, caffeine reduces the microglial activation, and the release of ROS and cytokines by blocking A2A receptors, thereby preventing damage of midbrain dopaminergic neurons. Additionally, caffeine may reduce proliferation and activity of astroglia by A2A receptor blocking, thereby controlling the level of neuroinflammation (Pierri et al., 2005). Pre-treatment with caffeine has been shown to be moderately helpful against the neurotoxic insults in the MPTP model of PD (Xu et al., 2010). However, it was still not clear if caffeine preserves the function of the GABAergic and glutamatergic neurons in PD.

We have addressed this issue in a recent article (Bagga et al., 2016), taking into account the following observations from the study. First, the MPTP neurotoxicity led to a severe impairment in the motor and neural functions, which were alleviated by the pre-exposure of caffeine (**Figure 1**). Second, MPTP exposure led to a perturbation in the striatal metabolite homeostasis that was un-altered in caffeine pre-treated mice. Thirdly, the mice treated with MPTP showed perturbed excitatory and inhibitory neuronal activity in the cortical and basal ganglionic regions, and caffeine treatment prior to MPTP showed preservation of the neuronal function in the cortex and olfactory bulb while that in the striatum and thalamus was partially protected.

The primary goal of this study was to measure the neural activity in the mid brain regions, which is affected in PD. The metabolic activity of inhibitory and excitatory neurons in the cortex, cerebellum, olfactory bulb and basal ganglia was measured across brain using ^{1}H-[^{13}C]-NMR spectroscopy together with an infusion of ^{13}C labeled glucose in mice. The oxidative metabolism of [1,6-$^{13}C_2$]glucose in GABAergic and glutamatergic neurons labels GABA-C_2 and glutamate-C_4, respectively. Hence, the kinetics of labeling of GABA-C_2 and glutamate-C_4 provides a measure of GABAergic and glutamatergic neuronal metabolic activity (Patel et al., 2005).

In an earlier study, we showed that MPTP exposure in mice led to the reduced ^{13}C labeling of GABA-C_2, glutamate-C_4 and glutamine-C_4 from [1,6-$^{13}C_2$]glucose indicating reduction in neuronal activity (Bagga et al., 2013). We also found elevated

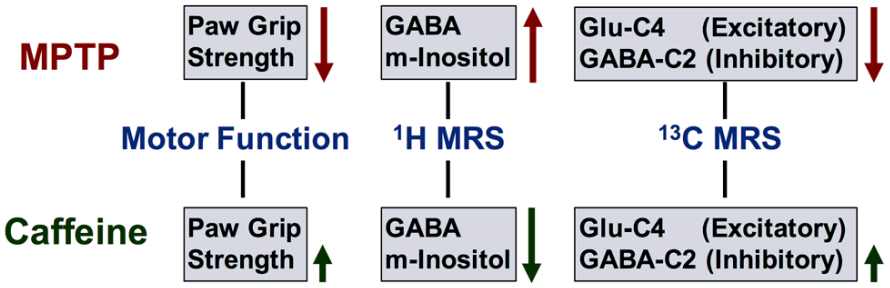

Figure 1 Summary of the physiological and neural findings with caffeine in MPTP treated mice.
MPTP exposure in mice leads to loss of motor function, increased in the levels of GABA and myo-inositol in striatum and olfactory bulb, and reduction in labeling of Glu-C_4 and GABA-C_2 from [1,6-$^{13}C_2$] glucose. Metabolism of [1,6-$^{13}C_2$]glucose in brain leads to incorporation of ^{13}C label into glutamate and GABA, which is used to measure excitatory and inhibitory neuronal activity, respectively. Pretreatment with caffeine partially improved the motor function, and normalized the altered metabolite homeostasis in MPTP-treated mice. Most interestingly, caffeine pre-treatment was able to normalize the excitatory and inhibitory neuronal activity in most of the regions while it partially protected thalamus and striatum from MPTP neurotoxicity. MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GABA: gamma-aminobutyric acid.

glial activity in the striatum following the MPTP treatment. Corroborating with the previous study, the metabolic activity of glutamatergic neurons was found to be reduced in the cerebral cortex, striatum and olfactory bulb, and to a lesser extent in the cerebellum and thalamus following the MPTP exposure. Further, the activity of GABAergic neurons decreased significantly in all the brain regions but striatum, where it was slightly reduced in the MPTP treated mice. The reduction in metabolic rate in striatum could arise due to the decreased excitatory feedback from the motor cortical regions leading to a reduced stimulation of the striatal neurons. The reduction in the inhibitory and excitatory neuronal function in striatum may lead to impairment in the brain's capability to control the limb control. These outcomes are consistent with the observation of hypo-glucose metabolism in the cerebral cortex and caudate nucleus in PD patients. Additionally, the data suggest that caffeine pretreatment preserves neuronal function fully in the olfactory bulb, cerebellar and cortical areas, and partly in the mid brain regions.

Our study reveals a number of interesting perspectives in understanding the symptomatic evaluation of neuroprotective effects of caffeine. Deficit in the motor activity control is a pyramidal symptom of PD. MPTP exposure was shown to cause a dramatic decline in the paw-grip strength (Bagga et al., 2013). The reduced grip strength following the MPTP treatment in our study was in agreement with earlier literature. Further, the data indicated that pre-exposure with caffeine offers moderate protection against the loss of the grip strength due to MPTP neurotoxicity suggesting its role in preventing the diminishing of motor function control due to the MPTP neurotoxicity.

Another perspective arises from the evidence of altered metabolite homeostasis in the midbrain of mice treated with MPTP. The levels of GABA and m-inositol were found to be increased in the striatum of MPTP exposed mice. The level of m-inositol was found to be preserved to the control value in animals pretreated with caffeine preceding the neurotoxin administration. The activation of microglia using immunohistochemical markers has been shown in the striatum and SNc following the MPTP neurotoxicity in mice (Kohutnicka et al., 1998). m-Inositol is reported to be an *in vivo* biomarker of glial cells and inflammation (Chang et al., 2013). Higher m-inositol level in the MPTP treated animals is suggestive of increased number of activated microglia in the olfactory bulb and striatum due to MPTP neurotoxicity. Moreover, the preservation of level of m-inositol in the olfactory bulb and striatum in caffeine pre-exposed animals is suggestive of inhibition of the microglial activity and the protection of the basal ganglia dopaminergic neurons against MPTP neurotoxicity.

In the future, the long term intake of caffeine instead of acute treatment, should be evaluated, which may provide better neuroprotection against PD causing agents including MPTP. Further studies are needed to evaluate the duration and dose dependence of neurometabolites homeostasis and energetics maintenance with caffeine. Additionally, ^{1}H MRS and ^{13}C NMR spectroscopy can be coupled to monitor the etiopathology of PD and various therapeutic regimens on neurometabolite homeostasis and neurometabolic activity. In summary, ^{13}C NMR spectroscopy has shown a potential to monitor neuronal activity and neuro-inflammation, which may aid in early diagnosis of PD, and follow the efficacy of different interventions for alleviation of the disease.

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