In-vivo evidence that high mobility group box 1 exerts deleterious effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model and Parkinson’s disease which can be attenuated by glycyrrhizin

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Abstract
High-mobility group box 1 (HMGB1) is a nuclear and cytosolic protein that is released during tissue damage from immune and non-immune cells — including microglia and neurons. HMGB1 can contribute to progression of numerous chronic inflammatory and autoimmune diseases which is mediated in part by interaction with the receptor for advanced glycation endproducts (RAGE). There is increasing evidence from in vitro studies that HMGB1 may link the two main pathophysiological components of Parkinson’s disease (PD), i.e. progressive dopaminergic degeneration and chronic neuroinflammation which underlie the mechanistic basis of PD progression. Analysis of tissue and biofluid samples from PD patients, showed increased HMGB1 levels in human postmortem substantia nigra specimens as well as in the cerebrospinal fluid and serum of PD patients. In a mouse model of PD induced by sub-acute administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), systemic administration of neutralizing antibodies to HMGB1 partly inhibited the dopaminergic cell death, and reduced the increase of RAGE and tumour necrosis factor-alpha. The small natural molecule glycyrrhizin, a component from liquorice root which can directly bind to HMGB1, both suppressed MPTP-induced HMGB1 and RAGE upregulation while reducing MPTP-induced dopaminergic cell death in a dose dependent manner. These results provide first in vivo evidence that HMGB1 serves as a powerful bridge between progressive dopaminergic neurodegeneration and chronic neuroinflammation in a model of PD, suggesting that HMGB1 is a suitable target for neuroprotective trials in PD.

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1. Introduction
Occurrence of chronic inflammation in Parkinson’s disease (PD) is increasingly recognized (Hirsch and Hunot, 2009, Teismann and Schulz, 2004), and recent studies argue in favour of a central role for chronic inflammation in disease progression in different PD models (Gao et al., 2003, Zhang et al., 2010). The basic idea emerging from these observations is as follows: neurons can sustain a certain level of inflammatory challenge; however, if the inflammatory process is prolonged and levels of neurodegeneration exceed a certain threshold; death of neurons, accumulation of protein aggregates, and unregulated neuroinflammation potentiate each other almost independently of the initial cause of neurodegeneration (Gao et al., 2011, Hirsch et al.,

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High-mobility group box 1 (HMGB1) is a non-histone DNA binding protein, which stabilizes nucleosome formation indirectly, and regulates the interaction of transcription factors with DNA (Bustin and Reeves, 1996). HMGB1 can be secreted actively from inflammatory cells but is released in greater amounts passively from dying cells during tissue damage and inflammatory disease and can then act as a damage associated molecular pattern (DAMP) to initiate inflammatory responses (Andersson and Tracey, 2011). This mechanism may be particularly relevant for PD as a recent study elegantly demonstrated that: by use of cell models reflecting PD pathophysiology HMGB1 signalling indeed triggered progressive dopaminergic neurodegeneration by uncontrolled chronic inflammation (Gao et al., 2011). The underlying mechanisms of action are the increased expression of HMGB1-binding multi-ligand receptors, which are mainly located on the surface of cells of the innate immune system (Andersson et al., 2008) and the activation of these receptors due to translocation of HMGB1 to the cytosol (Lu et al., 2014). HMGB1 binds to receptor for advanced glycation end products (RAGE) (Andersson et al., 2008, Scafﬁdi et al., 2002), Toll-like receptors 2 (TLR2) and 4 (TLR4) (Andersson et al., 2008, Wahamaa et al., 2011) and macrophage antigen complex 1 (Mac1) (Gao et al., 2011). Of these, the RAGE-HMGB1 interaction has been shown to be pathogenetic, e.g. in models of ischemic injury (Kim et al., 2006) and Alzheimer’s disease (Mazzari et al., 2011).

Most recently, a study focusing on the cause of tissue damage during subarachnoid haemorrhage (Sun et al., 2013) suggested that the deleterious effects of released HMGB1 can be modulated by naturally occurring anti-inflammatory compounds such as glycyrrhizin. Glycyrrhizin may be a particularly interesting target compound for neuroprotection in PD, as it is a commonly used sweetener and generally recognized as safe when not excessively consumed (Isbrucker and Burdock, 2006). The European Union suggests that up to 100 mg of glycyrrhizin acid a day, which equals approximately 50 g of liquorice sweets (Stormer et al., 1993) do not represent any health hazard for humans.

Based on these data, and on our recent observation that a second RAGE ligand, S100B, has deleterious effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-model (Sathe et al., 2012), we have explored the role of HMGB1 in the MPTP model as well as in MPTP-treated mice. In addition, blockade of HMGB1 with a neutralizing antibody (200 μg) (Yang et al., 2004) i.p. prior to treatment with MPTP and every third day afterwards. Control mice received saline only. Glycyrrhizin (16.8 or. 50 mg/kg, Sigma) was administered i.p. 30 min after the first injection of MPTP once daily at 24 h intervals over the course of the study, the dose was based on previous studies (Abe et al., 2008, Kim and Lee, 2008).
Animals were sacrificed at selected time points after the last injection of MPTP (0, 1, 2, 4, 7, 14, 21 days). For HPLC measurements and tyrosine hydroxylase (TH) immunohistochemistry, mice were sacrificed 21 days after the last MPTP injection. All protocols were in accordance with the Home Office regulations.

2.4. Western blot analysis

Mouse and human brain extracts were prepared and Western blot analyses performed as described earlier (Sathe et al., 2012). Primary antibodies were: HMGB1 (1:1000, Millipore UK); RAGE (1:1000, Millipore); S100B (1:1000, Sigma); cyclooxygenase-2 (COX-2) (1:250, BD Bioscience, UK); tumour necrosis factor-alpha (TNF-α) (1:1000, Abcam, UK); β-actin (1:25,000, Sigma). Blots were incubated at 4 °C overnight. Horseradish peroxidase-conjugated secondary antibodies (anti-rabbit or anti-mouse 1:10,000, Amersham) and ECL solution (Luminol sodium salt in 0.1 mM Tris HCl and Para-hydroxycoumarin in DMSO) were used for chemiluminescence detection. Bands were quantified using the FluorChem 8800 digital image system (Alpha Innotech, UK).

2.5. Total RNA extraction and RT-PCR

Total RNA from the ventral midbrain was isolated in Trizol (Invitrogen, UK). First strand cDNA was analysed using the Superscript...
II kit (Invitrogen) following manufacturer’s instructions. The cDNA was then amplified by polymerase chain reaction in a 10 μl total reaction volume using the Roche Light cycle 480. The primer mouse sequence used was as follows: HMGB1 5′-TCGCTTTGATTTTGGGGCGGT-3′ (forward) and 5′-AGCTGAGAATGGCTTGGGTCGT-3′ (reverse). As internal control, β-actin cDNA was co-amplified using primer sequences 5′-TGTTGGTGGAATGGGTCAG-3′ (forward) and 5′-TTTGATGTCACGCACGATTTCC-3′ (reverse). All primers were mouse-specific and intron-spanning and were designed based on reported sequences available from the GENE BANK database. The PCR products were all of the expected size, and their proper identities were confirmed by automatic sequencing performed by DNA Sequencing & Services (MRCPPU, College of Life Sciences, University of Dundee, Scotland, www.dnaseq.co.uk) using Applied Biosystems Big-Dye Ver 3.1 chemistry on an Applied Biosystems model 3730 automated capillary DNA sequencer.

2.6. Immunohistochemistry

Immunostaining was performed according to standardized in-lab protocols (Sathe et al., 2012). Primary antibodies (in PBS-Triton-NGS) were: HMGB1 (1:1000, Sigma); glial fibrillary acidic protein (GFAP) (1:1000, DAKO); TH (1:1000, Millipore) and Iba1 (1:500, Wako Chemicals, Germany). The sections were incubated in anti-rabbit (1:200, cy3, Jackson Immuno Research, UK) or anti-mouse (1:300, Alexa Fluor 488 nm, Molecular Probes, UK) fluorescein-conjugated antibodies for visualization using confocal microscopy. Immunohistochemistry was carried out as described earlier (Sathe et al., 2012) and counterstained for Nissl (Thionin, Sigma). TH-, GFAP-, Iba1- and Nissl-positive cells in the SN pars compacta (SNpc) were counted using the optical fractionator method with the examiner being blinded towards treatment group. Striatal density of TH immunoreactivity was measured as described elsewhere (Sathe et al., 2012). For double immunofluorescence microglia were incubated with first biotinylated Lycopersicon esculentum (tomato) lectin. (Vector Laboratories, Peterborough, UK) and revealed with streptavidin conjugated with DyLight 488 (Vector Laboratories).

2.7. HPLC

Levels of 1-methyl-4-phenylpyridinium (MPP⁺) dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in striatal samples as described (Sathe et al., 2012).

![Fig. 4. Double immunofluorescence reveals localization of HMGB1 in the nuclei with translocation to the cytosol after MPTP in GFAP-positive astrocytes (E–H) and Iba1-positive microglia (M–P) in the substantia nigra pars compacta (2d after MPTP). Data are mean ± SEM, n = 4–6 mice per group. Scale bar = 20 μm.](image-url)
2.8. Data analysis

Data were analysed with Graphpad Prism (version 5.04) and JMP software (version 10.0, SAS). All values are expressed as means ± the standard error of the mean (SEM) or frequency unless stated otherwise. Differences between means were analysed using Student’s t-test (2 groups) and the one-way ANOVA (> 2 groups). When ANOVA showed significant differences, pair-wise comparisons between means were

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**Fig. 5.** HMGB1 is translocated into the cytoplasm 1 day after MPTP treatment in dopaminergic neurons. Double immunofluorescence in mouse midbrain reveals localization of HMGB1 in the nuclei of TH-positive dopaminergic neurons after saline (A–D, white arrow), with a clear translocation to the cytosol after MPTP (E–H, white arrows), which is significantly reduced in mice receiving HMGB1-neutralizing antibody (I–L) or glycyrrhizin (M–P) (2d after MPTP). (Q) Stereological cell counting in epifluorescence field was performed for TH-positive neurons in SNpc. TH-positive cells presenting staining for HMGB1 in the cytoplasm portion were considered positive to HMGB1 translocation from nuclei to cytoplasm. *p < 0.05, ***p < 0.001 compared to MPTP mice group treated with saline (Newman-Keuls post-hoc test). Data are generated with 5 mice per group. Value are presented with mean ± SEM. Scale bar = 20 μm.
assessed using the Newman–Keuls post-hoc test. Pearson correlation coefficient was used for correlation analyses, and the Fisher’s exact test for comparison of categorical data. Differences of HMGB1 levels between PD patients and controls were calculated using a linear regression model (two effects: PD yes/no, and age), with the likelihood ratio as outcome. Differences were assumed to be significant at p < 0.05 (two-sided).

3. Results

3.1. HMGB1 is detectable in neuronal and glial cells of human post-mortem tissue, and higher levels are present in PD patients than controls

Immunostaining in the post-mortem midbrain slices of PD and control subjects revealed that HMGB1 protein is consistently expressed in TH-positive neurons within the SNpc (Fig. 1B–I). PD patients regularly showed cytosolic location of HMGB1 but not controls, indicating translocation of HMGB1 into the cytosol in TH-positive neurons (Fig. 1F–I, white arrow). Immunoblotting yielded significantly higher HMGB1 protein levels in the SNpc of PD cases than in those of controls (Fig. 1A). For additional information double immunofluorescence labelling of HMGB1 along with three different cell markers, TH (Supplemental Fig. 1), GFAP and Microglial cells (using tomato lectin) (Supplemental Fig. 2) in human brain tissue was performed.

3.2. Heightened serum and CSF HMGB1 levels in PD

In both serum and CSF of the 122 sample pairs investigated, HMGB1 levels were significantly higher in PD patients than in controls (Fig. 2A, B). In PD patients, serum (p = 0.035, Fig. 2C), but not CSF HMGB1 levels (p = 0.75) correlated negatively with age at onset of the disease. Serum HMGB1 levels showed a significant positive correlation with disease duration (p = 0.037, Fig. 2D) which was not the case in CSF samples (p = 0.80).

3.3. HMGB1 RNA and protein levels are increased after MPTP treatment

In mice, mRNA and protein levels of HMGB1 in the ventral midbrain (the brain region containing the SNpc) are increased one day after MPTP administration and then returned back to baseline levels (Fig. 3A, B). Immunohistochemistry confirmed HMGB1 as a protein widely expressed in astrocytes and microglia (Fig. 4). Dopaminergic neurons clearly showed enhanced staining for HMGB1, due to increased levels in the cytosol, indicating that HMGB1 is more actively translocated from the nucleus to the cytoplasm one day after in MPTP-treated mice than in saline-treated mice (Fig. 5A–H, white arrows), which is significantly reduced in mice receiving HMGB1-neutralizing antibody (1–L, Q) or glycyrrhizin (M–P, Q).

3.4. Neutralizing HMGB1 antibody reduces MPTP neurotoxicity

Next we assessed the effects of blocking HMGB1 on the level of disease in the MPTP mouse model. Mice were administered anti-HMGB1 antibody as described in Methods. Significantly more TH-positive neurons (58% in mice receiving neutralizing HMGB1 antibody versus 44% in control mice) as well as Nissl-stained neurons (79% in mice receiving neutralizing HMGB1 antibody versus 59% in control mice) survived after MPTP treatment compared to untreated (Fig. 6A, B and supplemental table 1). This difference was reflected by a significantly less marked decrease in both the density of TH-positive fibres (44 versus 14%, Fig. 6C), and in dopamine levels (30% versus 18%, Fig. 6D) in the striatum of mice treated with neutralizing HMGB1 antibody (Fig. 6C and supplemental table 1). Determination of 1-methyl-4-phenylpyridinium (MPP+) levels in the striatum after MPTP treatment with and without concomitant
HMGB1 antibody administration indicated that the antibody injection did not impair MPTP metabolism (Table 2).

3.5. Neutralization of HMGB1 lowers RAGE and TNF-α levels in MPTP-treated mice

Assessment of the influence of HMGB1 neutralizing antibody on MPTP-induced effects in the mouse midbrain by use of immunoblotting (Fig. 7A) revealed significantly lower RAGE protein levels in mice treated with HMGB1 antibody, compared to mice only receiving MPTP (Fig. 7B). Comparable results were obtained for TNF-α (Fig. 7C). In contrast, COX-2 protein levels were not significantly different between the groups after MPTP administration (Fig. 7D). There were no significant differences in RAGE and TNF-α levels between saline treated groups (data not shown).

3.6. Neuronal damage and microglial cell activation in MPTP-treated mice is reduced when HMGB1 is neutralized

As HMGB1 is linked to inflammation, we assessed whether the protective effect of a neutralizing HMGB1 antibody is associated with a reduction of MPTP-induced microglial and astroglial cell activation. Stereological counting showed that, compared to untreated mice, treatment of MPTP-mice with neutralizing antibody to HMGB1 prevented both microglial cell activation (Iba1 positive cells) and gliosis (GFAP positive cells) in the SNpc (Table 3).

3.7. Glycyrrhizin reduces MPTP toxicity dose-dependently

We next assessed whether the natural liquorice extract glycyrrhizin, which is known to bind to HMGB1 had any neuroprotective effect in the MPTP mouse model. Glycyrrhizin had a dose-dependent effect on MPTP-induced toxicity in mice after three weeks of treatment (Fig. 8). MPTP + saline treatment 12.31 ± 2.9 MPTP + HMGB1 antibody treatment 10.49 ± 1.9 MPTP + glycyrrhizin 11.30 ± 1.4

MPP⁺ levels in striatum 90 min after MPTP treatment. Values are means ± SEM for four mice per group. Groups were compared with Newman–Keuls post-hoc test (n.s.).

3.7. Glycyrrhizin reduces MPTP toxicity dose-dependently

We next assessed whether the natural liquorice extract glycyrrhizin, which is known to bind to HMGB1 had any neuroprotective effect in the MPTP mouse model. Glycyrrhizin had a dose-dependent effect on MPTP-induced toxicity in mice after three weeks of treatment (Fig. 8). In mice treated with MPTP alone, 32% of TH-positive neurons (Fig. 8A, C) in the SNpc, and 74% of the Nissl-positive neurons survived. In MPTP-treated mice who received daily doses of 16.8 mg/kg glycyrrhizin, 66% of TH- and 84% of Nissl-stained neurons survived. Daily i.p. injection of 50 mg/kg glycyrrhizin led to a survival rate of 75% of the TH-positive neurons (Fig. 8A, C), and 84% of the Nissl-stained neurons. These results were confirmed by optical density measures of TH-positive fibres in the striatum. In untreated mice, MPTP caused 80% loss of TH-positive striatal fibres, but in glycyrrhizin-treated mice this was reduced to 53% and 37% loss of fibres at doses of 16.8 mg/kg and 50 mg/kg respectively. (Fig. 8B, D).

To assess whether glycyrrhizin also influences striatal dopaminergic dysfunction after MPTP treatment, dopamine, DOPAC and HVA levels were determined in mouse striatal tissue (see supplemental table 2). The data support a role for HMGB1 in causing damage to the dopaminergic system in MPTP treated mice, since application of glycyrrhizin with increasing doses led to a dose dependent recovery of metabolites levels aforementioned. Control experiments revealed that glycyrrhizin per se did not influence the dopaminergic system. Detailed information about MPP⁺ levels are provided in Table 2.

The effect of glycyrrhizin also appeared to be mediated via blockade of HMGB1 protein is supported by the evidences of reduced HMGB1 and RAGE protein levels in the midbrain of MPTP mice treated with glycyrrhizin (Fig. 9). Interestingly, levels of a RAGE co-ligand, S100B (Sathe et al., 2012), were not significantly altered.

4. Discussion

This is, to the best of our knowledge, the first study (i) showing in vivo evidence that HMGB1 protein levels are altered in patients with PD and that blocking HMGB1 in an animal model of PD is neuroprotective, (ii) providing evidence of how HMGB1 contributes to the progression of the disease by causing activation of microglia and increased gliosis in the SNpc, and (iii) demonstrating that the damaging effects of HMGB1 can be reduced by administration of the naturally occurring HMGB1-binding compound glycyrrhizin.

Increased levels of HMGB1 protein in the SN, as well as in the CSF and serum of PD patients support the hypothesis that HMGB1 is...
involved in the pathogenesis of PD. The negative correlation of HMGB1 serum levels with age at onset of the disease suggests a specific effect of the HMGB1 in PD pathogenesis; as, even though indirectly, earlier occurrence of the disease is associated with a more "specific" pathology leading to clinical symptoms (de la Fuente-Fernández et al., 2011). However, age at onset parameter shows only 1% ($R^2 = 0.01$) of the variance of the HMGB1 serum levels, so the negative correlation is weak. The positive correlation of HMGB1 serum levels ($R^2 = 0.06$) with PD duration, shows a variance of 6% of HMGB1 levels. This provide evidences in favour of an involvement of the protein in the previously proposed uncontrolled inflammatory process in PD which, in concert with dying neurons and pathological protein aggregates, leads to a

![Fig. 8. Glycyrrhizin rescues dopaminergic neurons dose-dependently from MPTP toxicity.](image)

![Fig. 9. Glycyrrhizin significantly reduces MPTP-induced HMGB1 and RAGE but not S100B levels.](image)
vicious cycle of continuous neurodegeneration (Gao et al., 2011, Hirsch et al., 2013). Moreover, this positive association of HMGB1 levels with disease duration argues for the existence of additional sources beyond damaged and dying neurons in particular at advanced disease stage where the number of potentially HMGB1-delivering (dopaminergic) neurons is reduced as the disease progresses. HMGB1 was consistently observed in the cytosol of TH-positive neurons in PD tissue, but not in control tissue. Additionally, MPTP-treated but not saline-treated mice also regularly showed HMGB1 located in the neuronal cytoplasm, indicating that translocation of HMGB1 from the nucleus to the cytoplasm is increased in these animals. Although the process of HMGB1 secretion is not entirely clarified yet, we consider this process paramount for the interpretation of our findings (Lu et al., 2014). HMGB1 in the ageing brain has been considered as an inflammatory mediator, and high HMGB1 levels are associated with diverse mechanisms eventually leading to progressive neuronal damage (Kim et al., 2006, Fang et al., 2012, Muhammad et al., 2008, Takata et al., 2012).

The deleterious effects of HMGB1 induced by MPTP can be inhibited by blocking HMGB1, supporting a role for HMGB1 in causing damage to the dopaminergic system after MPTP treatment. In a recent study, Sasaki and colleagues showed that HMGB1 inhibition via monoclonal antibody administration protects against 6-OHDA neurotoxicity in a rat model. Moreover in support of our findings they showed reduced translocation of HMGB1 from nuclei to cytoplasm after treatment with neutralizing antibody (Sasaki et al., 2016). The observed effects in this study were, at least partly, driven by upregulation and activation of the multi-ligand receptor RAGE. We found that neutralization of HMGB1 in the chronic MPTP mouse model led to reduced levels of RAGE and TNF-α in combination with reduced dopaminergic damage, which indicates that the downstream signalling cascades of RAGE, involving NF-kB, contribute to the damaging effect of HMGB1 (Ramasamy et al., 2005). Also, prior studies have implicated HMGB1 exposure in up-regulating RAGE (Andersson and Tracey, 2011, Schmidt et al., 2001), thus inhibition of HMGB1 was likely the cause for the observed reduced expression of RAGE.

Further downstream cascades known to be activated upon ligand–RAGE interaction – such as erk1/2 (p44/p42) MAP kinases, p38 and SAPK/JNK MAP kinases and the JAK/STAT pathway – as well as additional multi-ligand receptors (e.g. TLR 2 and 4, Mac1 receptor (Gao et al., 2011, Andersson et al., 2008, Wahamaa et al., 2011)) are likely to contribute to the HMGB1-mediated effect (Ramasamy et al., 2005). Indeed, Mac1 expressed on the surface of microglia is a promising candidate for additional HMGB1-mediated dopaminergic toxicity, as HMGB1 has recently been shown to induce the production of multiple inflammatory and neurotoxic factors via microglial Mac1 (Gao et al., 2011), and neutralization of HMGB1 as performed in this study dramatically reduced MPTP-induced glialosis. The cytosolic localization of HMGB1 in microglia also suggests that not only HMGB1 released from dying dopaminergic neurons but HMGB1 released from microglia contribute to the observed HMGB1-induced cell death.

HMGB1 mediated activation of RAGE leads to microglia recruitment in a model of stroke (Muhammad et al., 2008). Additionally, a recent work on HMGB1 implicates its oxidation state in inflammatory pathogenesis, such that the oxidative damage associated with PD onset may lead to conversion of the nuclear thiol HMGB1 to oxidized HMGB1, which would enhance its inflammatory potential (Andersson and Tracey, 2011).

Moreover, mechanisms beyond receptor activation may contribute to the deleterious effects of HMGB1. HMGB1 is detectable in beta amyloid (Aβ) plaques, and high levels of HMGB1 are associated with impaired microglial phagocytosis of amyloid-beta–40 (Takata et al., 2012) and 1–42 (Takata et al., 2004) in the rat brain. Impaired microglial phagocytosis as shown in the above models leads to delayed clearance of the Aβ species and accelerated neurodegeneration. This is of interest as (i) HMGB1 has been demonstrated to bind to native (Song et al., 2014) and aggregated α-synuclein (Lindner et al., 2004), and to be present in α-synuclein filament-containing Lewy bodies (Lindner et al., 2004), and (ii) α-synuclein can be released from neurons, and this extracellular α-synuclein may even more effectively contribute to progressive neurodegeneration, spreading of α-synuclein pathology, and increased neuroinflammation, than intracellular α-synuclein does (Lee et al., 2014). Although the interaction of (secreted) HMGB1 and (secreted) α-synuclein is still hypothetical, we feel that, with the influence of HMGB1 on neuroinflammation and neurodegeneration in the MPTP model shown here, further in-depth studies on the interaction of HMGB1 with different α-synuclein species are urgently needed.

5. Conclusion

In agreement with the results of previous studies showing a protective effect of glycyrrhizin in HMGB1-mediated injury (Musumeci et al., 2014), our findings show a substantial reduction of MPTP-induced dopaminergic neurodegeneration, due to inhibition of HMGB1 expression and translocation, accompanied by reduced RAGE levels in midbrain mouse tissue. The dose-dependency of the protective effect of glycyrrhizin observed here indicates that the compound is a finely tuneable inhibitor. This makes it, in our view, a promising candidate for future controlled trials on neuremodulation in PD.

This study shows that HMGB1 is involved in PD, and pharmacological inhibition of HMGB1 by use of neutralizing HMGB1 antibody as well as glycyrrhizin in the MPTP model is neuroprotective.

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Competing interests

Dr. Tracey has a patent Inhibitors of HMGB1 issued to Merck Serono. All other authors declare that they have no competing financial interests.

Author contributions

WM, PT conceived and designed the experiments, performed experiments, were involved in drafting and editing the manuscript, and interpreted primary data. MS, PS, HLM, MAH, TWR, performed the experiments. WM, TG, GR, JVF, DB, PT edited the manuscript. KJT contributed reagents. All authors read and approved the final manuscript.

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