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Research Report

The effects of dose and route of administration of PSI on behavioural and biochemical indices of neuronal degeneration in the rat brain

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ABSTRACT

Repeated subcutaneous administration of proteasome inhibitor 1 [PSI, Z-Ile-Glu(OtBu)-Ala-Leu-CHO] to rats causes progressive motor deficits and nigral dopaminergic cell loss in our laboratories, but this is controversial since others have not reproduced these findings. For this reason, we have investigated the role that the dose of PSI and its route of administration have on motor activity and neuronal loss in rat brain. PSI (8, 12 or 16 mg/kg, s.c.) was administered to female Wistar rats on 6 occasions on alternative days over 2 weeks. Subsequently PSI (8 mg/kg) was administered by oral, s.c. and i.p. routes on alternate days to separate groups of animals. Rats were assessed for motor function on a weekly basis up to 5 months after the end of PSI treatment. Locomotor activity was decreased following s.c. administration of 8 and 12 mg/kg PSI but not following 16 mg/kg. In subsequent experiments PSI (8 mg/kg) decreased motor activity after p.o. but not i.p. administration. PSI 8 mg/kg s.c. or p.o., but not i.p., caused neuronal loss in the substantia nigra, raphe nuclei, locus coeruleus, nucleus basalis of Meynert and dorsal motor nucleus of vagus. These data confirm that systemic administration of PSI reduces locomotor activity in rats and induces widespread neuronal degeneration in brain. However, the effects of PSI and its time course of action are dose and route dependent.

1. Introduction

Disruption of the ubiquitin proteasome system (UPS) occurs in the substantia nigra (SN) in Parkinson's disease (PD) as shown by decreased proteasomal activity and reduced expression of α-subunits and the PA28 and PA700 regulatory caps of the 26S proteasome (McNaught and Jenner, 2001). As a consequence, there has been interest in developing models of cell death in PD based on the use of proteasomal inhibitors which have been shown to induce dopaminergic cell death. For example, proteasomal inhibition with either lactacystin or PSI [Z-Ile-Glu(OtBu)-Ala-Leu-CHO] causes a concentration dependent decrease in the survival of catecholaminergic PC12 cells (Nair et al., 2006; Rideout et al., 2001) and selective toxicity to dopaminergic neurones in rat primary ventral mesencephalic cultures (McNaught et al., 2002b; Reaney et al., 2006). In the rat, stereotaxic injection of lactacystin or MG-132 into the SN or epoxomicin into the striatum of rats resulted in inhibition of the UPS and a loss of dopaminergic neurones in SN accompanied by the appearance of intracytoplasmic inclusions in SNpc (McNaught et al., 2002a; Sun et al., 2006). These investigations support the concept that proteasomal inhibition is a key pathogenic mechanism in PD.

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This led to a series of studies that appeared to show that the systemic administration of proteasomal inhibitors led to a model of progressive neuronal degeneration in the rat resembling events occurring in PD. McNaught et al. (2004) reported that subcutaneous (s.c.) administration of both epoxomicin and PSI resulted in progressive motor deficits and nigral dopaminergic neuronal death. Importantly, PSI also caused neuronal loss in the locus coeruleus (LC), dorsal motor nucleus of the vagus (DMNV) and nucleus basalis of Meynert (NBM) accompanied by inclusion body formation which is a key feature of the pathology of PD (McNaught et al., 2004). However, these effects of PSI have proved difficult to reproduce in the rat and in other species (Bove et al., 2006; Kordower et al., 2006; Manning-Bog et al., 2006). However, we managed to partially replicate the findings of McNaught et al. (2004) in the rat using subcutaneous administration of PSI (Zeng et al., 2006). Nigral dopaminergic cell death occurred that was accompanied by decreased motor activity and these effects lasted up to 11 months after PSI administration (Zeng et al., 2006). But we did not observe the range or magnitude of biochemical and behavioural changes originally reported by McNaught et al. (2004). In addition, this finding study did not help explain why others were unable to detect nigral cell loss following systemic proteasomal inhibitor administration. A criticism of this study was that the degree of nigral dopaminergic cell loss was evaluated by counting the number of tyrosine hydroxylase (TH)+ cells at the level of the 3rd cranial nerve. This may misrepresent cell loss in SN and may only indicate decreased expression of TH. However, in a subsequent study, we compared unbiased stereological estimates of the number of TH or fluorogold (FG)-labelled nigral neurones with standard manual counting methods in PSI-treated rats and showed the same persistent nigral dopaminergic neuronal loss (Bukhatwa et al., 2009). Indeed, others have also reported approximately 40% loss of dopaminergic neurones in SN after PSI treatment of rats using a similar stereological approach (Schapira et al., 2006).

This leaves the conundrum of why the effects of systemic PSI administration are so difficult to reproduce. Another explanation may lie in a lack of data on the pharmacokinetic profile of PSI and the relationship between dose and effect. We have routinely used doses of PSI that are higher than those originally reported by McNaught et al. (2004) and various dosage regimens have been employed by others in their studies (Bove et al., 2006; Manning-Bog et al., 2006; Kordower et al., 2006). As a consequence, we now report on the effect of a range of doses of PSI administered by the subcutaneous route and their effect on motor function, nigral cell loss and degeneration in RN, LC, NBM and DMNV. There is also nothing known about the bioavailability of PSI and as a consequence we have compared the effects on dopaminergic function after subcutaneous, intraperitoneal and oral administration.

2. Results

2.1. PSI dosage assessed by sc administration

2.1.1. Behavioural assessment

Vehicle treatment of rats had no effect on spontaneous locomotor activity over the course of the study (Fig. 1). Administration of PSI (8 or 12 mg/kg, s.c.) reduced spontaneous locomotor activity and this became significant 5 weeks after the last dose of PSI when compared to vehicle-treated animals ($F=33.03$, $DF=3$, 156, *$P<0.05$, †$P<0.05$ respectively, Fig. 1). By contrast, the highest dose of PSI (16 mg/kg, s.c.) had no effect on locomotor activity compared to vehicle-treated animals (Fig. 1).

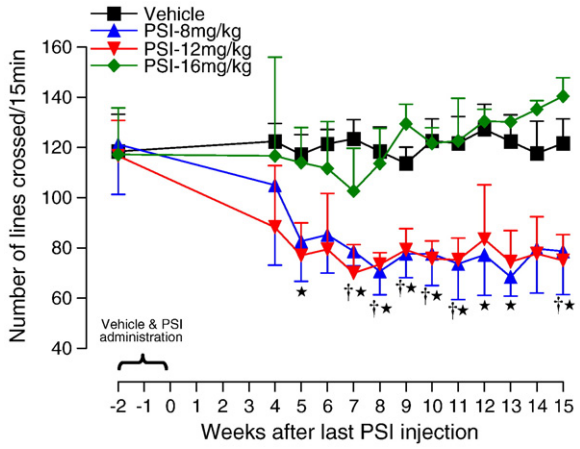

Fig. 1 – Spontaneous motor activity by 70% DMSO (control group) or PSI (8, 12 or 16 mg/kg, s.c.) treated female Wistar rats measured weekly over 17 weeks after administration. PSI with 8 and 12 mg/kg but not 16 mg/kg showed a gradual decrease in spontaneous motor activity which became significant at 5 weeks after the last PSI injection compared to control group. The decrease in locomotor activity remained consistent up to 15 weeks following PSI administration. Values shown are mean ± SEM (n = 6–7/group). *P and †P < 0.05 vs. control and PSI-16 mg/kg groups.

2.1.2. Immunohistochemical analysis

PSI (8, 12 and 16 mg/kg, s.c.) reduced the number of TH+ cells in the SNpc compared to vehicle-treated animals respectively but the loss became less marked as the dose of PSI was increased ($F=10.5$, $DF=3$, 14, *$P<0.05$ and **$P<0.01$ respectively, Fig. 2).

Similarly the number of 5-HT+ cells in the RN and DBH+ cells in LC decreased at all doses of PSI but this was only statistically significant at 8 mg/kg s.c. when compared to vehicle-treated animals ($F=22.3$, $DF=4$, 17, *$P<0.05$, Table 1). The loss of neurones declined with increasing PSI dosage (Table 1).

ChAT+ cell number in the NBM and DMNV were reduced following PSI treatment but this was not dose-related (Table 1). In the NBM, only 12 mg/kg PSI caused a significant loss of ChAT+ cells (40%) while 8 and 16 mg/kg PSI induced a non-significant loss (18% and 25% loss respectively) compared to vehicle-treated animals ($F=22.3$, $DF=4$, 17, *$P<0.05$, Table 1). In the DMNV, PSI (8, 12 and 16 mg/kg) induced significant losses of ChAT+ cells (21% with 8 mg/kg, 36% with 12 mg/kg and 40% with 16 mg/kg respectively) compared to vehicle-treated animals ($F=22.3$, $DF=4$, 17, *$P<0.05$, Table 1).

2.2. Route of PSI administration

2.2.1. Behavioural assessment

There was no change in spontaneous locomotor activity during vehicle treatment or for 22 weeks after its i.p., s.c. or