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Pharmacokinetic, neurochemical, stereological and neuropathological studies on the potential effects of paraquat in the substantia nigra pars compacta and striatum of male C57BL/6J mice

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ABSTRACT

The pharmacokinetics and neurotoxicity of paraquat dichloride (PQ) were assessed following once weekly administration to C57BL/6J male mice by intraperitoneal injection for 1, 2 or 3 weeks at doses of 10, 15 or 25 mg/kg/week. Approximately 0.3% of the administered dose was taken up by the brain and was slowly eliminated, with a half-life of approximately 3 weeks. PQ did not alter the concentration of dopamine (DA), homovanillic acid (HVA) or 3,4-dihydroxyphenylacetic acid (DOPAC), or increase dopamine turnover in the striatum. There was inconsistent stereological evidence of a loss of DA neurons, as identified by chromogenic or fluorescent-tagged antibodies to tyrosine hydroxylase in the substantia nigra pars compacta (SNpc). There was no evidence that PQ induced neuronal degeneration in the SNpc or degenerating neuronal processes in the striatum, as indicated by the absence of uptake of silver stain or reduced immunolabeling of tyrosine-hydroxylase-positive (TH^+) neurons. There was no evidence of apoptotic cell death, which was evaluated using TUNEL or caspase 3 assays. Microglia (IBA-1 immunoreactivity) and astrocytes (GFAP immunoreactivity) were not activated in PQ-treated mice 4, 8, 16, 24, 48, 96 or 168 h after 1, 2 or 3 doses of PQ.

In contrast, mice dosed with the positive control substance, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 10 mg/kg/dose × 4 doses, 2 h apart), displayed significantly reduced DA and DOPAC concentrations and increased DA turnover in the striatum 7 days after dosing. The number of TH^+ neurons in the SNpc was reduced, and there were increased numbers of degenerating neurons and neuronal processes in the SNpc and striatum. MPTP-mediated cell death was not attributed to apoptosis. MPTP activated microglia and astrocytes within 4 h of the last dose, reaching a peak within 48 h. The microglial response ended by 96 h in the SNpc, but the astrocytic response continued through 168 h in the striatum.

These results bring into question previous published stereological studies that report loss of TH^+ neurons in the SNpc of PQ-treated mice. This study also suggests that even if the reduction in TH^+ neurons reported by others occurs in PQ-treated mice, this apparent phenotypic change is unaccompanied by neuronal cell death or by modification of dopamine levels in the striatum.

1. Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride, [PQ]) is a non-selective herbicide that interferes with photosynthesis (photosystem I) and damages plant membrane proteins by the production of oxygen free radicals. PQ is used pre-planting or pre-emergence on a variety of crops, and post-emergence around fruit

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trees, vegetables, vines, and sugar cane (Lock and Wilks, 2001). Although occupational exposure to PQ occurs mainly by the dermal route, PQ is poorly absorbed through intact human skin (0.03 μg/cm^2 over 24 h), with only ~0.3% of the applied dose absorbed within 24 h (Wester et al., 1984).

The association between pesticide use, rural living or living on a farm and the occurrence of Parkinson's disease (PD) has been reviewed (Wirdefeldt et al., 2011) and evaluated in several meta-analyses (Priyadarshi et al., 2000; Brown et al., 2006; Van der Mark et al., 2012) but reports of an association between PQ use and PD (Liou et al., 1997; Tanner et al., 2011) have been questioned (Berry et al., 2010; Mandel et al., 2012; Van Maele-Fabry et al., 2012). In addition, although PQ is frequently stated to be a structural analog of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a chemical frequently used as an animal model of PD (Jackson-Lewis and Smeyne, 2005; Jackson-Lewis et al., 1995, 2012; Jackson-Lewis and Przedborski, 2007), there is doubt that the reported effect of PQ on dopaminergic neurons could be mediated by the same mechanism as described for 1-methyl-4-phenylpyridinium (MPP$^+$), the active metabolite of MPTP (Miller, 2007; Richardson et al., 2007; Ramachandiran et al., 2007).

When PQ was administered by intraperitoneal (ip) injection to male C57BL/6J mice, a reduction in the number of tyrosine-hydroxylase-positive (TH$^+$) neurons in the substantia nigra pars compacta (SNpc) has been reported (Brooks et al., 1999; McCormack et al., 2002; Jiao et al., 2012). However, because the detection of dopaminergic neurons depends on TH$^+$ immunoreactivity, a reduction in the number of TH$^+$ neurons is not conclusive evidence that dopaminergic neurons have actually died. Although some authors have reported that the total number of neurons in the SNpc was reduced concomitantly with the reduction in the number of TH$^+$ neurons in Nissl-counterstained sections (McCormack et al., 2002), few investigators have assessed the effect of PQ on total neuronal count in the SNpc using Nissl-only stain. Furthermore, because the reduction in the number of TH$^+$ neurons has been reported to occur only after the second or third ip injection of PQ, but not after the first injection (McCormack et al., 2005), it is important to consider the timing of dose and the number of doses administered when assessing effects of PQ on the SNpc.

There is limited supporting evidence (McCormack et al., 2002, 2006; Purisai et al., 2007; Mitra et al., 2011) and some contradictory evidence (McIntosh et al., 2010) that dopaminergic neurons die following either PQ (McCormack et al., 2002, 2006) or PQ plus maneb (McIntosh et al., 2010) treatment, as indicated by the uptake of amino cupric silver stain and the activation of microglia and astrocytes in response to the appearance of dying and degenerating neurons. In one study, it was suggested that an observed reduction in TH$^+$ neurons in the SNpc in mice administered 10 doses of PQ (7 mg/kg/dose) over a 20-day period was due to cell death by an apoptotic mechanism (Peng et al., 2004).

The concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as DA turnover in the striatum of C57BL/6J mice, have been reported to be either unaffected (Woolley et al., 1989) or only marginally affected by PQ treatment (McCormack et al., 2002, 2005). Others have reported reductions in DA levels (Kang et al., 2009) and/or increases in DA turnover (Shepherd et al., 2006; Songin et al., 2011) in adult mice when the animals were exposed to PQ prenatally (Barlow et al., 2004), or when PQ was administered daily for 24 days prior to an evaluation which occurred 7 days after the last dose (Prasad et al., 2009).

There is indirect evidence that PQ may be transported across the blood–brain barrier *via* the neutral amino acid transporter (Shimizu et al., 2001; McCormack & Di Monte, 2003) or the organic cation transporters, OCT-2 and OCT-3 (Chen et al., 2007; Rappold et al., 2011). While it has been postulated that PQ is taken up into dopaminergic neurons by the dopamine transporter (DAT), the divalent paraquat cation does not appear to be a substrate for DAT (Foster et al., 2004; Richardson et al., 2005). Recent studies have suggested that the paraquat monovalent cation, which could form in a reduced anoxic environment, is transported into dopamine neuron terminals by DAT (Rappold et al., 2011) in a manner analogous to the transport of MPP$^+$ (Cui et al., 2009). However, direct measurement of [^{14}C] PQ in the brain has consistently shown low-level, homogenous labeling with no evidence of tissue localization, except in pigmented structures (Lindquist et al., 1988) or in structures outside the blood–brain barrier, such as the olfactory bulb and the tissues lining the lateral ventricles of the brain (Lindquist et al., 1988; Widdowson et al., 1996).

The pharmacokinetics of PQ in plasma and the brain have been described for male C57BL/6J mice (Prasad et al., 2007, 2009), the animal model that is most frequently used to assess the effect of PQ on dopaminergic neurons (McCormack et al., 2002). Prasad et al. (2009) reported that it took approximately 18 days of PQ treatment to reach a steady-state concentration in the frontal cortex, striatum, hippocampus and cerebellum of male C57BL/6J mice, and that PQ was slowly eliminated from the brain after the last dose.

The present series of studies were conducted to further investigate the mechanisms of action underlying the reported neurochemical and stereological effects of 1, 2 or 3 ip doses of 10 mg PQ/kg/occasion. Initial findings failed to confirm stereological results reported in the literature. In order to understand the basis for this, the potential dose-response was evaluated using an intermediate (15 mg PQ/kg/occasion) and a maximum-tolerated dose (25 mg PQ/kg/occasion) in young adult male C57BL/6J mice. In addition, the effects of highly-purified, analytical-grade PQ (supplied by Syngenta) were compared to the effects of PQ obtained from Sigma–Aldrich, a source of PQ commonly used by other investigators. Histopathological correlates of the reported loss of TH$^+$ stained dopaminergic neurons and neuronal processes in the SNpc and striatum were evaluated by conducting detailed, blinded, intensive time-series evaluation of indicators of cellular necrosis, apoptosis and glial activation in the midbrain and striatum. Pharmacokinetic parameters were determined in blood, plasma and brain to relate any effect of PQ on stereological or pathological endpoints to the concentration of PQ in the brain. A relatively low dose of MPTP (10 mg/kg administered 4 times in a single day with a 2 h inter-dose interval) was used as a positive control throughout this series of studies to determine if the methodology employed was sensitive enough to detect small changes in the number of TH$^+$ neurons in the SNpc.

2. Materials and methods

2.1. Study conduct

The in-life phase of studies reported herein were conducted in the AAALAC-accredited (Association for Assessment and Accreditation of Laboratory Animal Care International) laboratory of WIL Research Laboratories, LLC. The pharmacokinetic study (Study 7) was a non-GLP investigative study conducted at Syngenta's Central Toxicology Laboratory, UK. The other studies were conducted according to Good Laboratories Practices Regulations (US EPA, 1989; OECD, 1997) and the protocols for these studies were approved by the Institutional Animals Care and Use committee at WIL Research Laboratories. Brain neurochemistry investigations were performed by RTI International (Research Triangle Park, NC). Microscopic slides of brain sections for pathology evaluation were prepared by Neuroscience Associates Inc., Knoxville, TN and examined by a pathologist (Tox Path Specialists, Frederick, MD)