

File PARAQUAT
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29/6/95

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29-Jun-1995

COMPARISON OF THE TOXICOLOGY OF MPTP AND PARAQUAT

At the RIM meeting on paraquat earlier in the year, there was strong agreement in principle and funding for a comparative study, in the appropriate animal model, of paraquat with MPTP to put the two chemicals into perspective with regard to their toxicity to the central nervous system.

We recommend the marmoset monkey as the species of choice and enclose our proposals for the study which has been assembled by Peter Widdowson and which I and the CTL paraquat project team own. We wish to get the study, its planning and Home office licence approval as soon as is practicable.

I gather there is a Ag Chem liaison meeting on 21 July and we will be looking for any business comments and the go ahead at that meeting, I cannot attend but Bob Scott will put the item on the agenda and John Ishmael is well briefed on the study and objectives.

Kind regards,

Ted

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SYNG-PQ-24176710

Proposal to Compare and Contrast the Neurotoxicity of Paraquat and MPTP in Marmosets Focusing on the Nigrostriatal Dopamine System.

Proposer: Dr. P.S. Widdowson, Neurotoxicology Research Group, ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire.

Background.

The chemical similarity between the herbicide paraquat (1,1'-dimethyl-4,4'-dipyridylum) and MPP⁺ (1-methyl-4-phenylpyridinium), a metabolite of MPTP (N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine), which is a potent neurotoxin has led some research groups to suggest that paraquat may be neurotoxic to primates including humans with a similar neuroanatomical target for its effects. We have recently reported that paraquat is largely unable to circumvent the blood/brain barrier to enter the brain where it can damage neurons and astrocytes (Naylor et al., 1995) owing to its highly charged and hydrophilic nature. The inability of paraquat to enter the brain is in marked contrast to MPTP which due to its non-polar chemical nature diffuses across the blood/brain barrier and in doing so is converted to the neurotoxic metabolite, MPP⁺ (1-methyl-4-phenylpyridinium) by the monoamine oxidase enzyme (type B) found in astrocytes and 5-HT-containing neurons (Burns, 1991). We have suggested that the blood/brain barrier restricts entry of paraquat into the brain and that high paraquat exposure does not pose a neurotoxic risk since the concentration of paraquat in the brain is sub-toxic. We have noted however, that following median lethal doses of paraquat to rats (20 mg/kg/s.c.), there is a small penetration of paraquat into brain regions which lack a blood/brain barrier, including the posterior hypothalamus, anterior olfactory bulb and area postrema of the medulla oblongata. Over time, the amount of paraquat entering the brain through these routes gradually increases allowing some paraquat to be detected in and around the substantia nigra and striatum 24 h following administration. It should be noted, however, that these concentrations entering the brain were extremely small and well below the 'no effect level' required for neuronal cell damage as measured by the direct administration of paraquat into the brain (Bageetta et al., 1992).

We are proposing to compare the effects of paraquat and MPTP administration in a primate species, to evaluate whether paraquat will enter into the brain in sufficient quantities to alter the functioning of the nigrostriatal system. We have chosen to focus on two neurochemical approaches which we feel will clearly demonstrate that paraquat administration does not affect the nigrostriatal system. The insensitivity of rodents to the neurotoxicity of paraquat, owing to the low levels of monoamine oxidase B in the brain, means that this type of work cannot be performed in smaller animals such as rats or mice. Numerous reports have demonstrated only transient neurotoxicity (24-48 h) following MPTP administration consisting of a limited ataxia and small decreases in dopamine turnover. There are no reports that MPTP can selectively destroy dopaminergic neurons in rodents as has been reported in higher species, such as monkeys and humans. Due to cost restrictions, and new animal experimental legislation, we are proposing to perform this work on marmoset monkeys (*Callithrix jacchus*) rather than larger, more expensive monkeys which are becoming more difficult to obtain. There are a number of advantages and disadvantages of working with marmosets. The advantages are that we maintain a colony of these monkeys at Alderley Park, thereby circumventing any need to import animals. Their cost is not restrictive and will allow us to use group sizes of five animals to obtain data which may be statistically relevant. The animals are not of such a large size as to pose major difficulties with regards to MPTP or paraquat administration and handling. The major disadvantage is that the marmoset is less sensitive to MPTP neurotoxicity than larger monkeys such as cynomolgus (Crossman, 1987; Mitchell et al., 1989) vervet (Burns, 1991) or rhesus (Burns, 1991). The degree of damage to cells in the substantia nigra is less than in larger monkeys and humans (Burns, 1991; Waters et al., 1987). Despite this problem, we believe that we can obtain meaningful data

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from a study using a limited number of marmoset monkeys using the technique established by Professor Jenner's group at Kings College Hospital, London (Waters et al., 1987).

We propose to divide the study into two components, the first will address the neurochemical changes to dopamine and excitatory amino acid receptors in brain areas containing dopamine-containing axonal projections in marmoset brain following administration of MPTP or paraquat. Evidence for cell damage in the substantia nigra will be sought using neuropathology techniques such as staining for a reactive gliosis (GFAP staining) and counting the number of tyrosine hydroxylase positive neurons. Waters et al., (1987) has reported a reduction of 82% in tyrosine hydroxylase immunoreactive neurons in the substantia nigra following multiple MPTP injections in the marmoset monkey. The second study will examine other measures of neuronal cell damage including evidence that MPTP, but not paraquat, produces free radical induced lipid peroxidation in the substantia nigra and causes the activation of calcium dependent neutral protease (calpains) which degrade the cytoskeletal matrix in neurons (myosin light chains, filamin, talin, fodrin/spectrin, ankyrin etc.) (Croall and DeMartino, 1991).

Both studies will involve detailed evaluation of the marmosets for clinical signs of neurotoxicity, again comparing the effects seen with MPTP with paraquat. We propose to use Professor Alan Crossman as an external consultant to help with this study. Professor Alan Crossman (Chairman of the Division of Neuroscience at the University of Manchester) has agreed to participate in this study and to provide a consultative input into assessment of the nigrostriatal damage based upon the marmoset behaviour (locomotor activity). Prof. Crossman has an international reputation for his work on Parkinson's disease models in primates and has been at the forefront of research into examining the neurochemistry and neuroanatomy of the MPTP model in primates. His agreement to participate in the study will be an advantage for the assessment of the degree of damage produced by MPTP and to assess whether there is any locomotor dysfunction produced by paraquat. This would also add impact to any scientific publication of the findings.

Experimental Methodology.

Animal Treatment.

Female marmosets (ZENECA Pharmaceuticals small primate breeding unit) (300-390 g) will be group housed (n = 5) under a 12 h light/dark cycle and free access to water and food. The animals will be administered daily intraperitoneal doses of vehicle (sterile saline; 2 ml/kg/day), MPTP in saline (2 mg/kg/day) or paraquat ion (0.5 to 1 mg/kg/day) for 5 days. This dosing regime of MPTP has been demonstrated to result in a Parkinson-like hypokinesia (Waters et al., 1987; Close and Elliott, 1991) which persists from days 2 for approximately 30 days post dosing after which there is some degree of behavioural recovery (Waters et al., 1987). The dose of paraquat chosen is below the median lethal dose measured in humans (30 mg/kg/po.) but may result in lung and/or kidney damage. Initially, we propose to use two marmosets for dosing setting to assess whether 0.5 or 1 mg/kg/day is adequate to achieve neurotoxicity and that these doses do not result in fatalities. The tissues from these paraquat treated animals i.e. liver, kidneys, lungs and brain will be fixed in buffered formyl saline and processed for neuropathological examination. The animals will be closely inspected twice daily for signs of akinesia and signs of general toxicity.

One month following the first dose of MPTP or paraquat, the animals will be killed by barbiturate overdose (pentobarbitone 50 mg/kg) and the brains removed.

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Assessment of Parkinsonian Akinesia.

We propose to use a modified version of the methodology described by Close and Elliott (1991) and Smith et al (1993) which measures changes in general activity, stereopathy, skeletal muscle tone, changes in gait and any other signs of general toxicity, such as increased defecation/urination, vocalization, vomiting or general agitation/irritation. The animals will be scored according to the scale below with maximum Parkinson like ataxia receiving a score of 13 and controls receiving a score of 0. The marmosets will be observed twice daily and scored between 1000 and 1200 daily throughout the study period. The scoring will be performed blind as to which monkey receives saline, MPTP or paraquat.

Parkinsonian Features.

1. Gait.

- 0 - Normal; uses all four limbs smoothly.
- 1 - Mild reduction in ability to jump and walk normally.
- 2 - Moderate reduction in ability to jump and walk normally.
- 3 - Marked impaired ability to ambulate normally.
- 4 - Unable to ambulate.

2. Bradykinesia.

- 0 - Normal speed and facility of movements.
- 1 - Moderate slowing of overall movements.
- 2 - Severe slowing of movements; movement is slow, laboured and difficult to initiate and maintain.
- 3 - No movement, akinetic.

3. Balance and Coordination.

- 0 - Normal balance.
- 1 - Mild loss of balance and coordination occurring during movement.
- 2 - Severe loss of balance and coordination.

4. Level of Spontaneous Movements.

- 0 - Normal levels of spontaneous movements or sustained activity.
- 1 - Mild reduction in spontaneous movements.
- 2 - Severe reduction in spontaneous movements; essentially no movement; tends to remain stationary for long periods.

5. Gross Motor Skills. (All Limbs).

- 0 - Uses limbs normally.
- 1 - Decrease in capacity to use limbs; rarely uses limbs.
- 2 - No use of limbs.

Stage 1. Neurochemical/neuropathological studies.

Lungs, liver, kidneys and adrenal glands will be removed at post-mortem and fixed in neutral buffered formyl saline for a pathological examination. It is expected For the first study, the midbrains containing the substantia nigra will be dissected free from the rest of the brain and immersed in ice cold 4 % paraformaldehyde containing lysine (1.36%) and periodate (0.31%) in 0.1 M phosphate buffer (pH 7.4). After 72 h, the midbrains will be transferred to phosphate buffer

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containing 30 % sucrose (w/v) and 0.02 % sodium azide (w/v). for 24 h at 4 °C. Three sets of sections will be cut on a freezing microtome (30 µm) through the substantia nigra collecting sections every 120 µm). Sections will be collected in phosphate buffer containing 5% sucrose and 0.2% sodium azide and mounted on chrome-alum-gelatin coated slides. The sections will be stained for Nissl substance using cresyl violet and a second set of slides stained for tyrosine hydroxylase. A third set of slides will be stained for glial fibrillary acidic protein (GFAP). The forebrain containing caudate nucleus, putamen and other dopamine-containing terminal projections will be placed in ice-cold 0.9 % saline solution for 5 min and then frozen in isopentane cooled to - 40 °C on dry ice. The tissue will be stored at - 70 °C until sectioned at - 20 °C in a cryostat. Twenty micron sections will be collected through the caudate/putamen region of each brain and thaw mounted on gelatin coated slides. The slides will be dehydrated overnight (4 °C) and stored at - 70 °C. Quantitative receptor autoradiography will be performed on the sections for dopamine receptors, reuptake sites, cholinergic and glutaminergic receptors.

Receptor	Ligand	Non specific defined with:
Dopamine -D1	[³ H]SCH23390	SCH23390/ binding to 5-HT receptors blocked with mianserin.
Dopamine -D2	[³ H]YM-09151-2	(-)Sulpride
Dopamine reuptake	[³ H]WIN 35,428	GBR-12909
Muscarinic	[³ H]N-methyl-scopolamine	Atropine
Glutamate/NMDA	[³ H]MK-801	MK-801

Stage 2. Evidence for Oxidative Stress.

The second study will deal with the search for free radical damage by measuring the degree of lipid peroxidation and calpain-induced activation. These techniques will examine in closer detail evidence of MPTP neurotoxicity using new techniques (calpain activation) which will support the data for publication in a good scientific journal. The striatum (caudate nucleus and putamen plus globus pallidus) will be dissected out of each brain as will the substantia nigra. One striatum and substantia nigra from each brain will be homogenized in 150 mM KCl and the degree of lipid peroxidation will be examined in each brain region from one half of the brain using the thiobarbituric acid reaction. The substantia nigra will be dissected out of the second half of the brain and the degree of calpain I activity estimated by measuring the amount of spectrin/fodrin breakdown products formed following quantitative Western blotting as described by Lee et al. (1991).

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Budget.

The animal numbers required for both studies are as follows:

Stage 1. Neurochemical markers.

5 control marmosets plus two animals for dose setting.

5 marmosets treated with MPTP

5 marmosets treated with paraquat

Total 15 animals @ £600/animal = £10,200

Stage 2. Evidence for free radical induced damage

5 control marmosets

5 marmosets treated with MPTP

5 marmosets treated with paraquat

Total 15 animals @ £600/animal = £9,000

Animal housing, care and monitoring (costing £2/day/animal)= £2,000

Consumables, drugs, radioligands and chemicals for both studies = £2,500

Consultancy contract covering Prof. Alan Crossman's input into the project =£3,000

Estimated total cost for the project = £ 26,700.

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