Axon outgrowth and neurofilament protein expression in mouse neuroblastoma cells exposed to the neurotoxin MPTP.

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The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces a CNS pathology in mammals similar to that observed in Parkinson's Disease [1,2]. MPTP itself, is found not to be toxic. The widely accepted mechanism of MPTP-induced neurotoxicity, in vivo, appears to be through deamination by monoamine oxidase-B (MAO-B), localised in the mitochondrial membrane, which results in the formation of 1-methyl-4-phenylpyridinium (MPP\textsuperscript{+}) [3]. This toxic metabolite is selectively accumulated in dopaminergic neurones by way of a high affinity dopamine re-uptake system [4]. It is further concentrated in mitochondria, where it generates free radicals, impairing mitochondrial respiratory chain through the inhibition of Complex I of the electron transport chain [5]. This results in a depletion of ATP leading to cell death.

In this study we have attempted to identify an early cellular marker of neurotoxicity. To achieve this we have examined the morphological and biochemical effect of sublethal doses of MPTP on immortalised mouse neuroblastoma N2a cells. Initially sublethal doses of MPTP were established through the use of the trypan blue exclusion assay. Cells were plated out at a density of 50,000 cells/ml in growth medium (Dulbecco's Modified Eagles Medium supplemented with 10% foetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 10 μg/ml streptomycin) prior to differentiation. Differentiation of N2a cells was induced by removing serum withdrawal and the addition of 0.3 mM cAMP. Cells were incubated for a further 24 hours prior to further experimental assessment.

The projection of axons from N2a cell bodies is characteristic of healthy differentiated cells. Therefore the effect of sublethal doses of MPTP on these cells was morphologically assessed. This was achieved by fixing differentiated cells, exposed to the neurotoxin for 24 hours, with 90% (v/v) methanol in Tris buffered saline and staining with Coomassie Blue R250. Cells were then viewed microscopically and the number of axons counted in five random fields per well (Fig 1). An axon was defined as a cell projection greater than two cell bodies in length.

Fig 1. Effect of sub-lethal doses of MPTP on axon outgrowth in differentiated N2a cells.

Cells were plated at a density of 50,000 cells/ml prior to differentiation and exposure to the neurotoxin for 24 hours. Data expressed as mean axon number/100 cells ± standard deviation, where n=3 with 4 replicates per experiment. Statistical analysis was carried out using the two sample t-test. All values < 0.005.

Fig 2. Effect of sub-lethal doses of MPTP on neurofilament protein in differentiated N2a cells.

Equal cell aliquots were loaded on a 7.5% SDS-PAGE gel and then transferred to a nitrocellulose membrane prior to probing with RMd09 [Blot A], directed to neurofilament protein and Ta51 [Blot B], directed to phosphorylated neurofilament protein. 1. control (24 hr). 2. 5μM MPTP (24 hr). 3. 10μM MPTP (24 hr).

Differentiated cells challenged with 5μM and 10μM MPTP expressed a decrease in axon number (Fig 1).

In order to characterise this morphological effect biochemically, extracts of differentiated cells exposed to the neurotoxin were electrophoresed on a 7.5% SDS-PAGE gel. Protein was then transferred to a nitrocellulose membrane and probed with monoclonal antibodies raised against neurofilament protein. Neurofilaments were chosen as they are major cytoskeletal components of neuronal cells and are involved in the architecture of axons. A monoclonal anti-neurofilament antibody (RMd09) and monoclonal anti-phosphorylated neurofilament antibody (Ta51) were used as probes to reveal any changes.

A net increase in phosphorylated neurofilaments was observed in MPTP treated extracts of cells probed with Ta51 (Fig 2B). In contrast, extracts of exposed cells probed with RMd09 showed a decrease in neurofilament protein corresponding to the inhibition of axon outgrowth described earlier (Fig 2A). If these changes were the direct result of the neurotoxic action of MPTP, and occur prior to the depletion of cellular ATP, it may indicate an early marker of toxicity.

We conclude that the toxicity of MPTP towards differentiated mouse neuroblastoma cells is associated with altered cell morphology together with decreased neurofilament synthesis and increased phosphorylation state.