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COMMENTARY

BIOCHEMICAL MECHANISMS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE TOXICITY

COULD OXIDATIVE STRESS BE INVOLVED IN THE BRAIN?

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The parkinsonogenic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is known to be a mitochondrial toxin *in vitro*. However, MPTP and a toxic metabolite, 1-methyl-4-phenylpyridine (MPP^+), are also known to produce oxidative stress in the lung just like the MPP^+ structural congener, paraquat [1, 2]. MPP^+ stimulates the release of glutathione disulfide into the plasma, probably after release from lung cells. This rise in glutathione disulfide levels is a fairly specific indicator of oxidative stress induction and of the action of glutathione peroxidase to detoxify hydrogen peroxide formed during oxidative stress [3, 4]. In addition, both MPTP and MPP^+ are much more toxic to selenium-deficient mice than normal mice [1, 2], which indicates the importance of the selenoenzyme, glutathione peroxidase, in the pulmonary toxicity of these compounds. This evidence is very strong support for the induction of oxidative stress by MPTP in the lung. A more detailed discussion of the biochemistry of oxidative stress is provided below.

The question that will be asked here is what is the importance of oxidative stress in the brain during MPTP-induced toxicity. Oxidative stress would be induced by the redox cycling of MPP^+ which involves the one electron reduction of MPP^+ by cytochrome P450 reductase, which is present in dopaminergic neurons and their projections in the striatum [5], or some other reductive process. The MPP radical formed must be stable enough to reduce oxygen which produces superoxide radical anion and leads to the production of active oxygen species which are toxic to cells. The selective toxicity of MPP^+ to dopaminergic neurons is due to its specific uptake by the dopamine uptake system following its release from astrocytes [6].

Parkinson's disease and MPTP

MPTP produces a clinical syndrome in humans very similar to Parkinson's disease [7, 8]. Although the neuropathology of the two conditions is not identical [7, 9], both conditions result in extensive losses of dopaminergic neurons from the zona compacta of the substantia nigra. The induction of Parkinson's disease may involve oxidative stress generation in the substantia nigra. Evidence for this includes: higher than normal levels of iron in the substantia nigra in Parkinson's disease [10]; elevated levels of lipid peroxides in the midbrain in Parkinson's disease [11]; and changes in defensive mechanisms against oxidative stress in Parkinson's disease, such as peroxidase, catalase, superoxide dismutase and glutathione [12–14]. It should also be mentioned that recent evidence implicates changes in mitochondrial function in the induction of Parkinson's disease [15]. Many of the changes associated with Parkinson's disease described above have been investigated in MPTP toxicity as will be discussed.

Mitochondrial toxicity and MPTP

MPTP is known to produce mitochondrial toxicity by virtue of its inhibition of NADH dehydrogenase [16]. Of course the bioactivation of MPTP by monoamine oxidase B (MAOb) takes place in the mitochondria which then actively sequester MPP^+, which requires ATP, such that the intramitochondrial concentrations of MPP^+ may reach the millimolar range. This high concentration is required for the inhibition of NADH dehydrogenase by MPP^+ which is a weak, reversible inhibitor of the enzyme. Upon inhibition of NADH dehydrogenase, the levels of ATP fall which might lead to cell death. However, it is important to remember that the inhibition of NADH dehydrogenase by MPP^+ is reversible, such that as the levels of ATP fall and the uptake of MPP^+ slows down, lower intramitochondrial levels of MPP^+ may allow the regeneration of NADH dehydrogenase activity. Therefore, the mitochondrial toxicity of MPP^+ may be self-limiting depending on the interaction between MPP^+ and ATP concentrations.

Few experiments have investigated the *in vivo* effects of MPTP on mitochondrial function. However, the *in vivo* inhibition of mitochondrial respiration by MPTP may be of short duration (a few hours) with total return of normal function within a few days of treatment [17]. One study has reported changes in the appearance of brain mitochondria after MPTP administration to monkeys [18]. These changes were not seen in a similar study conducted in mice [6]. It is possible that mitochondrial changes

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induced by MPTP are transient and may not explain entirely the long-term effects of MPTP.

Hydrogen peroxide and MPTP toxicity

It is well established that MPTP is metabolized in the brain by mitochondrial MAOb which produces MPP^+ and hydrogen peroxide in astrocytes, serotonergic neurons and endothelial cells [19, 20]. This hydrogen peroxide is toxic to brain cells because it can produce active oxygen species such as hydroxyl radicals [21]. Hydrogen peroxide is detoxified by cytosolic glutathione peroxidase with the production of glutathione disulfide which must be detoxified itself by glutathione disulfide reductase. Glutathione disulfide is toxic to cells by virtue of its ability to alter the enzymatic function of a number of proteins by forming protein-glutathione mixed disulfides [22]. The peroxidative stress induced by hydrogen peroxide generation may explain the toxicity to astrocytes and endothelial cells produced by MPTP [6]. Of course these cells form the biochemical blood–brain barrier against MPTP brain penetration which, if damaged, may allow increased penetration of MPTP into the neurons.

MPTP and exogenous antioxidants

The most confusing aspect of oxidative stress induction by MPTP is the conflicting data in the literature about the use of antioxidants to protect against MPTP toxicity. A number of groups have found no protection by antioxidants against MPTP toxicity whereas a few have reported some protection. In order to use antioxidants, it must first be shown that the antioxidants penetrate into the brain and either increase the brain levels of the antioxidant or increase the turnover of the antioxidant. This has been performed by only one group, Wiener *et al.* [23], using the glutathione precursor, (−)-2-oxo-4-thiazolidine carboxylate. It was found that administration of the antioxidant increased the brain levels of glutathione and attenuated the MPTP-induced depletion of striatal dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). This demonstrates that glutathione is a critical factor in MPTP toxicity. All data in the literature on vitamin E, vitamin C, β-carotene, *N*-acetylcysteine and other antioxidants must be regarded as preliminary since assessment of the penetration of the antioxidants into the brain was not performed before testing them against the toxicity of MPTP. However, vitamin C is known to penetrate into the brain very effectively [24]. Since vitamin C may be capable of protecting the brain from MPTP toxicity [25–27], vitamin C could also be a critical factor in the mechanism of toxicity of MPTP. One study could not verify this finding [28] perhaps because the MPTP given was dissolved in ethanol, which potentiates the toxicity of MPTP as will be discussed.

Antioxidants have also been tested in isolated cell preparations against MPTP toxicity. In isolated hepatocytes, antioxidants are without effect [29]. However, the rationale for testing antioxidants in these cells is minimal since they display no oxidative damage from MPTP (see below). Cultured midbrain preparations have also been used in tests with antioxidants and MPTP or MPP^+ with no protective effects [30, 31]. Since these systems should have no barrier to the penetration of antioxidants such as occurs *in vivo* with the blood–brain barrier, this may be evidence against oxidative stress induction in the midbrain by MPTP. To be sure of this conclusion the stability of the antioxidants used will have to be assessed in the culture systems. MPTP must be present in the culture medium for hours to express toxicity. Whereas MPTP may be stable this long, some antioxidants, such as vitamin E, may not be. In addition, the efficacy of the doses of antioxidants used should be verified by testing with direct acting oxidants such as *t*-butylhydroperoxide.

MPTP and free radicals

Confusion also exists over reports that it is chemically impossible for MPP^+ to redox cycle, which would produce oxidative stress, since the electrochemical potential for reduction of MPP^+ to form a free radical is too great [32, 33]. However, there are a number of reports that, in fact, free radicals are formed in the presence of MPTP and its MAO metabolites, 1-methyl-4-phenyl-2,3-dihydropyridine ($MPDP^+$) or MPP^+. For instance, MPP^+ is known to catalyze the formation of superoxide radical anion and hydroxyl radical in the presence of cytochrome P450 reductase [34]. This process probably involves the reduction of MPP^+ to produce MPP radical which donates its electron to oxygen. The ability of cytochrome P450 reductase to reduce MPP^+ must indicate that the enzyme can effectively lower the threshold barrier to the one electron reduction process. MPP radical has been shown to reduce oxygen to form superoxide radical anion [35] which regenerates MPP^+ and is the basis of redox cycling. In addition, MPDP radical can reduce oxygen to form superoxide radical anion [36]. In fact, MPTP itself in the presence of iron or metal ions [37] or mitochondria [38] is associated with the production of superoxide radical anion. This may be mediated by MPDP radical or perhaps by MPP radical. $MPDP^+$ is known to produce MPP radical in the presence of MPP^+ [38] or iron ions [39]. Finally, $MPDP^+$ interacts with synthetic melanin to catalyze the formation of superoxide radical anion [40]. In summary, MPP^+ is capable of redox cycling, albeit not as well as paraquat, which produces superoxide radical anion. $MPDP^+$ may be involved in the initiation of this redox cycling.

Diethyldithiocarbamate pretreatment enhances the striatal toxicity of MPTP [41, 42]. This compound is an inhibitor of some copper- or zinc-containing enzymes such as superoxide dismutase, aldehyde dehydrogenase and alcohol dehydrogenase. The enhancement of MPTP toxicity by diethyldithiocarbamate may indicate that superoxide dismutase is critical to the protection of cells from MPTP toxicity, presumably by detoxifying superoxide radical anion. However, the inhibition of superoxide dismutase by diethyldithiocarbamate was not assessed in these experiments, which may be important in light of the low doses used. Similarly, aldehyde or alcohol dehydrogenase activities were not assessed after diethyldithiocarbamate treatment. However, ethanol or

acetaldehyde administration prior to MPTP treatment was found to enhance MPTP striatal toxicity [43], perhaps by competitive inhibition of alcohol dehydrogenase or aldehyde dehydrogenase. MPTP or an active metabolite could be deactivated, in part, by aldehyde dehydrogenase in the brain or liver [44]. This may explain in part the increased brain delivery and retention of MPTP and MPP^+ elicited by diethyldithiocarbamate as reported by Irwin *et al.* [45]. Clearly, more work is needed to assess the importance of superoxide dismutase, alcohol dehydrogenase and aldehyde dehydrogenase in the toxicity of MPTP.

MPTP and hepatocyte toxicity

Another point of confusion is that that MPTP or MPP^+ does not appear to induce oxidative stress in hepatocytes [29]. This may be because of the high levels of cytochrome P450 in these cells which may detoxify cytosolic MPTP [44] and prevent or diminish the production of cytosolic oxidative stress. Therefore, MPTP toxicity appears in these cells as a strictly mitochondrial event. Of course, the dopaminergic neurons of the substantia nigra possess little or no cytochrome P450 [46], which makes them fundamentally different from hepatocytes. Clearly, the toxicity of MPTP is expressed differently in various types of cells such as pulmonary and hepatic cells. In addition, the pathology of MPTP and MPP^+ is different in the lung and liver [1]. In the lung these agents induce periarteriolar edema, which is identical to the pathology of paraquat, and may produce a severe loss of pulmonary function. In the liver, a transient vacuolation of hepatocytes is seen which may not result in significant pathology. This may indicate that MPTP has more than one mechanism of toxicity, perhaps involving a mitochondrial mechanism and an oxidative stress mechanism. Investigations of MPTP toxicity in the brain should take this into account.

MPTP and vitamin E

Oxidative stress is usually magnified in vitamin E deficient animals since vitamin E is critical for the protection of lipid membranes from active oxygen species. In addition, the brains from vitamin E deficient animals have significantly higher superoxide radical anion basal generation rates than controls [47]. The toxicity of MPTP has been found to be potentiated by vitamin E deficiency [48]. Mice which are about 70% depleted of vitamin E in various brain regions were found to be more sensitive to the toxicity of MPTP in the substantia nigra in terms of depletion of DOPAC and lowering the ratio, DOPAC/dopamine. This may indicate that MPTP induces oxidative stress in the midbrain. However, the striatum was not more sensitive to MPTP toxicity in these animals. This suggests biochemical differences between the midbrain and the striatum in terms of MPTP toxicity.

MPTP is capable of producing changes in vitamin E levels in the brains of normal mice [48]. There are significant, although transient, increases in vitamin E levels in the striatum and the cerebellum, 1 and 5 hr after MPTP treatment respectively. In the cerebral cortex, vitamin E levels are transiently depleted 1 hr after MPTP treatment. The midbrain is slow to change with an increase in vitamin E levels noted 48 hr after MPTP treatment, which return to normal 2 days later. These changes in vitamin E levels show that MPTP produces an oxidative challenge to membranes throughout the brain, which is reacted to differently by the various brain regions. Some regions, such as the striatum, may exhibit a rapid compensatory increase in vitamin E levels, whereas the midbrain is much slower to respond in this manner. This may make the midbrain more susceptible to damage to lipid membranes through lipid peroxidation than the striatum.

MPTP and lipid peroxidation

Lipid peroxidation can be one sequela of oxidative stress especially in vitamin E deficient animals, since active oxygen species can damage lipids. The brains of normal mice treated with MPTP do not form lipid peroxides in any brain region examined [49, 50]. However, vitamin E deficient mice are susceptible to lipid peroxidation in the midbrain but not the striatum or any other brain region examined [50]. This suggests that oxidative stress may be induced more significantly by MPTP in the midbrain than in the striatum or any other brain region. If lipofuscin accumulation is a measure of lipid peroxidation *in vivo*, then MPTP may induce a certain amount of lipid peroxidation in the brains and retinas of normal mice. In both the substantia nigra and the retina, MPTP treatment produces an increased deposition of lipofuscin over a short time period [51, 52].

Lipid peroxidation has also been examined *in vitro* with MPTP or MPP^+. Using homogenized brain preparations, MPP^+ was found to stimulate lipid peroxidation [53], whereas MPTP was found to inhibit lipid peroxidation [53, 54]. However, in the presence of iron, MPTP induces lipid peroxidation or dopamine oxidation perhaps by forming MPDP radical as discussed above [54, 55].

MPTP and glutathione

Glutathione has been shown to be a critical antioxidant in the toxicity of MPTP, as mentioned before. Glutathione is also critically involved in oxidative stress since it is a cosubstrate for glutathione peroxidase. Experiments have been performed with mice depleted of brain glutathione by intracerebroventricular injections of diethyl maleate [56] which depletes glutathione in the striatum and midbrain to the same extent (about 60% depletion from control levels). MPTP is much more toxic to these mice in terms of lethality and depletion of dopamine in the substantia nigra. There was no potentiation of dopamine depletion in the striatum by diethyl maleate pretreatment.

MPTP is also reported to deplete glutathione in the midbrain but not the striatum 24 hr after treatment [57, 58]. In addition, glutathione is depleted transiently in the striatum, 2 hr after MPTP treatment [56]. Gluthatione depletion can be a trait of some oxidative stress-inducing agents, but is more common with agents which deplete glutathione by forming glutathione conjugates. However, MPTP does not appear to form glutathione conjugates. Some of the glutathione depletion produced by MPTP may

be caused by decreased ATP levels, since ATP is needed by glutathione synthetase. Depletion of ATP is produced by the mitochondrial toxicity of MPTP. As mentioned before, glutathione disulfide reductase is a critical enzyme in the regeneration of glutathione during oxidative stress. This enzyme can be inhibited irreversibly by carmustine which potentiates the toxicity of oxidative stress-inducing agents [59]. In experiments with carmustine, the inhibition of brain glutathione disulfide reductase by carmustine alone was about 54%, whereas the combination of carmustine and MPTP produced about 69% inhibition, which was significantly more inhibition than with carmustine alone [56]. Carmustine also potentiates the toxicity of MPTP in terms of depletion of dopamine in the substantia nigra [56]. However, in the striatum carmustine protects against the depletion of HVA by MPTP. This further implies biochemical differences between the striatum and the substantia nigra in terms of MPTP toxicity.

MPTP toxicity in the striatum and midbrain

One of the assumptions that has been made by a number of investigators is that the dopaminergic neurons in the substantia nigra are biochemically identical to their nerve terminal projections in the striatum. However, the data reported so far in the literature seem to indicate that the midbrain is biochemically different from the striatum in terms of its response to MPTP toxicity. In fact, there are some known and putative differences between the dopaminergic terminals in the striatum and the dopaminergic neurons in the substantia nigra. For instance, glutathione levels are low in the neurons and somewhat higher in their terminals [60]. This may make the neurons more susceptible to oxidative stress than their terminals. In addition, vitamin C levels may not be present in high amounts in the substantia nigra [61]. Vitamin E levels are somewhat higher in the striatum than the substantia nigra [48]. Catalase, which normally detoxifies peroxisomal hydrogen peroxide, may be present in lower levels in the striatum than the substantia nigra [62]. Catalase also decreases with age [63] which may partially explain the increased sensitivity of older mice to MPTP toxicity [6]. Cytochrome P450, which deactivates MPTP, is present in higher amounts in the striatum than the substantia nigra [46, 64]. Other critical defensive enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione disulfide reductase may be present in similar levels in the striatum and substantia nigra [65]. In addition, aldehyde dehydrogenase levels are similar in the midbrain and the striatum [66]. The differential levels of glutathione and cytochrome P450 in the terminals and neurons may be major factors in the differential susceptibility of the striatum and substantia nigra to oxidative stress.

Quite a bit of discussion is present in the literature about iron levels in the substantia nigra and MPTP toxicity [37, 39, 54, 55]. Intracellular iron may be toxic to neurons by virtue of the ability of iron to produce toxic oxygen species [67]. Iron is present in the zona reticulata in high amounts, but not in the dopaminergic neurons of the zona compacta [68]. In fact, iron is found in the oligodendrocytes of the zona reticulata and the striatum and does not appear to be associated with neuronal processes [68]. Therefore, the involvement of iron in MPTP toxicity needs to be addressed carefully, taking into account the low levels of iron in the neurons. However, if MPTP were to induce increases in neuronal iron, this would undoubtedly be a significant aspect of the neurotoxicity of MPTP.

MPTP and mitochondrial oxidative stress

There are reports of oxidative stress induction by MPTP in the midbrain and even in the striatum, such as changes in glutathione and vitamin E levels. Could there also be some induction of oxidative stress in mitochondria? Mitochondria have long been used in investigations of oxidative stress [69]. The glutathione pool in mitochondria may be distinct from the cytoplasmic pool [70] which may make mitochondrial susceptibility to oxidative stress different from cytoplasmic susceptibility. In addition, mitochondria are rich sources of oxygen radicals, chiefly by virtue of the presence of ubisemiquinone which readily induces oxidative stress [69]. It is fascinating that the work of Fariello *et al.* [71] has shown recently a transient depletion in the reduced form of coenzyme Q10 (ubiquinol), in nigral tissue, 1 hr after treatment of mice with MPTP. They found no change in ubiquinol in tissue from the striatum. A portion of ubiquinol is in mitochondria where it is involved in mitochondrial respiration. The depletion of ubiquinol may be due to the inhibition of NADH-ubiquinone oxidoreductase by MPP^+ [72]. However, the depletion of ubiquinol may produce a depletion of its one electron oxidation product, ubisemiquinone. Since MPTP is known to stimulate oxygen radical formation in the presence of mitochondria [38], the depletion of ubiquinol may be a compensatory mechanism which would decrease oxygen radical formation. Alternatively, MPTP or MPP^+ could be involved in a redox cycle with ubisemiquinone which would preempt its reduction to ubiquinol. In addition, ubiquinol has antioxidant properties due to its ability to accept electrons. Depletion of the antioxidant, ubiquinol, could make mitochondria more susceptible to oxidative damage. Finally, just as with other oxidative stress-inducing agents, MPP^+ in combination with 6-hydroxydopamine is associated with calcium efflux from mitochondria as the result of pyridine nucleotide oxidation and hydrolysis [73]. Of course, elevated cytosolic calcium levels can be toxic to cells and may be involved in the cellular toxicity of MPTP [74].

MPTP and dopamine release

MPP^+ administration by *in vivo* microdialysis is associated with the release of dopamine from storage vesicles [75]. Increases in intracellular levels of dopamine are associated with the production of hydrogen peroxide through the action of MAOa [76] since dopamine is a substrate for the enzyme, although a poor substrate. As discussed before, hydrogen peroxide generation may lead to neurotoxicity. MPTP may also cause the release of dopamine through another mechanism involving hypoxia. MPTP is known to damage endothelial cells which

may produce a decrease in blood flow through the affected areas of the brain [6, 77]. This could lead to decreased oxygen delivery to these areas. Hypoxia is known to release dopamine from storage vesicles [78]. Therefore, hydrogen peroxide generation may be involved in MPTP toxicity through two mechanisms.

In conclusion, there is quite a bit of evidence showing that MPTP induces oxidative stress in the brain. This oxidative stress seems to involve somewhat different biochemical mechanisms in the striatum and the midbrain. Or perhaps the two brain regions respond differently to oxidative stress. Mitochondria may also be involved in MPTP-induced oxidative stress in the brain. MPTP is a complex toxin which appears to have at least two mechanisms of toxicity: inhibition of mitochondrial respiration and induction of oxidative stress. In the lung, oxidative stress may be the predominant mechanism of toxicity of MPTP. However, in the liver, mitochondrial toxicity may be more important. Perhaps both mechanisms occur in the brain where oxidative stress may be critical in the midbrain and perhaps mitochondrial toxicity is more important in the striatum.

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