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# MPTP FAILS TO INDUCE LIPID PEROXIDATION *IN VIVO*

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**Abstract**—It has been speculated that the conversion of MPTP to $MPP^+$ destroys dopaminergic neurons by promoting the generation of hydroxyl radicals and causes lipid peroxidation. The results obtained in the present work indicate that the primary products of lipid peroxidation are not detectable in MPTP treated animals and thus other mechanisms besides lipid peroxidation should be considered to explain the cytotoxicity of this neurotoxin.

Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is neurotoxic to nigrostriatal dopaminergic neurons in monkeys and mice [1–3] and causes parkinsonism in man [4, 5].

Different authors have described the presence of specific high affinity binding sites for $^3$H-MPTP in rat, monkey and human brains with evidence of a correlation to monoamine oxidase (MAO) [6–9]. MPTP is metabolized by brain MAO forming the electrophilic 1-methyl-4-phenyl pyridinium ion ($MPP^+$) [10–12] which may inter-react with nucleophilic functionalities of neuronal macromolecules.

It has been demonstrated that such bioactivation may be the initial chemical reaction in the chain of biochemical events leading to the neurotoxic action of MPTP. In fact, it has been shown that its toxicity can be blocked by MAO inhibitors [13–15].

It has been speculated that the conversion of MPTP to $MPP^+$ is essential in damaging dopaminergic neurons since this may promote the production of hydroxyl radicals thus causing lipid peroxidation leading to membrane destruction [14, 16, 17].

Since only indirect evidence [18–20] suggests that the neurotoxicity of MPTP may be dependent on the formation of lipid peroxidation-inducing products we investigated whether MPTP can induce brain lipid peroxidation *in vivo*.

## MATERIALS AND METHODS

Male C57 BL/6 mice (20–22 g) were housed in groups of 10 under an artificial light–dark cycle (light from 8 p.m. to 8 a.m.) and at a constant temperature (23°) and a relative humidity (55%). Mice were maintained on *ad libitum* food and water intake. Mice were randomly divided into two groups, the first (N = 20) received intraperitoneal injections of 0.2 ml MPTP–HCl (30 mg/kg of the free base dissolved in 0.9% saline) at 12-hr intervals for five injections. Mice in the second group (N = 20) received 0.2 ml of saline with the same experimental design. Animals were sacrificed by cervical dislocation either 1 hr after the first injection or 2 hr and 1 week after the last injection. The brain and striatum were rapidly removed, the striatum dissected and immediately used for the assay.

The neurotoxicity of MPTP treatment in these animals was evidenced by HPLC-EC dosage of striatal dopamine (DA). The decrease of striatal DA levels in treated animals was in a range between 50 and 80% respect to control values.

The occurrence of lipid peroxidation was evidenced directly by detection of the absorbance at 233 and 242 nm due to the conjugated-diene function using the second-derivative absorption spectrophotometry method [21, 22].

Determination of the diene conjugate signal was performed on brain and striatal microsomes extracts according to reference [23]. The lipid/chloroform extracts were placed in glass test tubes with ground glass stoppers. The solvent was removed under vacuum at 42°. The lipids were then dissolved in cyclohexane in order to obtain a lipid concentration of 100 μg/ml. The lipids were then immediately scanned from 300 to 220 nm and the absorbance and the second derivative spectrum were recorded. Before the scanning operation, a memorized background-correction scan among quartz cells containing cyclohexane was performed to avoid spectral differences between sample and reference cells. Autoxidation experiments were performed by using total lipids extracted from striatum microsomes. Total lipids were placed in opened flasks and left to autoxidize in the air. After 1 hr, samples were treated for scanning operations as previously described.

In the figures based on the second derivative spectra, an absorption peak in the conventional sense appears as an absorption minimum.

## RESULTS AND DISCUSSION

The lipid peroxidation is commonly described as an oxidative oxygen-dependent deterioration of unsaturated fatty acids. The peroxidative breakdown of membrane polyunsaturated fatty acids is involved

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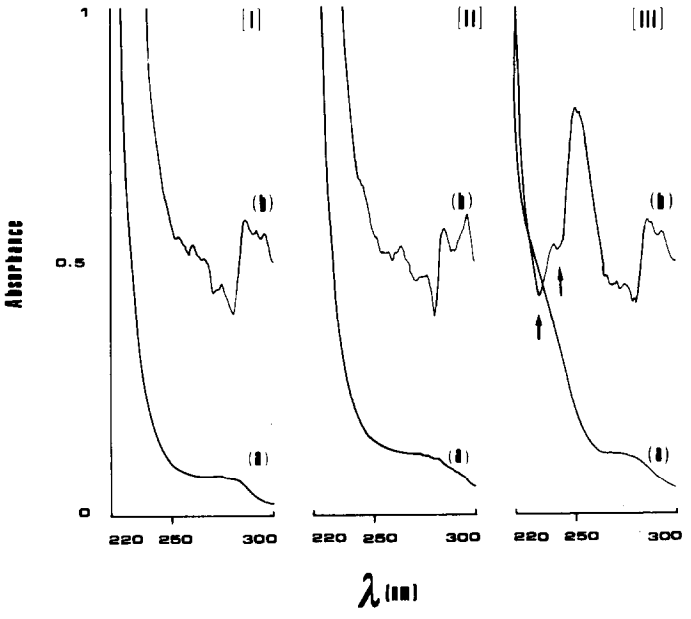


Fig. 1. U.v. (a) and second derivative spectra (b) of lipid extracted from mouse striatum microsomes: untreated (I), MPTP treated (II) 1 hr after autoxidation (III). The arrow shows in the second derivative spectra the minimum at $\lambda = 233$ nm and $\lambda = 242$ nm.

in the pathogenesis of tissue injury induced by several chemical agents [24].

The hydroxyl radical is considered one of the most potent oxidants for its ability to react at high rates with virtually any organic compound. Hydroxyl radical-induced lipid peroxidation proceeds by way of hydrogen atom abstraction from the bis allylic carbon of unsaturated fatty acids and by formation of hydroperoxides possessing the *cis*, *trans* (c,t) and *trans*, *trans* (t,t) isomeric conjugated diene systems which strongly absorbs at 233 and 242 nm [22, 25].

Figure 1 shows the spectra of lipid extracts of striatum microsomes prepared during MPTP intoxication. No modification to the second derivative spectrum occurred after MPTP administration. The second derivative spectra from both brain and striatum MPTP treated animals 2 hr and one week after the injection of the drug similarly do not present a signal (results not shown).

The second derivative spectrum modification occurs only with autoxidized lipids and results in the appearance of a minima peaks at 233 and 242 nm that characterize specifically the t,t and c,t conjugated diene hydroperoxides. These signals have been described and characterized previously in relation to autoxidation of linolenic acid [21] and as a consequence of $CCl_4$ intoxication *in vivo* in rat liver lipids [22, 23]. Several authors have suggested that the neurotoxic effect induced by MPTP administration is related to the biotransformation of MPTP to $MPP^+$ or the biotransformation of $MPP^+$ itself which both may produce hydroxyl radicals thus causing lipid peroxidation leading to membrane destruction [10, 11, 14, 16, 17, 26].

The results obtained in the present work indicate that the primary products of lipid peroxidation are not detectable in MPTP treated animals and that the relative u.v. and second derivative spectra are substantially similar to those of normal tissues.

A possible explanation for these negative results is that in mice the MPTP-induced lesion is limited to striatal dopaminergic nerve endings alone, thus the entity of lipid peroxidation could not be sufficient for our method.

In conclusion, our data indicating that MPTP does not promote lipid peroxidation in addition to other evidence which indicates that induced lipid peroxidation is inhibited by MPTP in brain homogenates [16], suggest that mechanisms other than lipid peroxidation have to be considered to explain the cytotoxicity of this neurotoxin.

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