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COMPARATIVE STUDIES ON THE MECHANISMS OF PARAQUAT AND 1-METHYL-4-PHENYLPYRIDINE (MPP^+) CYTOTOXICITY

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SUMMARY. 1-methyl-4-phenylpyridine (MPP^+) is the putative toxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and is structurally similar to the herbicide paraquat (PQ^{++}). We have therefore compared the effects of MPP^+ and PQ^{++} on a well characterized experimental model, namely isolated rat hepatocytes. PQ^{++} generates reactive oxygen species within cells by redox cycling and its toxicity to hepatocytes was potentiated by pretreatment with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), an inhibitor of glutathione reductase. In BCNU-treated cells, PQ^{++} caused GSH depletion, lipid peroxidation and cell death. These cytotoxic effects were prevented by the antioxidant N,N'-diphenyl-p-phenylenediamine (DPPD) and the iron-chelating agent desferrioxamine. MPP^+ also caused GSH depletion in BCNU-treated hepatocytes but its cytotoxicity was not markedly affected by BCNU, nor was it accompanied by significant lipid peroxidation. DPPD and desferrioxamine also failed to prevent MPP^+-induced cell death. We conclude that the production of active oxygen species is likely to play a major role in PQ^{++} cytotoxicity, while MPP^+-induced cell damage may involve additional, more important toxic mechanisms. © 1986 Academic Press, Inc.

1-methyl-4-phenylpyridine (MPP^+) has recently been shown to be markedly neurotoxic on the nigrostriatal dopaminergic system (1). Its effects were more severe than those induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the Parkinsonism-causing compound which is oxidized to MPP^+ by type B monoamine oxidase in brain mitochondria (2). MPP^+ is structurally similar to the herbicide paraquat (PQ^{++}) (Fig. 1), and the two compounds also share certain common toxic properties in in vivo animal systems (3,4). PQ^{++} has been proposed as a model compound for oxidant-initiated toxicity, involving the production of reactive oxygen species (5). On the other hand, the toxicity of MPTP and its oxidized metabolite MPP^+ occurs via unknown mechanisms. In this study, we compare the toxic effects of PQ^{++} and MPP^+ in isolated rat hepatocytes, a system shown to be par-

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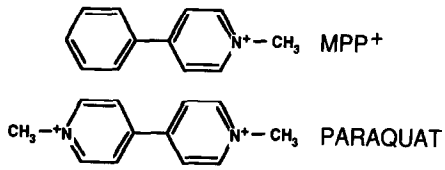

Figure 1

ticularly suitable for studying different mechanisms of toxic cell damage, including that caused by oxidative stress (6).

MATERIALS AND METHODS

Hepatocytes were isolated from male Sprague-Dawley rats (180-250g) by perfusion of the liver with collagenase and incubated in Krebs-Henseleit buffer (pH 7.4) as previously described (7). When inhibition of glutathione reductase (EC 1.6.4.2) was desired, the cells were pretreated for 20 min with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and then incubated for 75 min in fresh medium supplemented with 1mM methionine as in (8). The extent of enzyme inhibition was assessed by measuring maximal glutathione reductase activity in Triton-treated samples of hepatocytes (9); approx. 90% inhibition was consistently observed.

Aliquots of the cell incubations were taken at various times for measurement of cell viability, glutathione and lipid peroxidation. Cell viability was assessed under the light microscope as exclusion of a 0.16% solution of Trypan blue dye. Reduced (GSH) and oxidized (GSSG) glutathione was measured by HPLC as described by Reed et al. (10). Spectrophotometric analysis of thiobarbituric acid-reactive products provided an index of lipid peroxidation (11). Results from these assays shown here are typical of at least three separate cell preparations. 1-Methyl-4-phenylpyridinium iodate was a kind gift of Professor Neal Castagnoli, Jr., Department of Pharmaceutical Chemistry, University of California, San Francisco. BCNU was supplied by the National Cancer Institute, Bethesda, Maryland. Pure PQ^{++} was a gift from Dr. L. L. Smith, CTL, I.C.I. (U.K.). Other chemicals were of the highest available commercial quality.

RESULTS AND DISCUSSION

As mentioned above, the similarity in structure between PQ^{++} and MPP^{+} has prompted the suggestion that the two compounds might also be alike with regard to their mechanisms of toxicity (3,4). PQ^{++} can undergo a cyclic single electron reduction/oxidation, which in the presence of O_2 generates highly reactive species (e.g. superoxide anion, $O_2^{\cdot-}$, hydrogen peroxide, H_2O_2, hydroxyl radical, $OH^{\cdot}$) and ultimately leads to a condition of oxidative stress (5). When H_2O_2 is formed, a rapid oxidation of reduced glutathione (GSH) results from the activity of glutathione peroxidase. Consequently, an increased concentration of GSSG in cellular systems or in the plasma *in vivo* after exposure to a variety of drugs has been interpreted as an indicator of oxidative stress (12,13). Recently, a rise in plasma

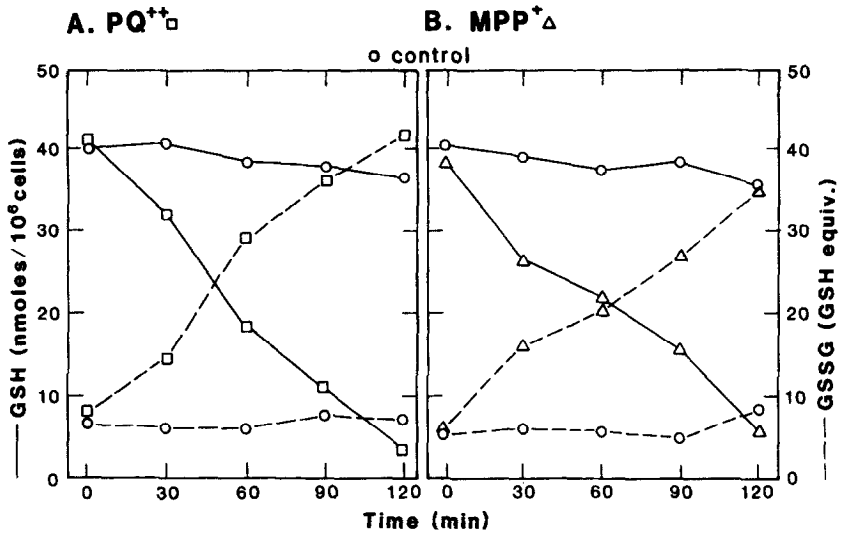

Fig 2 Intracellular GSH consumption (—) and total, intracellular + extracellular, GSSG formation (----) in hepatocytes pretreated with BCNU and then incubated without (o) or with 1.5 mM PQ^{++} (□) or 1.5 mM MPP^{+} (△).

GSSG has been reported after systemic administration of MPP^{+} in rats, and therefore a free radical-mediated mechanism of toxicity has been proposed for this compound as well (4).

Isolated rat hepatocytes pretreated with BCNU have been shown to be a useful model to study the toxicity of redox-cycling compounds. These cells are markedly more sensitive to oxidative stress, since the enzymatic reduction of GSSG to GSH is inhibited and the activity of glutathione peroxidase impaired (9). BCNU pretreated hepatocytes exposed either to PQ^{++} or MPP^{+} show a progressive decrease in GSH and a parallel rise in GSSG (Fig. 2A and B). This seems to support the possibility of enhanced H_2O_2 formation in these cells and prompted us to investigate the role of oxidative stress in PQ^{++} and MPP^{+}-induced cytotoxicity. When glutathione reductase activity is inhibited by BCNU, a dramatic potentiation of PQ^{++} toxicity occurs, confirming the oxidative nature of the damage (Fig 3A and B). A similar pattern has been observed under identical experimental conditions with diquat, another bipyridyl herbicide capable of redox cycling with molecular oxygen even more efficiently than PQ^{++} (8). The comparison of cell toxicity in non-treated vs. BCNU-treated hepatocytes incubated in the presence of MPP^{+}

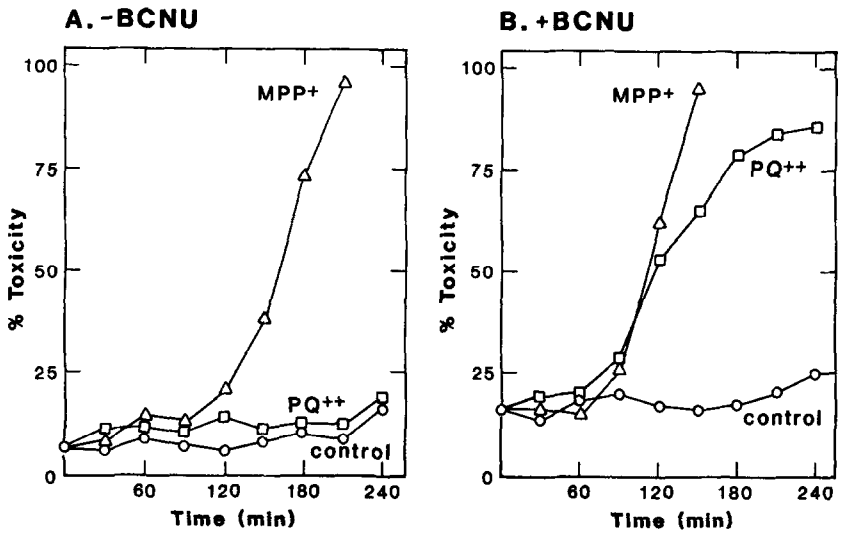

Fig 3 Cell viability after exposure of heptocytes to 1.5 mM PQ^{++} (□) or 1.5 mM MPP^{+} (△). Hepatocytes were preincubated without (A) or with (B) BCNU, as described in Materials and Methods.

gives different results (Fig. 3). The pattern of cell death is not substantially modified in contrast to the potentiation seen with PQ^{++} and other redox-cycling compounds (8,9); the slightly earlier onset of toxicity is likely to be a consequence of enhanced hepatocyte vulnerability after the BCNU pretreatment procedures (e.g. centrifugations and washings). A direct inhibition of glutathione reductase activity by MPP^{+} was ruled out as the cause of lack of potentiation of MPP^{+} toxicity by BCNU. In fact, the maximal enzyme activity in the hepatocytes was unaffected by MPP^{+} exposure (results not shown).

One of the consequences of oxygen radical production is the initiation and propagation of lipid peroxidation. As shown in Fig 4A, PQ^{++} toxicity in BCNU-treated hepatocytes is accompanied by increasing formation of lipid peroxidation products. The importance of this toxic event with regard to cell death was tested using the potent antioxidant N,N'-diphenyl-p-pheylenediamine (DPPD), which completely blocks PQ^{++}-induced lipid peroxidation (Fig. 4A). DPPD also clearly prevents PQ^{++} cytotoxicity (Fig. 4B). On the other hand, MPP^{+} is significantly less efficient in stimulating lipid

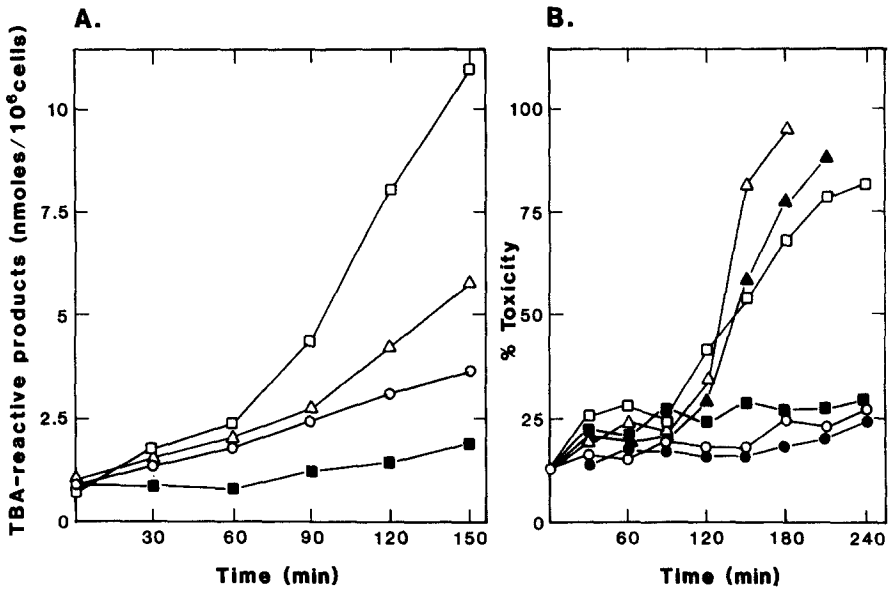

Fig 4 Formation of lipid peroxidation products (A) and effect of the antioxidant DPPD on cell viability (B) during incubation of hepatocytes in the absence of (o) or presence of 1.5 mM PQ^{++} (□) or 1.5 mM MPP^{+} (△). Filled symbols indicate the presence of 10 μM DPPD in the incubations.

peroxidation (Fig. 4A) and DPPD is unable to protect against its toxicity (Fig. 4B).

The interaction of $O_2^{\cdot-}$ and H_2O_2 in the presence of transition metals can lead to the formation of even more deleterious active oxygen species, such as $OH^{\cdot}$, which may be responsible for the toxicity of redox-cycling compounds. This reaction may also be of particular interest in the case of MPP^{+}-induced neurotoxicity, since the high concentration of transition metals in the substantia nigra has been hypothesized to be a major factor responsible for the specific vulnerability of neurons in that nucleus (14). We have compared the toxicity of PQ^{++} and MPP^{+} in BCNU-treated hepatocytes in the presence and absence of desferrioxamine, which should prevent OH formation by chelating iron (15). The results again show a clear difference: in the case of PQ^{++}, desferrioxamine completely prevents the occurrence of cell death, while MPP^{+}-induced cytotoxicity does not seem to be significantly affected by the presence or absence of free iron (Fig. 5).

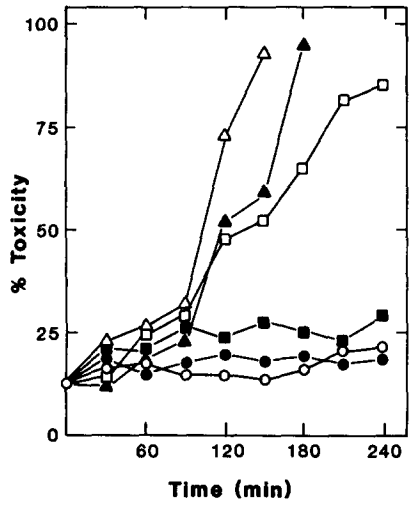

Fig 5 Cell toxicity induced by 1.5 mM PQ^{++} (□) or 1.5 mM MPP^{+} (△) in hepatocytes incubated in the absence or presence (filled symbols) of 100 µM desferrioxamine. (o) control.

In conclusion, the comparison of mechanisms of PQ^{++} and MPP^{+} toxicity in isolated hepatocytes has revealed some similarities but also several important differences. The cytotoxic effects of PQ^{++} are clearly related to oxygen radical production (e.g. H_2O_2 and $OH^{\cdot}$) and can be potentiated or prevented by making the cells more or less sensitive to the generation of "activated oxygen." In contrast, MPP^{+} cytotoxicity seems to be relatively independent of oxygen radical-induced oxidative stress, and the results presented here lead to the possibility of the involvement of other toxic mechanisms. Exposure of hepatocytes to both of these compounds results in oxidation of glutathione, but the significance of this effect may be substantially different in each case. GSSG formation after exposure to MPP^{+} might be the result of direct interaction of GSH with free radicals via generation of the glutathionyl radical ($GS^{\cdot}$) (16). However, some evidence for the production of oxygen radicals during MPP^{+} toxicity has been reported (17,18). Thus, it is possible that GSH oxidation indeed reflects oxygen radical formation during incubation of hepatocytes with MPP^{+}, but this event is likely to play only an augmentative and in any case less critical role in cell death as compared to PQ^{++}-induced toxicity.

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