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Comparison of Paraquat Actions on Oxygen Radical Generation and Lipid Peroxidation between Submitochondrial Particle and Microsome of Mouse Liver[1]

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ABSTRACT

In order to evaluate a potential role of mitochondria in the mediation of toxicity of paraquat (PQ), submitochondrial particle and microsome of mouse liver were compared by oxygen radical generation and lipid peroxidation.

With NADH in submitochondrial particle and NADPH in microsome as electron donors, PQ stimulated production of superoxide anion and H_2O_2 in both fractions. Under the same conditions, PQ enhanced the generation of ethylene from methional suggestiong stimulation of OH• production by PQ. But these effects by PQ were somewhat lower in submitochondrial particle than in microsome. In addition, lipid peroxidation(measured as MDA production) was stimulated by PQ in both fractions. The stimulation of lipid peroxidation in both fractions seemed to occur by the same mechanism probably through perferryl ion. This was supported by the following findings: i) The lipid peroxidation in both fractions was partially inhibited by SOD and completely inhibited by DETAPAC(an iron chelator) but not by catalase or OH• scavenger. ii) Addition of ADP-Fe^{3+} further increased PQ-induced lipid peroxidation but decreased ethylene production from methional suggesting no correlation between OH• production and lipid peroxidation.

The redox-cycling of PQ in mitochondria appeared to be linked to NADH dehydrogenase, not to CoQ since all of the observed stimulations by PQ in submitochondrial particle were inhibited by p-hydroxymercuribenzoate(a NADH dehydrogenase inhibitor) but not affected by other respiratory chain blockers.

The above results demonstrate that redox-cycling properties of PQ leading to oxygen radical generation and lipid peroxidation can also occur in mitochondria in the same manner as in microsome. Therefore, the observed actions of PQ in mitochondria suggest that mitochondria may also contribute to toxicity of this drug in vivo.

Key Words: Paraquat(PQ), Oxygen radical, Lipid peroxidation, Submitochondrial particle, Microsome

Abbrevation: PQ; paraquat, PQ• : paraquat radical, OH• ; hydroxyl radical

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INTRODUCTION

Paraqat(PQ: 1-1′-dimethyl-1, 4′-bipyridium; methyl viologen) is widely used and most potent bipyridyl herbicides which have been shown to be highly lethal to man and animals(Klaassen, 1985). The primary target organ for paraquat appears to be the lung and the time course of its pulmonary toxicity are edema, hemorrhage, and fibrosis in diffuse area of the lung with subsequently occurrence of respiratory failure and death(Von der Hardt & Cardeas, 1971). This high susceptability of the lung results from an energy-dependent accumulation of paraquat into the lung(Michael *et al.*, 1976). However, pathological changes are also seen in kidney, liver, thymus, adrenals and brain of the paraquat-poisoned animals(McDonagh & Martin, 1970; Nagi, 1970; Fennelly *et al.*, 1971).

The precise mechanism underlying toxicity of this agent has not been elucidated until now. Some investigators proposed that a deficiency of surfactants due to the inhibition of fatty acid synthesis by NADPH depletion is a possible mechanism of PQ-induced toxicity(Macklin, 1954; Wakil, 1962; Rose *et al.*, 1976). However, it has been recently suggested that the toxicity may be mediated by oxygen free radicals(Dodge, 1971; Ilett *et al.*, 1974; Shu *et al.*, 1979; Kornbrust & Mavis, 1980). Paraquat can be reduced to PQ radical($PQ+e^{-}\rightarrow PQ^{\cdot}$), which is rapidly reoxidized to the parent molecule with production of superoxide anion under aerobic conditions ($PQ^{\cdot}+O_2\rightarrow PQ+O_2^{-\cdot}$) in a cyclic manner and this redox-cycling property of paraquat is important in toxicity development.

It was reported that NADPH-dependent cytochrome P_{450} reductase which used NADPH as an electron donor in microsome catalyzed reduction and autooxidation of PQ and stimulated the production of the oxygen free radicals($PQ+NADPH+H^{+}\rightarrow PQ^{\cdot}+NADP^{+}$)(Christian & Karl, 1979; Ronald *et al.*, 1979; Michael *et al.*, 1982). But, various enzymatic and non-enzymatic systems as well as NADPH-dependent cytochrome P_{450} reductase have been known to reduce univalently molecular oxygen to superoxide anion(Kappus, 1981; 1986). Among them the respiratory chain of mitochondrial inner membrane also has reductase activity which is capable of catalyzing one electron reduction of xenobiotics(Takeshige *et al.*, 1980; Takayanagi *et al.*, 1980; Sate *et al.*, 1983). In mitochondria, actaully, 2 to 5 percent of consumed oxygen molecules can be redued to oxygen free radicals by NADH dehyrogenase and CoQ during the transportation of electrons through the respiratory chain(Boveris *et al.*, 1978; Takeshige & Minakami, 1979; Turrens & Boveris, 1980. Therefore, it is possible that the mitochondrial respiratory chain may be another source for the generation of oxygen free radicals mediating the toxicity of paraquat than microsomal NADPH-dependent cytochrome P_{450} reductase.

In the present study, we tried to evaluate a potential role of mitochondria in the mediation of toxicity of paraquat. For this purpose, we used submitochondrial particle and microsome from mouse liver and compared the capability of the oxygen radical generation and lipid peroxidation by PQ between both fractions. Furthermore, to identify the redox-cycling site of paraquat in the respiratiory chain, we tested the effects of respiratory-chain blockers and succinate on the productions of oxygen free radicals and lipid peroxidation.

MATERIALS AND METHODS

Tris, HEPES, paraquat(PQ), NADPH, NADH, ADP, epinephrine, adrenochrome, methional, thiobarbituric acid, superoxide dismutase(SOD), catalase, p-hydroxymercuribenzoate, rotenone, antimycin A, EDTA were purchased from Sigma Chemical Co.(St. Louis, MO., U.S.A). Trichloroacetic acid, sodium azide, dimethyl sulfoxide(DMSO), DETAPAC were purchased from E. Merck(Darmstadt, Germany) and other chemicals were reagent grade.

Preparation of mouse liver microsome and submitochondrial particle

Microsomal and mitochondrial fractions were isolated from fresh mouse liver by differential centrifugation. Liver tissues obtained from mice were chopped into small pieces and homogenized in 10 volumes of cold homogenizing buffer solu-

tion(250 mM sucrose, 0.5 mM EDTA, 10 mM Tris /HCl, pH 7.4) with a polytron homogenizer (PCU-I, Brinkman Inc.) at rheostat 6 for 15 sec(5 sec×3). After the homogenate was centrifuged at 1,000 g for 10 min, the supernatant was filtered with 4 layers of cheese cloth. The filtrate was centrifuged at 10,000 g for 15 min and then the pellet was used as mitochondrial fraction afterwards. To isolate microsomal fraction the supernatant was centrifuged again at 10,000 g for 5 min and the resulting supernatant was centrifuged at 100,000 g for 60 min. The resulting pellet was suspended in 20 volumes of cold buffer solution containing 150 mM KCl and 10 mM Tris/ HCl(pH 7.4) and the suspension was recentrifuged at 100,000 g for 60 min to wash contaminants out.

Submitochondrial fraction was prepared from mitochondrial fraction described above. The mitochondrial fraction which was obtained as pellet from centrifugation at 10,000 g for 15 min was suspended in cold buffer solution(250 mM sucrose, 2 mM EDTA, 10 mM Tris/HCl, pH 7.4). After the suspension was centrifuged again at 10, 000 g for 15 min, the pellet was mixed to 20% suspension with cold buffer solution(250 mM sucrose, 2 mM EDTA, 10 mM Tris/HCl, pH 8.5). This mitochondrial suspension was sonicated with Branson sonicator at 3 amp for 1 min. The pellet obtained from centrifugation(100,000 g, 30 min) of the sonicated suspension was washed with cold buffer solution containing 150 mM KCl and 10 mM Tris/HCl(pH 7.4) and centrifuged at 100,000 g for 30 min(Lee & Ernster, 1967).

All procedures were carried out below 4°C and the fractions of microsome and submitochondrial particle were suspended with buffer solution containing 150 mM KCl and 10 mM Tris /HCl(pH, 7.4), and stored at −20°C until used. The concentration of protein was determined by the method of Lowry *et al.* (1951).

Measurement of superoxide anion production

The generation of superoxide anion was determined by measuring adrenochrom formed from the oxidation of epinephrine by superoxide anion(Misra and Fridovich, 1972). 3 ml of reaction mixture containing 150 mM KCl, 20 mM HEPES/ NaOH(pH 7.4), 1 mM epinephrine, 0.2 mg/ml microsome or 0.25 mg/ml submitochondrial particle and various concentration of paraquat was incubated in a spectrophotometry cuvette at 37°C for 5 min. The reaction was started with the addition of 0.5 mM NADPH in microsome and 0.5 mM NADH in submitochondrial particle. The oxidation of epinephrine to adrenochrome was traced with a dual-wavelength spectrophotometer(Amico-Chance) equipped with a thermostatted cell compartment at 575 nm wavelengh (reference wavelength, 485 nm). The concentration of adrenochrome was estimated by absorbance of the standard solutions of adrenochrome.

Measurement of hydrogen peroxide(H_2O_2) production

The generation of hydrogen peroxide was estimated by measuring the formation of $Fe(SCN)_3$ from ferrous ammonium sulfate and potassium thiocyanate upon oxidation of Fe^{2+} to Fe^{3+} by hydrogen peroxide(Hildebrandt & Roots, 1975). 1 ml of reaction mixture containing 150 mM KCl, 20 mM HEPES/NaOH(pH 7.4), 0.5 mg/ml microsome or submitochondrial particle and paraquat was incubated at 37°C for 5 min in a shaking water bath. The reaction was started with the addition of 0.5 mM NADPH and 0.5 mM NADH in microsome and in submitochondrial particle, respectively. After 10 min, the reaction was terminated by the addition of 1 ml of cold 4% trichloroacetic acid and the mixture was centrifuged at 3,000 g for 10 min. 0.2 ml of 10 mM ferrous ammonium sulfate and 0.1 ml of 2.5 M potassium thiocyanate were added to 1 ml of the resulting supernatant. The production of hydrogen peroxide was calibrated from absorbance at 480 nm by the known amount of hydrogen peroxide. To inhibit the activity of catalase which is contaminated in both membrane fraction 1 mM and 0.2 mM sodium azide were added to the microsomal and submitochondrial reaction mixtures respectively.

Mesaurement of hydroxyl radical (OH•) production

The generation of hydroxyl radical was estimated from determination of ethylene formed by interaction of methional and hydroxyl radical (Beauchamp & Fridovich, 1970). 1 ml of reaction mixture containing 150 mM KCl, 20 mM HEPES/

NaOH(pH 7.4), 0.5 mg/ml microsome or submitochondrial particle, paraquat, 5 μl methional was transferred in a 12 ml glass vial sealed with a gas-tight rubber cap. The reaction was started by the addition of 0.5 mM NADPH or NADH and incubated at 37°C in shaking water bath. After 60 min of incubation 1 ml aliquot of gas phase in the reaction vial was obtained with a gas-tight syringe and injected into gas chromatograph(GCV, PYE-Unicam) to analyze ethylene. Gas chromatograph was equipped with a 1/8 inch×3 meter stainless steel column filled with chromosorb 101 and a flame ionization detector. The temperature of column, detector and injector were 70°C, 120°C and 80°C, respectively. The gas flow was 36 ml/min for nitrogen carrier, 35 ml/min for hydrogen gas and 400 ml/min for air. Amount of ethylene production was calculated from integrated areas of chromatogram prepared by known amount of pure ethylene gas.

Measurement of lipid peroxidation

Lipid peroxidation was measured by modified thiobarbituric acid method(Bidlack & Tappel, 1973). With 0.5 mM NADPH in microsome and 0.5 mM NADH in submitochondrial particle, the reaction of lipid peroxidation was initiated in the condition of 150 mM KCl, 20 mM HEPES/NaOH(pH 7.4), 0.5 mg/ml microsome or submitochondrial particle, paraquat at 37°C in shaking water bath. The reaction was terminated by the addition of volume of 20% TCA containing 1.7 N HCl after 60 min and the resulting mixture was centrifuged at 3,000 g for 10 min. 1 ml of the supernatant was mixed with 1 ml of 0.67% thiobarbituric acid, boiled in a tight-capped glass tube for 10 min. After cooling the mixture at room temperature, absorbance at 532 nm was measured with a spectrophotometer(Pye-Unicam). Amount of malondialdehyde(MDA) was calculated from the molar extinction coefficient of MDA, $1.53\times10^5\ M^{-1}\ cm^{-1}$(Placer *et al.*, 1966)

RESTULTS

Stimulatory effect of paraquat on superoxide anion and H_2O_2 production

Paraquat stimulated the oxidation of epinephrine to adrenochrome in submitochondrial parti-

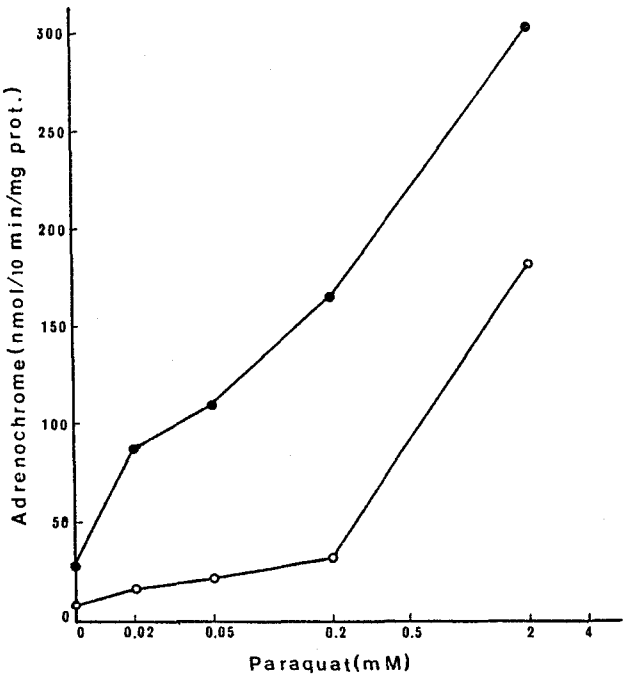

Fig. 1. Effects of PQ on adrenochrome formation from epinephrine in microsom and submitochondrial particle. Microsome(0.2 mg protein/ml) or submitochondrial particle(0.25 mg protein/ml) were preincubated for 5 min in the solution containing various concentrations of PQ, 1 mM epinephrine, 150 mM KCl and 20 mM HEPES-NaOH, PH 7.4. Reactions were started with the addition of 0.5 mM of either NADPH(when microsome was used) or NADH(when submitochondrial particle was used.) total reaction volumes were 1.0 ml and the reaction were performed at 37°C. The determination of adrenochrome was done as described in the Materials and methods.
●; microsome
○; submitochondrial particle

cle as well as in microsome. Adrenochrome formation was enhanced about 3-fold to 12-fold when 20 uM to 2 mM paraquat was incubated with mouse liver microsomes and NADPH. In submitochondrial particle, remarkable stimulation of the adrenochrome formation was observed between 200 uM and 2 mM paraquat(Fig. 1). The amount of adrenochrome formed in submitochondrial particle, however, was less than that in microsome. The oxidation of epinephrine to adrenochrome by paraquat in both fractions was completely inhibited by the addition of 15 units/ml SOD in the reaction mixture(data not shown).

Also NADPH-dependent and NADH-dependent H_2O_2 production were stimulated by paraquat in concentration dependent pattern Addition of paraquat(20 μM to 2 mM) caused 2 to 10 fold increase of H_2O_2 production in microsome and 1.5 to 6-fold in submitochondrial particle(Fig. 2). The production of H_2O_2 in submitochondrial particle was lower by 40% than that in microsome.

These results indicated that paraquat can stimulate the production of superoxide anion and H_2O_2 in submitochondrial particle as well as in microsome. And the stimulatory effect of paraquat on the production of oxygen radicals is much higher in microsome than that in submitochondrial particles.

Stimulatory effect of paraquat on OH· production

Paraquat stimulated the production of ethylene from methional in microsome. The stimulation in the presence of paraquat from 20 uM to 2mM was enhanced progressively to 7-fold. In submitochondrial particle, however, the stimulatory effect of paraquat was much higher than that in microsome. 4 mM paraquat caused 30-fold enhancement of ethylene formation in submitochondrial particle(Fig. 3).

Stimulatory effect of paraquat on lipid peroxidation

To evaluate the stimulation of lipid peroxidation by paraquat, microsome and submitochodrial particle were incubated with paraquat

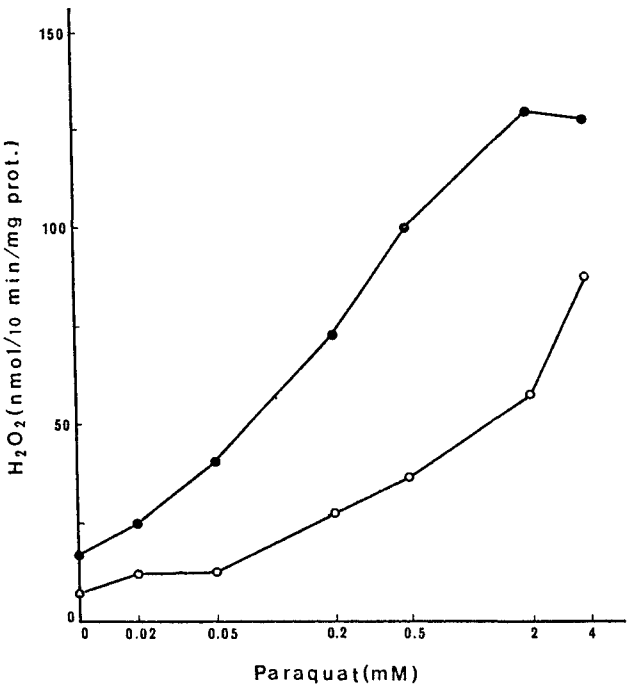

Fig. 2. Effects of PQ on H_2O_2 production in microsome and submitochondrial particle. Microsome or submitochondrial particle(0.5 mg protein/ml each) were treated for 10 min with PQ in the reaction medium omitting epinephrine and containing azide(1 mM in microsome medium or 0.2 mM in sub-mitochondrial particle medium). All other experimental conditions were the same as in Fig. 1. H_2O_2 was assayed as described in the Materials and Methods.
●; microsome
○; submitochondrial particle

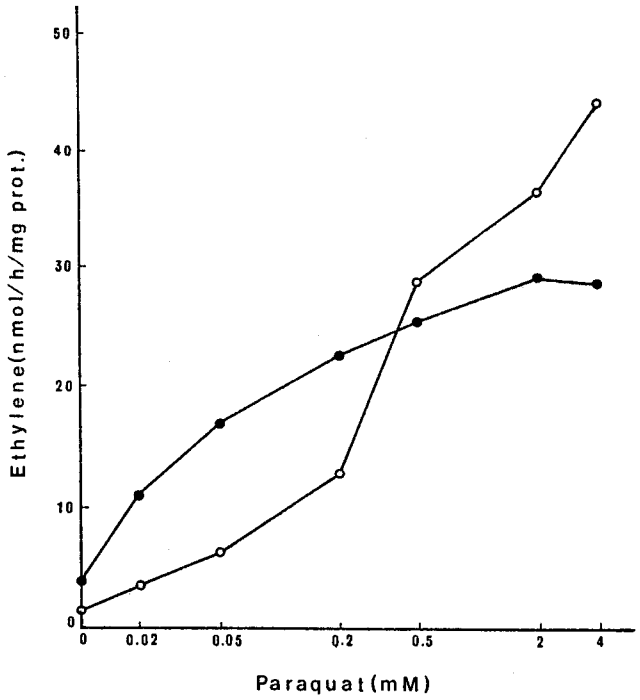

Fig. 3. Effects of PQ on ethylene formation from methional in microsome and submitochondrial particle. Microsome or submitochondrial particle(0.5 mg protein/ml each) were incubated for 1 h at 37°C in sealed glass vial(12 ml) with varous concentrations of PQ, 5 μl of methional, 150 mM KCl, 0.5 mM of NADPH(in the microsme medium) or NADH(in the submitochondrial particle medium) and 20 mM HEPES-KOH, PH7.4. The volume of reaction mixture was 1.0 ml. 1.0 ml aliquots from the gas phase were assayed for ethylene as described in the Materials and Methods.
●; microsome
○; submitochondrial particle

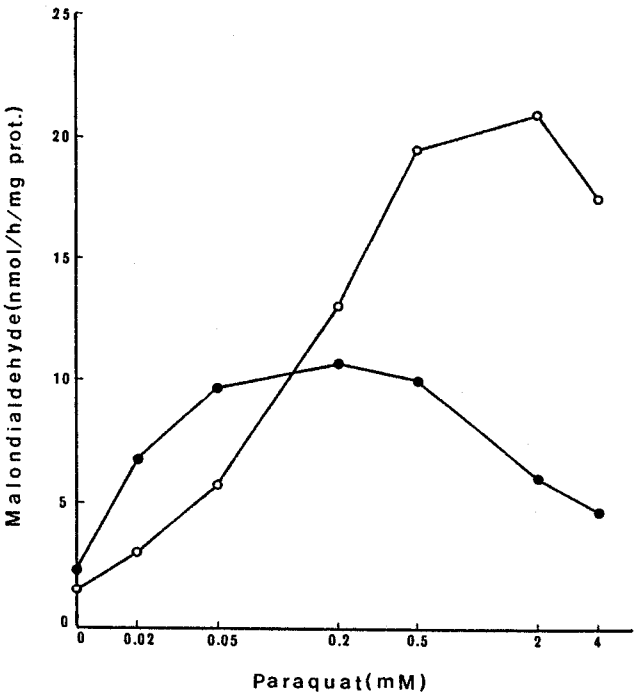

Fig. 4. Effects of PQ on MDA formation in microsome and submitochondrial particle. the reaction mixture(1.0 ml) containing 0. 5 mg protein/ml of microsome or sub-mitochondrial particle, various concentration of PQ, 0.5 mM NADH(in microsome medium) NADH (in the submitochondrial particle medium), 150 mM KCl and 20 mM HEPES-KOH, PH 7.4 were incubated for 1h at 37°C. MDA produced in the reaction mixture were determined as described in the Materials and Medhods.
●; microsome
○; submitochondrial particle

and NADPH or NADH for 60 min. The formation of malondialdehyde in microsome was increased dose-dependently by the addition of up to 200 uM of paraquat in the reaction mixture, but decreased by the addition of paraquat above 500 uM. On the other hand, the stimulatory action of paraquat in submitochondrial particle was observed continuously to 2 mM paraquat (Fig. 4).

Effects of oxygen radical scavengers on PQ-induced lipid peroxidation

To investigate the mechanism of the stimulatory action of paraquat on lipid peroxidation, we tried to evaluate effects of oxygen radical scavengers(SOD against superoxide anion, catalase against H_2O_2, DMSO and mannitol against OH·) and an iron-chelator(DETAPAC) on PQ-induced lipid peroxidation in both fractions was partly inhibited by SOD(15 unit/ml) and almost completely inhibited by DETAPAC(0.1 mM), but not by H_2O_2 and OH· scavengers. The addition of ADP-Fe^{3+} reduced the inhibitory action of SOD on PQ-induced lipid peroxidation but did not affect the action of DETAPAC. Therefore, it was suggested that only superoxide anion among the various oxygen radicals may contribute to PQ-induced lipid peroxidation and iron may play a major role in the lipid peroxidation(Table 1, 2).

Effect of ADP-Fe^{3+} on lipid peroxidation and OH· generation stimulated by paraquat

Generally, it was thought that iron could contribute to lipid peroxidation by catalyzing the production of OH· from superoxide anion and hydrogen peroxide(Haber & Weiss. 1934) or by being converted to a reactive perferryl ion combined with molecular oxygen(Morio *et al.*, 1985). From the above results showing that OH· scavengers could not affect the lipid peroxidation, it is possible that OH· doesn't mediate PQ-induced lipid peroxidation. To study further the roles of OH· and perferryl iron in the lipid peroxidation, we tried to evaluate the effect of iron on lipid peroxidation and OH· genergation induced by paraquat. As shown in Table 3, the addition of ADP-Fe^{3+} enhanced the lipid peroxidation about 8-fold and 3-fold but suppressed OH· production by 50% and 40% in mi-

Table 1. Effects of oxygen radical scavengers on PQ-induced lipid peroxidation in microsome

		MDA production(%)	
		−ADP-Fe^{3+}	+ADP-Fe^{3+}
Control		100[a]	100[b]
+ SOD	(15 U/ml)	64	108
+ Catalase	(14U/ml)	106	109
+ Azide	(0.2mM)	93	96
+ DMSO	(10 mM)	101	105
+ Mannitol	(20 mM)	93	110
+ DEPAPAC	(0.1 mM)	19	30

Effects of the components shown in the table on MDA production were observe in the medium(1. 0 ml) containing microsome(0.5 mg protein/ml), 0. 2 mM PQ, 0.5 mM NADPH, 150 mM KCl and 20 mM HEPES-KOH, PH 7.4 with or without ADP-Fe^{3+}(0.6-0.06 mM). Other experimental conditions were the same as in Fig. 4.

a; 1.80±0.04 nmol/10 min/mg protein of MDA were produced and taken as 100%

b; 14.5±0.04 nmol/10 min/mg protein of MDA were produced and taken as 100%

Table 2. Effects of oxygen radical scavengers on PQ-induced lipid peroxidation in submitochondrial particle

		MDA production(%)	
		−ADP-Fe^{3+}	+ADP-Fe^{3+}
None		100[a]	100[b]
+ SOD	(15U /ml)	55	87
+ Catalase	(14U/ml)	95	101
+ Azide	(0.2mM)	99	97
+ DMSO	(10 mM)	106	103
+ Mannitol	(40 mM)	98	102
+ DEPAPAC	(0.2 mM)	5	N.D.[c]

Experimental conditions were the same as in Table 1 but 0.5 mg protein/ml of submitochondrial particle, 0.5 mM PQ and 0.5 mM NADH were used.

a; 3.3±0.01 nmol/10 min/mg protein of MDA were produced and taken as 100%

b; 9.7±0.3 nmol/10 min/mg protein of MDA were produced and taken as 100%

c; not detectable

Table 3. Effects of ADP-Fe^{3+} on PQ-induced MDA and ethylene production in microsome and submitochondrial particle

	Microsome		Submitochondrial particle	
	Malondialdehyde[a] (nmol/10 min/mg prot.)	Ethylene (nmol/h/mg prot.)	Malondialdehyde[a] (nmol/10 min/mg prot.)	Ethylene (nmol/h/mg prot.)
−ADP-Fe^{3+}	1.8±0.04	25.2±1.2	3.3±0.01	28.8±1.1
+ADP-Fe^{3+}	14.5±0.4	13.1±1.3	9.7±0.3	18.4±1.2

MDA and ethylene production from microsome or submitochondrial particle were observed at fixed concentration of PQ in the absence or presence of ADP-Fe^{3+}(0.6-0.06 mM). The experimental conditions for MDA and ethylene production were the same as in Fig. 3. and 4, respectively.
a: in the presence of 0.2 mM but 0.5 mM PQ in all other experiments.

Table 4. Effects of respiratory chain blockers on PQ-induced oxygen radical generation and lipid peroxidation in submitochondrial particle

	Adrencohrome(%)	H_2O_2(%)	Ethylene(%)	MDA(%)
Control[a]	100	100	100	100
+ 0.5 mMp-Hydroxymercuribenzoate	N.D.[b]	5.6	42	12
+ 0.04 mMRotenone	120	120	120	110
+ 0.04 mMAntimycin	110	90	100	37
+ 50 mM NaCN	−	−	−	6

a: Adrenochrome, H_2O_2, ethylene and MDA from submitochondrial particle(0.5 mg protein/ml) and 0.5 mM PQ were assayed in the reaction conditions as in Fig. 1, 2, 3, and 4, respectively. The production of each product were observed in the presence of respiratory chain blockers shown above.
b: not detecatable

Table 5. Effects of succinate on PQ-induced oxygen radical generation and lipid peroxidation in submito chondrial particle

Additions	Adrenochrome (nmol/10 min/ mg prot.)	H_2O_2 (nmol/10 min/ mg prot.)	Ethylene (nmol/h/ mg prot.)	MDA (nmol/h/ mg prot.)
(A). Succinate(5 mM) +Antimycin(0.04 mM)	N.D.[a]	N.D.	0.65±0.005	N.D.
(B).(A) +PQ(0.5 mM)	N.D.	N.D.	0.85±0.05	N.D.
(C). NADH(0.5 mM) +PQ(0.5 mM)	−	−	−	19.3±0.1
(D).(C) +Succinate(5 mM)	−	−	−	15.3±0.6

Productions of adrenochrome, H_2O_2, ethylene or MDA from submitochondrial particle(0.5 mg protein/ml) were obsedved in the presence of the components shown in the table. The reaction conditions for adrenochrome, H_2O_2, ethylene and MDA were described in Fig. 1, 2, 3 and 4, respectively.
a: not detectable

crosome and submitochnondrial particle, suggesting no correlation between OH• production and lipid peroxidation. The stimulation of lipid peroxidation, therefore, seems to occur in both fractions by the same mechanism probably through perferryl ion.

Effects of resipratory chain blockers and succinate on PQ-induced oxygen radical generation and lipid peroxidation in submitochondrial particle

Respiratory-chain blockers were used to find which factor within the respiratory chain had the potential for univalently reducting paraquat. PQ-incuced oxygen radical generation was dramatically suppressed by p-hydroxymercuri-benzoate, an inhibitor of NADH dehydrogenase (Takayanagi *et al.*, 1980), but not by rotenone and antimycin A (inhibitors of CoQ and cytochrome oxidase). On the lipid peroxidation, the NADH dehydrogenase inhibitor, p-hydroxymercuribenzoate significantly inhibited the lipid peroxidation induced by paraquat in submitochondrial particle. And cytochrome oxidase inhibitors(antimycin A and sodium cyanide) also inhibited.

But when rotenone was treated with submitochondrial particle the degree of lipid peroxidation induced by paraquat was not changed(Table 4). Succinate supplying electrons for CoQ, meanwhile, did not cause paraquat to stimulate oxygen radical generation and lipid peroxidation(Table 5). These observations indicate that paraquat receives an electron from an electron carrier between NADH dehydrogenase and CoQ in the mitochondrial respiratory chain.

DISCUSSION

The results presented in this report indicate that submitochondrial particle can be a site to generate oxygen radicals by paraquat and paraquat stimulated NADH-dependent lipid peroxidation in submitochondrial particle.

Redox-cycling properties of PQ leading to oxygen radical generation and lipid peroxidation can also occur in mitochondria in the same manner as in microsome. Although the amount of PQ-induced oxygen radical generation was more in microsome than in submitochondrial particle, the stimulatory patterns by PQ were similar in both fractions. The lipid peroxidation stimulated by PQ also seems to be mediated by the same mechanism probably through perferryl ion in both fractions. Perferryl ion can be formed through two pathways: one is the direct reduction of iron by paraquat radical($PQ\cdot + Fe^{3+} + O_2 \rightarrow PQ + Fe^{2}\text{-}O_2 \rightarrow Fe^{3+}\text{-}O_2^{-}\cdot$), the other is the indirect reduction of iron intermediated by superoxide anion($PQ\cdot + O_2 \rightarrow PQ + O_2^{-}\cdot + Fe^{3+} \rightarrow Fe^{3+}\text{-}O_2^{-}\cdot$) (Takeshige *et al.*, 1980; Morio *et al.*, 1985). With regarding SOD-inhibitable peroxidation(about 50%) as the portion mediated by the indirect reduction of iron, PQ-induced lipid peroxidation may be ascribed equally to both pathways in the absence of exogenous ADP-Fe^{3+}. With the addition of ADP-Fe^{3+}, however, the formation of perferryl ion may be progressed dominantly by the direct reduction of iron.

The respiratory chain transmitted an electron from NADH to paraquat forming paraquat radical(Turrens & boveris, 1980; Takayanagi *et al.*, 1980). The site where paraquat receives an electron could be identified as a region between the p-hydroxymercuribenzoate-sensitive site and the rotenone-sensitive site, because PQ-induced oxygen radical generation and lipid peroxidation were markedly suppressed by p-hydroxymercuribenzoate but not by rotenone. Furthermore, the above hypothesis of PQ's redox-cycling could be supported by the result that succinate, an electron donor for CoQ, could not cause the oxygen radical production and the lipid peroxidation.

PQ-induced oxygen generation was not affected by antimycin A but the lipid peroxidation was inhibited by antimycin A and cyanide. These observation suggest that the reduced form of CoQ increased by blocking respiratory-chain sites posterior to CoQ cannot transmit a single electron to paraquat but can act as an antioxidant on the lipid peroxidation as reported by Takayanagi *et al.* (1980). The action of reduced CoQ was re-examined by the addition of succinate which also could increase the concentration of reduced CoQ. The result that succinate suppressed NADH-dependent and PQ-induced lipid peroxidation supports the antioxidant effect of reduced CoQ.

These studies demonstrated that lipid peroxidation of mitochondrial membrane occurred together with the production of oxygen radicals when paraquat was incubated with submitochondrial particle in the presence of NADH. And mitochondria may also contribute to toxicity of this drug in vivo through the generation of oxygen radicals. But the role of the lipid peroxidation is not the only potentially destructive reaction and may not wholly account for the observed pulmonary toxicity. In addition oxygen radicals were highly reactive to intracellular macromolecules resulting in randomly destroying nucleic acids and proteins as well as lipids. In this report, only lipid peroxidation was observed as an index of PQ-induced toxicity. Therefore, these studies need to be further evaluated in connection with other indices for the toxicity and biological functions, especially including mitochondrial functions.

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= 국문초록 =

Paraquat에 의한 산소 Radical 생성 및 지질과산화 작용의 Mouse 간 Submitochondria Particle과 Microsome에서의 비교

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Paraquat 독성작용에 있어서 mitochondria의 잠재적인 역할을 평가하기 위하여, 이 약물의 산소 radical 생성과 지질과산화 반응에 미치는 영향을 mouse간의 submitochondrial particle과 microsome에서 비교하여 보았다.

Submitochondrial particle 사용시 NADH를, microsome 사용시 NADPH를 전자공여체로 이용한 경우 paraquat는 두 분획에서 superoxide anion과 hydrogen peroxide의 생성을 증가시켰다. 동일한 조건하에서 paraquat는 hydroxyl radical의 생성을 시사하는 methional로 부터 ethylene의 생성을 증가시켰다. 그러나, paraquat에 의한 이들 각각의 효과는 microsome에서 보다 submitochondrial particle에서 약간 낮았다. 한편, 두 분획 모두에서 paraquat는 지질과산화 반응을 촉진시켰다. Submitochondrial particle과 micorsome에서의 Paraquat에 의한 지질과산화반응은 i) 두 분획에서 지질과산화는 SOD에 의해서 부분적으로 억제됨을 보였고, DETAPAC(iron chelator)에 의해서는 완전히 억제되었고, catalase와 hydroxyl radical scavenger에 의해서는 억제되지 아니하였으며, ii) 반응내 ADP-Fe^{3+} 첨가로 paraquat에 의한 지질과산화는 더욱 증가되었지만 methional로 부터 ethylene 생성은 감소하여 hydroxyl radical 생성과 지질과산화 사이에는 상관성이 없음으로 보아 같은 기전을 통해 촉진됨을 알 수 있었고 이러한 촉진작용은 perferryl ion을 통하여 일어나리라 추측되었다.

Submitochondrial particle에서 paraquat에 의해 촉진된 산소라디칼 생성과 지질과산화 반응은 p-hydroxymercuribenzoate(NADH dehydrogenase 억제제)에 의하여 억제되었으나 다른 respiratory chain 차단제들에 의해서는 거의 영향을 받지 않음으로 보아 mitochondria에서의 paraquat의 redox-cycling은 CoQ 보다는 NADH dehyrogenase와 관련이 있음을 시사하였다.

이상의 결과로 보아 산소 radical의 생성과 지질과산화를 유도하는 paraquat의 redox-cycling은 microsome에서와 마찬가지로 mitochondria에서도 일어나며, 이결과 생체내에서의 paraquat의 독작용에 관여함을 짐작할 수 있다.