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MECHANISM OF PROTECTION OF ALVEOLAR TYPE II CELLS AGAINST PARAQUAT-INDUCED CYTOTOXICITY BY DEFEROXAMINE

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Abstract—Paraquat toxicity has been associated with the generation of free radicals in alveolar epithelial cells in which paraquat specifically accumulates via a polyamine uptake system. In the present study we investigated whether deferoxamine (DF), an iron chelator that has antioxidant capacity and that also has a polyamine-like structure, could protect alveolar type II cells (ATTC) against injury by paraquat. Radiolabeled [^{3}H]adenine ATTC were incubated in a medium containing 75 μM paraquat in the absence or presence of DF (500 μM). After 3 hr of incubation paraquat-mediated cytotoxicity of ATTC, as measured by [^{3}H]adenine release, was significantly ($P < 0.005$) decreased by addition of DF (26.6 ± 2.6% vs 7.4 ± 1.7%). Accumulation of radiolabeled [^{14}C]paraquat at a concentration of 75 μM was also decreased (70%) by 500 μM DF from 94.8 ± 2.1 to 28.9 ± 6.7 nmoles paraquat/2.5 × 10^5 ATTC. This effect of DF was dose dependent and comparable with the protective effect of equimolar concentrations of putrescine. However, per cent uptake of paraquat at a concentration of 500 μM was not significantly inhibited by DF (1 mM), whereas paraquat-induced injury was still markedly reduced (36.2 ± 2.5% vs 2.6 ± 4.2%). This indicated that the protective effect of DF could not be explained by its competition with paraquat on uptake alone. In the same series of experiments using another iron chelator, pyridoxal benzoyl hydrazone (PBH), which has antioxidant properties similar to DF but does not show its polyamine-like structure, ATTC lysis was also prevented although paraquat uptake was not reduced. These *in vitro* data indicate that the mechanism of protection by DF against paraquat toxicity in lung epithelial type II cells is two-fold: inhibition of paraquat uptake through its compliance with the structural requirements necessary for transport, and inhibition of paraquat-induced iron-catalysed free radical generation.

Paraquat (1,1'-dimethyl-4,4-bipiridylium dichloride; PQ^{2+}) is a bipyridium herbicide which after ingestion may cause acute respiratory failure [1, 2]. The specific toxicity of paraquat for lung tissue can be explained by its selective accumulation in Clara cells and in alveolar type I and type II epithelial cells [3]. There is substantial evidence that the toxicity of paraquat is due to generation of oxygen radicals involving the oxidation of NADPH [4, 5]. It has also been suggested that paraquat is toxic due to depletion of NADPH by its cyclical reduction and re-oxidation and thereby affects NADPH-dependent processes [3] such as fatty acid synthesis which is needed for surfactant production [6] and the redox-cycling of glutathione [7] which is important for the protection against hydrogen peroxide (H_2O_2) or lipid hydroperoxide.

In mammalian tissue, PQ^{2+} is redox-cycling continuously, by which it transfers electrons to oxygen, possibly by the activity of the cytochrome P450 reductase which serves as an electron "shuttle" between the reduced pyridine nucleotide to paraquat ($NADPH + PQ^{2+} \rightarrow NADP^+ + PQ^+.$) [1, 4, 5, 8]. The so formed paraquat cation radical ($PQ^+.$) is very rapidly reoxidized by oxygen with the concommitant production of superoxide (O_2^-) [9, 10]. Superoxide in turn is reduced, spontaneously or catalysed by superoxide dismutase (SOD), to form H_2O_2. Hydrogen peroxide may then be reduced to hydroxyl radical ($\cdot OH$) in an iron-catalysed Fenton type reaction [11]. The hydroxyl radical is an extremely toxic species [12] with a half life of 10^{-9} sec and a diffusion radius of 2.3 nm [13]. Damage depends upon the site of its production: if close to DNA or membranes it can be devastating to cells.

In examining the possible defense mechanism against paraquat toxicity, previous investigators have focussed on the competition between endogenous polyamines and diamines, e.g. putrescine, and paraquat for their transport system in lung slices [14, 15] and in isolated rabbit lung epithelial cells [16]. Polyamines are actively taken up, in a time and temperature dependent manner by a transport process also used by other endogenous substrates [17, 18]. It is assumed that paraquat is mistaken for such an oligoamine because of its chemical structure and therefore transported over the membrane [19].

We recently showed that DF, a chelator that blocks the catalytic activity of iron in oxygen radical generation [20], could reduce the mortality by paraquat in vitamin E-deficient rats [21]. In the same study it was shown that DF can block $\cdot OH$ generation from paraquat. In addition several other studies have

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described the protective effect of DF in oxygen radical-mediated injury [22–24].

In the present study we further investigated the mechanism by which DF interferes with paraquat toxicity for ATTC. By using putrescine and another iron chelator, PBH, with similar antioxidant properties as DF but without an inhibitory effect on paraquat uptake, we discriminated between interference of DF with paraquat on uptake and with paraquat-mediated cell injury via an intracellular cytotoxic mechanism.

MATERIALS AND METHODS

Animals. Pathogen free male Wistar rats weighing approximately 250 g were obtained from Harlan-Olac (Zeist, The Netherlands).

Isolation of alveolar type II cells. Alveolar type II cells (ATTC) were freshly isolated according to the method of Dobbs *et al.* [25]. Briefly, digestion of lung tissue using elastase was followed by panning of the resultant cell suspension on rat IgG coated plates. Rat IgG and elastase (4.3 units/mL porcine pancreas) were obtained from the Sigma Chemical Co. (St Louis, MO). ATTC were identified by modified Papanicolaou stain and placed in primary culture in fibronectin (Central Laboratory of the Blood Transfusion Service of the Netherlands Red Cross, Amsterdam, The Netherlands) coated 96-well tissue culture plates (Nunc, Roskilde, Denmark). The fibronectin solution was allowed to adhere to the microtiter plates for 1 hr at room temperature and then aspirated before the plates were used. During the overnight culture (37°, 5% CO_2) in RPMI 1640-M199 (1:1, v/v; Gibco, Biocult Ltd, Paisley, U.K.) supplemented with 10% foetal calf serum and gentamicin (10 μg/mL), ATTC (2.5×10^5/well) were labeled with [^{3}H]adenine (0.1 μCi [8-^{3}H]adenine per well; sp. act. 27 μCi/mmol; Amersham International, Amersham, U.K.). After overnight culture, the medium was discarded and the plates were washed three times with Hanks balanced salt solution containing 0.1% gelatin, (GHBSS, pH 7.4) to remove non-adherent and dead cells.

Combined cytotoxicity and paraquat-accumulation assay. Paraquat-mediated ATTC injury and the accumulation of paraquat in ATTC were simultaneously assayed using GHBSS as buffer. After the cells had been washed, deferoxamine mesylate (DF, Ciba-Geigy, Basle, Switzerland), putrescine dihydrochloride (Sigma), and pyridoxal benzoyl hydrazone (PBH, gift of Dr P. Ponka, McGill University, Montreal, Quebec, Canada) were added. This was followed by the addition of paraquat (ICI, Rotterdam, The Netherlands), and 0.1 μCi *methyl*-[^{14}C]paraquat chloride/well (sp. act. 111 mCi/mmol; Amersham International) in a total volume of 200 μL/well. After an incubation period of 3 and 6 hr (37°, 5% CO_2) the plates were centrifuged (175 *g* for 5 min) and 100 μL samples of supernatant were removed to determine the cytotoxicity by measurement of the radioactivity (Philips liquid scintillation counter, Model PW 4700, Philips Analytical, Almelo, The Netherlands). Per cent cytotoxicity was expressed using the formula $(A\text{-}S/T\text{-}S) \times 100$, where *A* represents counts per minute in the supernatant of samples containing paraquat and ATTC in the absence or presence of DF, PBH or putrescine, *S* is the spontaneous release (15.7 ± 2.3%), and *T* represents the maximal release from control cells treated with 1 N NaOH. Using this method the extent of cellular injury can be expressed as the cytotoxic index, where 0% represents the release of ^{3}H from control cells and 100% represents all radioactivity that was releasable from NaOH treated control cells.

Cellular accumulation of paraquat was determined by washing the plates with GHBSS to remove the remaining supernatant and the non-adherent and dead cells. A total volume of 200 μL 1 N NaOH/well was added to lyse the cells. After 15 min 100 μL samples of the disrupted ATTC remnants were removed and after addition of 2.5 mL scintillation liquid all samples were counted. After correction for the number of cells the uptake of paraquat was expressed as nmoles paraquat/2.5×10^5 ATTC.

Hydroxyl radical assay. The generation of ·OH was measured by the oxidation of 2-keto-4-methylthiobutryic acid (KMB; Sigma) to ethylene [26]. Briefly, ethylene was assayed using gas chromatography (Model GC-9a, Shimadzu, Japan) with a stainless steel column packed with Carbosieve G (Supelco, S.A., Gland, Switzerland) and a flame ionization detector. The oven was heated from 145° to 195° (programmed at 6°/min). N_2 gas was used as carrier (flow rate 50 cm^3/min). Hypoxanthine (Sigma; 300 μM), xanthine oxidase (from Buttermilk, grade I, Sigma; 18 mUnits/mL) and ferric citrate (FeIII-citrate; 50 μM), were incubated at 37° in a shaking water bath in a 5.0 mL glass tube. Incubation medium was GHBSS (final volume 0.5 mL) plus KMB (10 mM) in the absence or presence of chelator. The tubes were sealed with rubber stoppers. After 30 min a sample of 0.5 mL of headspace gas was taken using an airtight syringe and this gas sample was directly analysed. Ethylene production was determined by comparing it with a calibration chromatogram of a known amount of ethylene standard (Supelco S.A., Gland, Switzerland) injected onto the column.

Statistical analysis. Statistical significance was determined by Student *t*-test for paired samples, with significance defined as $P < 0.05$.

RESULTS

Cytotoxicity and accumulation of paraquat. Paraquat in a final concentration of 5 to 500 μM was tested for cytotoxicity against isolated ATTC. During the 3 hr incubation period the accumulation of paraquat inside cells increased in a dose dependent manner from 14.7 ± 2.8 to 296.6 ± 58.6 nmol/2.5×10^5 ATTC, respectively (Fig. 1A). The corresponding cytotoxic index increased from −0.2 ± 6.0% to 36.2 ± 4.9%, reaching a plateau at approximately 75 μM paraquat (Fig. 1B).

Inhibition of paraquat-mediated cell injury and intracellular accumulation of paraquat by DF. In the same series of experiments it was demonstrated that cell injury could be prevented by the addition of a non-toxic concentration of DF (1 mM). As can be seen in Fig. 1B, ATTC injury at all paraquat concentrations used (0–500 μM) was less than 5%. In

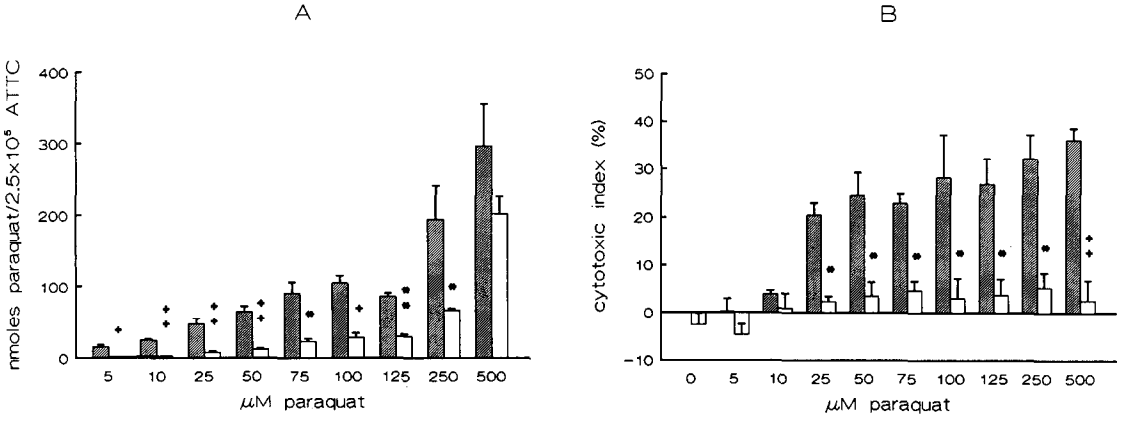

Fig. 1. Dose dependent effect of paraquat on inhibition of paraquat accumulation and ATTC injury by DF. ATTC were incubated for 3 hr at 37° in GHBSS with paraquat at various concentrations (5–500 μM) in the absence (hatched bars) or presence (white bars) of DF (1 mM). Accumulation of paraquat (A) and paraquat-induced ATTC injury (B) were measured as described in Materials and Methods. The extent of cellular injury is expressed as the cytotoxic index, where 0% represents the release of ^3H from control cells and 100% represents all radioactivity that was releasable from ATTC treated with 1 N NaOH. Bars denote mean ± SE of four experiments performed in triplicate. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.005$, and ** $P < 0.001$ compared to control values from ATTC incubated with paraquat alone.

agreement, the accumulation of paraquat in ATTC which were incubated in the presence of DF was also decreased compared to uptake by ATTC in medium with paraquat alone (Fig. 1A). This inhibition of paraquat uptake by DF was significant ($P < 0.05$) to a concentration of 250 μM paraquat. However, at 500 μM paraquat DF did not significantly inhibit paraquat uptake by ATTC (296.9 ± 58.6 vs 202.2 ± 24.1 nmoles paraquat/2.5×10^5 ATTC; $P > 0.05$).

Thus, at 500 μM paraquat there is a discrepancy between uptake and cell injury; at this concentration per cent inhibition by DF of paraquat uptake was only 30% vs > 70% at the lower concentrations of paraquat. In contrast, ATTC injury at the whole paraquat concentration range, including 500 μM, was almost completely inhibited (> 80%). Figure 2, showing a logarithmic curve of the accumulation of paraquat at all tested concentrations in the absence or presence of DF vs the corresponding cytotoxic index, illustrates that DF is acting two-fold. The following series of experiments was performed to elucidate the mechanisms responsible for these data.

Effect of DF and putrescine on paraquat accumulation in ATTC. To better characterize the mechanism by which DF inhibits paraquat uptake by ATTC we compared its effect with that of putrescine, a known potent agent of the energy dependent uptake of paraquat by alveolar epithelial cells [15]. Both DF and putrescine at an equimolar concentration of 0.1 mM significantly ($P < 0.01$ and $P < 0.005$, respectively) inhibited the accumulation of paraquat during 3 hr of incubation with 75 μM paraquat (Table 1). This effect was dose dependent. At a five-fold higher concentration of DF and putrescine (0.5 mM) per cent uptake of paraquat by ATTC decreased by 27 and 9%, respectively. Inhibition by putrescine was about three times stronger than the inhibition of paraquat uptake by

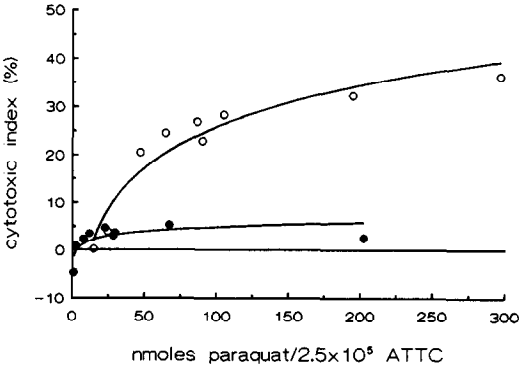

Fig. 2. Paraquat accumulation plotted vs ATTC cytotoxicity. ATTC were incubated for 3 hr at 37° in GHBSS with paraquat at various concentrations (5–500 μM) in the absence (open circles) or presence (closed circles) of 1 mM DF. Accumulation of paraquat and paraquat-induced ATTC injury were measured as described in Materials and Methods. The extent of cellular injury is expressed as the cytotoxic index, where 0% represents the release of ^3H from control cells and 100% represents all radioactivity that was releasable from ATTC treated with 1 N NaOH. Data represent mean ±SE of four experiments performed in triplicate. Nanomoles paraquat accumulation in 2.5×10^5 ATTC, horizontal axis, are plotted vs the corresponding cytotoxic index of the same cells, vertical axis. The curves of these data (also shown in Fig. 1) are interpolated in a logarithmic method.

DF. However, as shown in the same table, paraquat-mediated injury of ATTC was equally inhibited by DF and putrescine. This was about 30% at both 0.1 and 0.5 mM of the two compounds. Therefore at a similar molar basis with putrescine, DF has a less potent effect on paraquat accumulation but a comparable effect on cytotoxicity. These results indicate

Table 1. Effect of DF and putrescine on uptake and paraquat-mediated injury of alveolar type II cells

Test condition for ATTC	Cytotoxic index (%)	PQ accumulation (nmol/2.5 × 10^5 ATTC)
PQ alone	26.6 ± 2.9	94.8 ± 2.1
PQ+DF (0.1 mM)	9.4 ± 0.8‡	54.4 ± 8.1†
PQ+PC (0.1 mM)	8.3 ± 2.9*	18.6 ± 5.3§
PQ+DF (0.5 mM)	7.4 ± 1.7‡	28.9 ± 6.7§
PQ+PC (0.5 mM)	8.9 ± 2.5†	9.8 ± 2.6§

ATTC were incubated for 3 hr in GHBSS containing 75 μM paraquat (PQ) plus DF and putrescine (PC). Accumulation of paraquat and injury of ATTC were determined as described in Materials and Methods. The extent of cellular injury is expressed as the cytotoxic index, where 0% represents the release of ^3H from control cells and 100% represents all radioactivity that was releasable from ATTC treated with 1 N NaOH. Data represent mean ± SE of three independent experiments performed in triplicate.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.005$, and § $P < 0.001$ compared to ATTC incubated with paraquat alone.

that the action of DF is not limited to the cell membrane alone: when ATTC were exposed to 75 μM paraquat plus 0.1 mM DF, paraquat accumulated to 54.4 ± 8.1 nmol/2.5 × 10^5 ATTC whereas the cytotoxic index was only 9.8 ± 2.6%. As can be seen from Fig. 2 the amount of accumulated paraquat should have resulted in a cytotoxic index of at least 18%.

Effect of DF and PBH on paraquat-induced cytotoxicity and on paraquat accumulation. The preceding studies indicated that DF protects ATTC against paraquat toxicity, first by inhibition of uptake, and second by an undefined mechanism that operates intracellularly. Inasmuch as paraquat is toxic via oxygen radical generation [1, 9] and DF has antioxidant capacity by its particular iron chelating properties [20], we investigated whether this specific feature could be responsible for protection against intracellular paraquat. Therefore we compared the effect of DF with another iron chelator, PBH. This molecule differs from DF in its structural similarities with polyamines suggesting that it has no effect on paraquat uptake by ATTC. But at a 1:2 molar ratio, whereby it completely locks in the iron ion [27] binding to all of the six aquo positions which are important in transition metal catalysis [20, 28, 29], it may have antioxidant capacity. When ATTC were incubated with 75 μM paraquat in the absence or presence of DF (0.5 mM) and PBH (0.5 mM) we observed no effect on accumulation of paraquat by PHB (Fig. 3A). On the contrary, in the presence of PBH uptake of paraquat in ATTC was significantly ($P < 0.05$) enhanced compared to both ATTC in medium plus paraquat alone or in the presence of DF. This enhancement was even more pronounced after 6 hr of incubation ($P < 0.001$). Despite this increased uptake of paraquat there was no enhancement of its cytotoxic effects. Instead, cytotoxicity decreased, comparable to the inhibiting effect of DF on paraquat-mediated ATTC injury (Fig. 3B). This suggests an antioxidant effect of PBH.

Inhibition of hydroxyl radical production by DF and PBH. To address the possibility that the protective effect of both DF and PBH could be a result of their iron chelating capacity, drugs were tested for their ability to interrupt iron-catalysed hydroxyl radical generation. After 30 min (37°) incubation of hypoxanthine plus xanthine oxidase in the presence of FeIII-citrate, ·OH was indirectly detectable measuring the production of ethylene from KMB. When DF or PBH was added to the reaction mixture the peak heights, from which the amount of C_2H_4 was calculated, were approximately one third of the control (Table 2). To confirm the interpretation that this inhibition was indeed an effect of iron chelation, DF and PBH were deprived of their capacity to chelate iron. When the chelators were saturated with iron before incubation with KMB, the production of ethylene was not significantly ($P > 0.1$) inhibited (data not shown).

DISCUSSION

Several studies using lung slices or lung explants have shown that paraquat is preferentially taken up by alveolar type I and type II epithelial cells via a polyamine uptake system [1, 17]. These cells, which play a crucial role in maintaining the structure and functions of pulmonary alveoli can be damaged by paraquat [18, 30]. This may result in edema and pulmonary infiltration with neutrophils, followed by fibrosis of lung tissue and hypoxemia usually ending in death [21]. The mechanism of paraquat cytotoxicity involves the generation of toxic oxygen metabolites [8, 9]. Previously, we have shown that DF, by its antioxidant capacity, reduces mortality by paraquat in vitamin E-deficient rats [21].

The main objective of the present study was to assess the mechanism by which DF interferes with paraquat-induced cytotoxicity in ATTC. The accumulation of paraquat results from its transport by a process normally responsible for the uptake of the endogenous polyamines, which is an energy dependent system [14, 18, 31]. Since DF has antioxidant properties [22, 23] but also a polyamine-like structure with a primary amino group and several secondary amino and amide groups, we investigated its effect on paraquat-mediated lysis of ATTC as

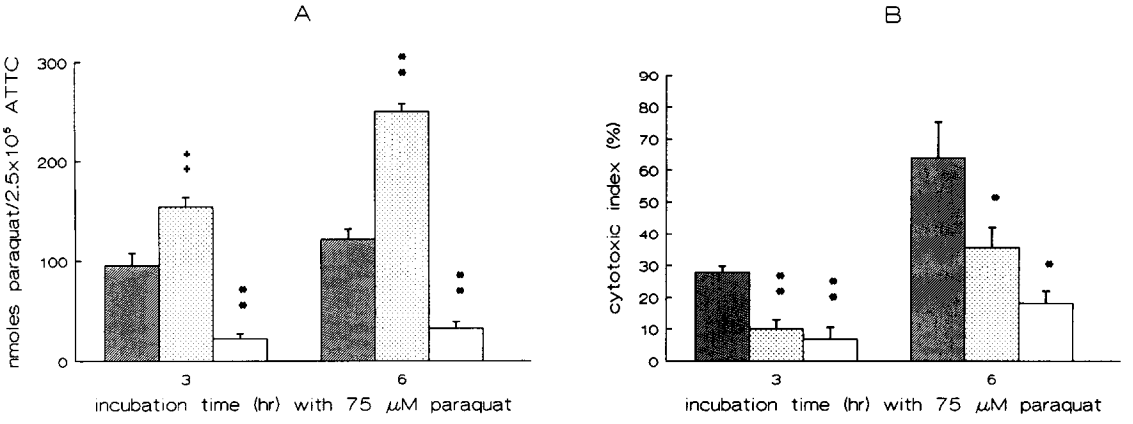

Fig. 3. Effect of DF and PBH on accumulation of paraquat in ATTC and on paraquat-induced cytotoxicity. ATTC were incubated for 3 and 6 hr at 37° in GHBSS with 75 μM paraquat in the absence (control; hatched bars) or presence of 0.5 mM DF (white bars) and 0.5 mM PBH (dotted bars). Accumulation of paraquat in ATTC (A) and the cytotoxic index (B) were measured as described in Material and Methods. Bars denote mean ± SD of three experiments in triplicate. * $P < 0.05$, ‡ $P < 0.005$, and ** $P < 0.001$ compared to control values from ATTC incubated with paraquat alone.

Table 2. Effect of DF and PBH on the iron catalysed generation of hydroxyl radical

Test conditions	C_2H_4 production (pmol/30 min)
HX + X + FeIII-citrate	1377 ± 45
HX + X + FeIII-citrate + DF	454 ± 38*
HX + X + FeIII-citrate + PBH	479 ± 15*

Generation of ethylene from KMB (10 mM) was assayed as described in Materials and Methods. Reaction mixtures containing KMB plus 300 μM hypoxanthine (HX) plus 18 mUnits xanthine/mL (X) in the presence of 50 μM FeIII-citrate were incubated for 30 min. The final concentration of DF and PBH was 500 μM. Data represent mean ± SE of three experiments performed in triplicate.

* $P < 0.001$ compared to control.

well as its effect on paraquat uptake by these cells. Because DF, by its structural similarities with polyamines probably blocks the accumulation system, uptake of paraquat in the presence of DF is likely to be an effect of diffusion only. Using this hypothesis we calculated that amount of paraquat that approximates to the uptake of paraquat by the accumulation system (assuming nmoles paraquat/cell + DF = diffusion only). It was found (not shown) that uptake of [^{3}H]adenine-labeled paraquat by ATTC obeys saturation kinetics and exhibits an apparent K_m value of 29 μM. Indeed, at low concentrations of paraquat (≤ 250 μM) both uptake and cell lysis were inhibited by deferoxamine. However, at a concentration of 500 μM the uptake of paraquat was not significantly reduced by DF whereas cell injury was almost (> 85%) completely inhibited. The accumulation of paraquat at this concentration in the presence of DF was as much as uptake at 250 μM paraquat in the absence of DF. From Fig. 2 it can be seen that the expected cytotoxicity at this amount of intracellular paraquat (200 nmol/2.5 × 10^5 ATTC) was 30%. Since the cytotoxic index was only approximately 5% it can be concluded that DF also protects against paraquat toxicity when paraquat has entered the cell. This conclusion is buttressed by the data in Table 1, demonstrating the effect of DF and putrescine on uptake and paraquat-mediated injury of ATTC. Previous studies have demonstrated that the energy-dependent process of paraquat accumulation in lung slices [15] and *in vivo* [3] can be blocked by the diamine putrescine. As shown here, putrescine at an equimolar basis as compared to DF is about 2–3 times more potent in blocking paraquat accumulation in ATTC. However, DF and putrescine are equipotent in reducing the cytotoxicity of paraquat.

Our results indicate that the protective effect of DF cannot be explained by its competition with paraquat on uptake alone. In support of the hypothesis that DF also protects via a direct effect on paraquat-mediated oxygen radical generation, we performed the same series of experiments with another iron chelator, PBH. Both DF and PBH were effective in decreasing the generation of ·OH by chelation of iron as measured indirectly by gas chromatography. Indeed, from Fig. 3 it can be seen that PBH inhibits injury of ATTC similarly to DF whereas it does not decrease the uptake of paraquat

in the lung cells. On the contrary, in the presence of PBH the concentration of intracellular paraquat significantly increased, mounting to 200 nmol/2.5×10^5 ATTC after 3 hr of incubation. Using Fig. 2, it can be seen that the expected cytotoxic index at this concentration should have been about 30%. However, it is possible that this discrepancy is a result of protection against cytotoxicity enabling the cells to accumulate paraquat.

Our tactic of using two compounds vs DF: (1) putrescine a molecule that specifically blocks paraquat uptake by ATTC [15, 32] but has no chelating properties, and (2) PBH which inhibits iron catalysed OH generation but does not compete with paraquat on uptake by ATTC, provides strong support for our conclusion that DF protects against paraquat toxicity by two separate mechanisms, namely: inhibition of paraquat uptake and a direct inhibitory effect on paraquat-mediated oxygen radical generation. With regard to the latter possibility it should be noted that DF must exert its antioxidant activity inside ATTC. We have no proof that DF can enter ATTC but it is possible that this occurs via the polyamine uptake system of these cells which is suggested by the fact that DF apparently blocks paraquat uptake via this system. Another argument for the possibility of an intracellular effect of DF is the observation that preincubation of endothelial cells with DF protects against neutrophil cytotoxicity [33], suggesting that DF chelates intracellular iron that would otherwise have been used for ·OH generation. We have similar observations with endothelial cells that were pretreated (30 min) with PBH (data not shown).

That an iron catalysed system is involved in paraquat toxicity is supported by our previous observation that DF inhibited paraquat-induced ·OH generation by *Escherichia coli* [21]. Addition of ferrous sulphate led to an enhancement of the killing of this microorganism by paraquat, whereas the administration of DF yielded a significant reduction of the bactericidal effect [34]. These data agree with the observation that paraquat by interaction with NADPH-cytochrome P450 reductase and several ferric complexes generates toxic oxygen radicals [35]. Furthermore, mice poisoned with paraquat survived better when they were treated with repeated injections of DF [36]. In contrast, iron loading of the animals prior to poisoning markedly reduced their lifespan. These data also point to iron-catalysed OH-mediated paraquat toxicity for lung tissue and are supported by the recent finding that ATTC are protected against paraquat by SOD and catalase [30].

In summary, our data indicate that DF protects lung epithelial type II cells against paraquat by two mechanisms, i.e. uptake blockade and chelation of iron which is corroborated by means of an iron chelator not interfering with paraquat uptake. It should be emphasized that our conclusions have been arrived at using *in vitro* studies. Whether the presented data can be extrapolated to the *in vivo* situation in man remains to be established by clinical trials.

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