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Protection against Hydrogen Peroxide-Mediated Cytotoxicity in Friedreich's Ataxia Fibroblasts Using Novel Iron Chelators of the 2-Pyridylcarboxaldehyde Isonicotinoyl Hydrazone Class

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ABSTRACT

Iron-loading diseases remain an important problem because of the toxicity of iron-catalyzed redox reactions. Iron loading occurs in the mitochondria of Friedreich's ataxia (FA) patients and may play a role in its pathogenesis. This suggests that iron chelation therapy could be useful. We developed previously the lipophilic iron chelators known as the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) ligands and identified 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH) as the most promising analog. Hence, this study assessed the efficacy of PCTH and other PCIH analogs compared with various chelators, including deferiprone and desferrioxamine (DFO). Age- and sex-matched control and FA fibroblasts were preincubated with iron chelators and subsequently challenged with 50 μM H_2O_2 for up to 24 h. The current study demonstrates an interesting structure-activity relationship among the closely related PCIH series of ligands, with only PCTH being highly effective at preventing H_2O_2-induced cytotoxicity. PCTH increased FA fibroblast cell viability by up to 70%, whereas DFO rescued viability by 1 to 5% only. Hence, PCTH, which was well tolerated by cells was far more effective than DFO at preventing oxidative stress. It is noteworthy that kinetic studies demonstrated PCTH to rapidly penetrate cells to induce ^{59}Fe efflux, whereas DFO, PCIH, 2-pyridylcarboxaldehyde benzoyl hydrazone, and 2-pyridylcarboxaldehyde *m*-bromobenzoyl hydrazone were far slower, indicating it is the rate of chelator permeation that is crucial for protection against H_2O_2. In addition, PCTH was found to be as effective as or more effective than conventional radical scavengers or the antioxidant idebenone (which has undergone clinical trials) at protecting cells against H_2O_2-mediated cytotoxicity. These findings further indicate the potential of PCTH for treatment of iron overload.

Development of novel orally effective iron chelators is vital for the treatment of iron-loading diseases, including β-thalassemia major (Kalinowski and Richardson, 2005). This remains an important research area because the clinically used chelator, desferrioxamine (DFO; Fig. 1), suffers several problems that are due, in part, to its hydrophilicity (Kalinowski and Richardson, 2005). This leads to poor absorption from the gut and poor penetration of cell membranes that prevents access to intracellular iron pools. Because of its short half-life, DFO requires subcutaneous administration for 12 to 24 h/day, five to six times/week to achieve a negative iron balance (Hershko et al., 2003). Together, these disadvantages lead to poor patient compliance (Kalinowski and Richardson, 2005).

Over the past 25 years, development of orally effective iron chelators has been ongoing (Kalinowski and Richardson, 2005). The most successful of these agents, deferiprone (L1; Fig. 1), is a small-molecular-weight chelator that is available in Europe for iron overload treatment (Kalinowski and Richardson, 2005). However, its safety remains controversial because of conflicting studies reporting liver fibrosis (Richardson, 2001). Novartis announced the development of the triazole ICL670 (deferasirox; Fig. 1), which gained U.S. Food and Drug Administration approval as an orally active iron chelator (Kalinowski and Richardson, 2005). However, the iron chelation efficacy and safety of ICL670 remains unclear. Hence, the development of new, efficient, and safe orally active iron chelators remains an important aim.

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ABBREVIATIONS: DFO, desferrioxamine; L1, deferiprone; ICL670A deferasirox; PCIH, 2-pyridylcarboxaldehyde isonicotinoyl hydrazone; PIH, pyridoxal isonicotinoyl hydrazone; Tf, transferrin; PCBH, 2-pyridylcarboxaldehyde benzoyl hydrazone; PCTH, 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone; PCBBH, 2-pyridylcarboxaldehyde *m*-bromobenzoyl hydrazone; FA, Friedreich's ataxia; BPS, bathophenanthroline disulfonate; Cat, catalase; DTPA, diethylenetriaminepentaacetic acid; DP, dipyridyl; MnTBAP, manganese(III) *meso*-tetrakis(4-benzoic acid)-porphyrin; SOD, superoxide dismutase; PCAH, 2-pyridylcarboxaldehyde *p*-aminobenzoyl hydrazone; PCHH, 2-pyridylcarboxaldehyde *p*-hydroxybenzoyl hydrazone.

We synthesized new aroylhydrazone iron chelators known as the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH; Fig. 1) analogs (Becker and Richardson, 1999). These chelators are tridentate (Bernhardt et al., 2007) and were derived from the highly effective pyridoxal isonicotinoyl hydrazone (PIH; Fig. 1) ligand (Ponka et al., 1979). The latter compound showed marked activity in vitro, in vivo, and in a clinical trial (Richardson and Ponka, 1998). However, the unfortunate failure to patent PIH led to a lack of commercial interest and the necessity to develop the PCIH series of ligands that maintain its optimal characteristics. Some of these chelators demonstrated high iron mobilization efficacy and effectively prevented iron uptake after it has been released from the iron transport protein, transferrin (Tf), in vitro (Becker and Richardson, 1999). Of the PCIH class, 2-pyridylcarboxaldehyde benzoyl hydrazone (PCBH; Fig. 1), 2-pyridylcarboxaldehyde *m*-bromobenzoyl hydrazone (PCBBH; Fig. 1), and 2-pyridylcarboxaldehyde thiophenecarboxyl hydrazone (PCTH; Fig. 1) were most effective at mobilizing intracellular iron and preventing iron uptake (Becker and Richardson, 1999).

For a ligand to be ideal for the treatment of iron overload disease, it must not exhibit marked redox activity or antiproliferative effects. Studies with the PCIH analogs examined this and demonstrated that these ligands did not potentiate ascorbate oxidation or benzoate hydroxylation, and they did not damage DNA in intact mammalian cells (Chaston and Richardson, 2003). Furthermore, these chelators had little antiproliferative activity (IC_{50} = 40–50 μM) in vitro against

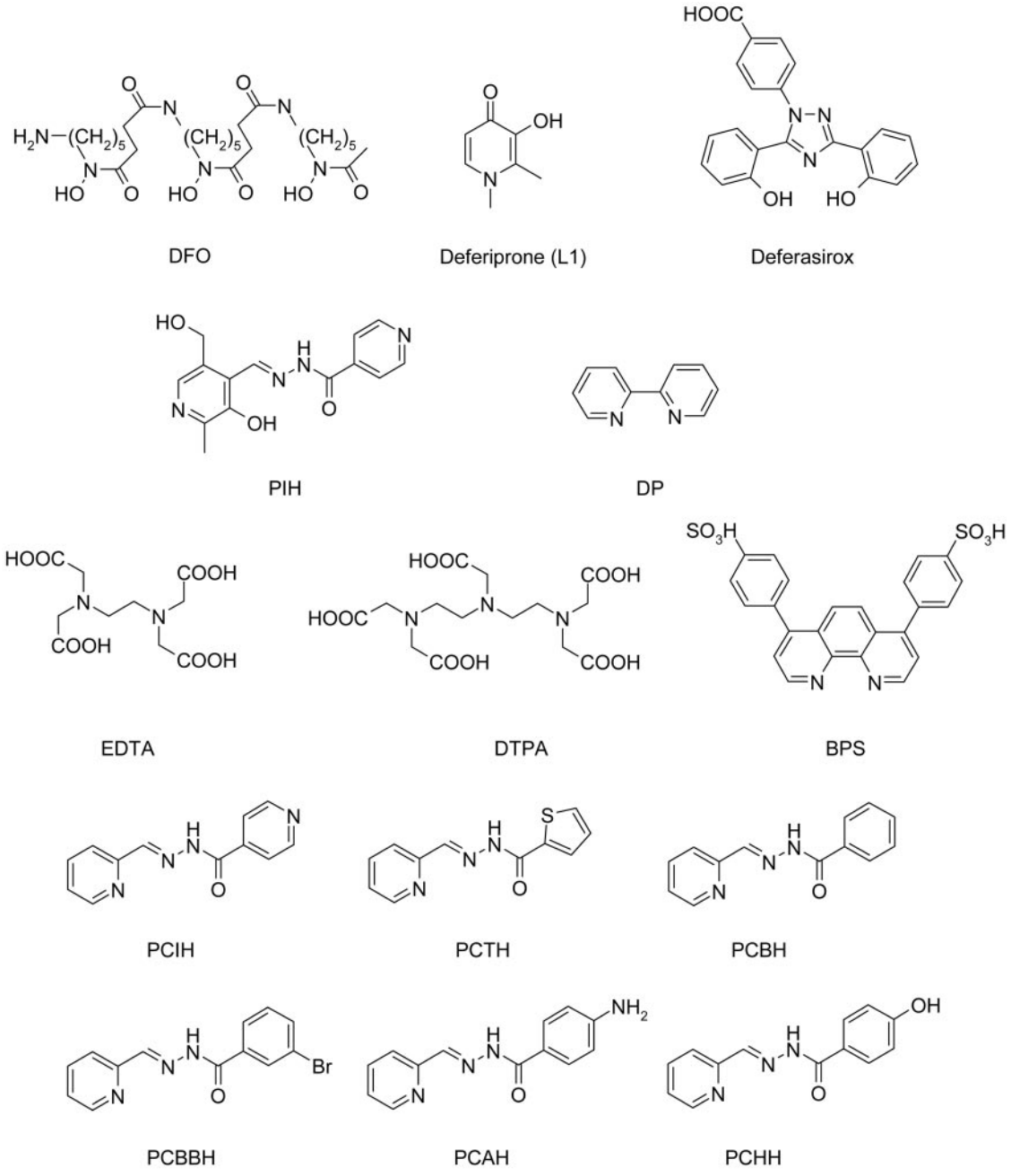

Fig. 1. Chemical structures of iron chelators described in this study: DFO, L1, ICL670A, PIH, DP, EDTA, DTPA, BPS, PCIH, PCTH, PCBH, PCBBH, PCAH, and PCHH.

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the SK-N-MC neuroepithelioma cell line, with an effect similar to or less than DFO (Becker and Richardson, 1999). Another in vitro study using a model of mitochondrial iron overload illustrated that several PCIH analogs were highly effective at increasing iron release from the iron-loaded mitochondrion, which could be an important factor in the treatment of the neurodegenerative and cardiodegenerative disease, Friedreich's ataxia (FA) (Richardson et al., 2001).

Considering its appropriate properties, an in vivo mouse trial investigated the iron chelation efficacy and tolerability of PCTH. This study demonstrated the ligand was well tolerated in mice at 100 mg/kg twice per day and could induce substantial iron excretion when administered orally (Wong et al., 2004). In fact, the efficacy of PCTH was comparable with L1 or PIH at the same dose (Wong et al., 2004). Taken together, these data suggested the PCIH series and particularly PCTH demonstrated properties suitable for treatment of iron overload disease.

In this study, we investigated the activity of the PCIH class of chelators at inhibiting H_2O_2-induced cytotoxicity in fibroblasts from patients with FA compared with sex- and age-matched control subjects. The study demonstrated an interesting structure-activity relationship among the structurally related PCIH series of chelators, with only PCTH being highly effective at preventing H_2O_2-induced cytotoxicity in fibroblasts from either control subjects or patients with FA. It is noteworthy that PCTH was found to rapidly penetrate cells to effectively induce ^{59}Fe efflux, whereas DFO, PCIH, PCBH, and PCBBH were significantly slower. This indicates it is the rate of permeation of the chelator that is a vital factor for protection against H_2O_2-mediated cytotoxicity. In addition, PCTH was as or more effective than conventional radical scavengers or the antioxidant idebenone at protecting fibroblasts against H_2O_2-mediated cytotoxicity. These results confirm the potential of PCTH as an agent for the treatment of iron-loading diseases.

Materials and Methods

Reagents. All commercial reagents were used without further purification. DFO was purchased from Novartis (Basel, Switzerland). The PCIH analogs and PIH were prepared using standard procedures (Bernhardt et al., 2007). Deferiprone was a gift from Prof. Roger Dean (Heart Research Institute, Sydney, Australia). Idebenone was obtained from Smart Nutrition (San Diego, CA). Bathophenanthroline disulfonate (BPS), catalase (Cat), diethylenetriaminepentaacetic acid (DTPA), EDTA, dipyridyl (DP), manganese(III) *meso*-tetrakis(4-benzoic acid)porphyrin (MnTBAP), and superoxide dismutase (SOD) were from Sigma-Aldrich (St. Louis, MO).

Cell Culture. Human FA fibroblasts and lymphoblasts and normal age- and sex-matched control fibroblasts and lymphoblasts were obtained from Corielle (Camden, NJ). Human SK-N-MC neuroepithelioma cells were purchased from the American Type Culture Collection (Manassas, VA). The cells were grown as described previously (Richardson and Baker, 1992; Richardson et al., 1995).

Effect of H_2O_2 in the Presence and Absence of Chelators on Fibroblast Viability. Fibroblasts were subcultured from culture flasks to 96-well plates and allowed to grow for 24 h at 37°C. The growth medium was then removed, and the cells were preincubated with control medium in the absence of fetal calf serum or this medium containing the agent(s) to be tested (i.e., chelators, antioxidants, or both) for 30 min or 12 h at 37°C. Medium alone or medium containing 50 μM H_2O_2 was then added to each of these conditions, and the incubation was continued for 1 min, 2 h, 12 h, or 24 h at 37°C. This H_2O_2 concentration was chosen after preliminary experiments in fibroblasts assessing its cytotoxicity. Viability was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium assay, as described previously (Richardson et al., 1995). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium color formation was directly proportional to the number of viable cells measured by direct cell counts using trypan blue staining (Richardson et al., 1995).

Preparation of ^{59}Fe-Transferrin. Human Tf (Sigma-Aldrich) was labeled with ^{59}Fe (PerkinElmer Life and Analytical Sciences, Boston, MA) to produce $^{59}Fe_2$-Tf (^{59}Fe-Tf), as described previously (Richardson and Baker, 1990; Richardson and Baker, 1992). In brief, apo-Tf was labeled with iron using the ferric-nitrilotriacetate complex at a ratio of 1 iron to 10 nitrilotriacetic acid. This complex was prepared in 0.1 M HCl, and then this solution adjusted to pH 7.4 using 1.4% $NaHCO_3$. This solution was added to apo-Tf and then incubated for 1 h at 37°C. Unbound iron was removed by exhaustive vacuum dialysis against 0.15 M NaCl adjusted to pH 7.4 using 1.4% $NaHCO_3$. The saturation of Tf with iron was monitored by UV-Vis spectrophotometry with the absorbance at 280 nm (protein) being compared with that at 465 nm (iron-binding site). In all studies, fully saturated diferric Tf was used.

^{59}Fe Efflux Assay from Fibroblasts. Iron efflux experiments examining the ability of various chelators to mobilize ^{59}Fe from sex- and age-matched control and FA fibroblasts were performed using established techniques (Baker et al., 1992; Richardson et al., 1995). In brief, to enable comparison with studies assessing the effects of H_2O_2 and chelators on viability, an analogous incubation protocol was followed. Fibroblasts were initially prelabeled with 0.75 μM ^{59}Fe-Tf for 30 h at 37°C and then washed four times with ice-cold phosphate-buffered saline. The cells were then incubated with either control medium or medium containing the chelator (50 μM) for 12 h at 37°C. After this, 50 μM H_2O_2 was then added to the medium in the presence and absence of the chelator for 24 h at 37°C. Subsequently, the overlying supernatant containing released ^{59}Fe was then separated from the cells using a Pasteur pipette and placed in a gamma-counting tube. The cells were removed from the plate in 1 ml of phosphate-buffered saline using a plastic spatula and added to a gamma-counting tube. Radioactivity was measured in both the cell pellet and supernatant using a gamma-scintillation counter (Wallac WIZARD 3; PerkinElmer Life and Analytical Sciences, Waltham, MA). In these studies, the novel ligands were compared with the well characterized chelators DFO and PIH (positive control subjects).

Statistical Analysis. Data were compared using the Student's t test. Results were considered statistically significant when $p < 0.05$.

Results

PCTH Rescues H_2O_2-Mediated Cytotoxicity in Fibroblasts from Control and Friedreich's Ataxia Patients. Previous studies demonstrated that the orally effective iron chelator PCTH has high iron chelation efficacy in vitro (Becker and Richardson, 1999; Richardson et al., 2001) and in vivo (Wong et al., 2004) and possessed properties suitable for the treatment of iron-loading diseases (Kalinowski and Richardson, 2005). FA is a condition in which iron loading occurs in the mitochondrion, and this could potentially play some role in the pathogenesis of this disease (Boddaert et al., 2007). Moreover, fibroblasts from patients with FA compared with control subjects were shown to be more sensitive to redox stress (Wong et al., 2000; Sturm et al., 2005). Considering this, the current study was designed to assess the ability of PCTH and other chelators to prevent H_2O_2-mediated toxicity in age- and sex-matched fibroblasts from control subjects and patients with FA.

Initial studies examined the susceptibility of control or FA

fibroblasts to a 1-min to 24-h incubation with 50 μM H_2O_2 after preincubation for 30 min (Fig. 2, A and B) or 12 h (Fig. 2, C and D) at 37°C with control medium or a variety of iron chelators, including DFO, PIH, or PCTH (50 μM). In this case, DFO and PIH were used as relative controls to judge the importance of membrane permeability. DFO is relatively hydrophilic and shows limited permeability and low iron chelation efficacy (Richardson and Ponka, 1994; Richardson et al., 1994), whereas PIH is considerably more lipophilic, permeating cells rapidly and having high activity at binding intracellular iron (Ponka et al., 1979; Richardson and Baker, 1991). Preincubation of control or FA fibroblasts with control medium for 30 min followed by the addition of H_2O_2 for up to 24 h led to a marked decrease in viability to less than 5% of the control after a 24-h incubation (Fig. 2, A and B). As shown by others (Sturm et al., 2005), fibroblasts from patients with FA were more sensitive to effects of H_2O_2 than control fibroblasts, the decrease in viability being more rapid in the former (Fig. 2, A and B). For example, after a 30-min preincubation with control medium followed by a subsequent 2-h reincubation with H_2O_2, viability of control and FA fibroblasts was equal to 49 ± 3% (n = 3) and 29 ± 3% (n = 3), respectively (Fig. 2, A and B).

Irrespective of the preincubation period, cells incubated with DFO, PIH, or PCTH alone in the absence of H_2O_2 had no effect on the viability of the fibroblasts relative to control medium (Fig. 2). This clearly demonstrates the low antiproliferative activity of these chelators, which confirms our earlier work (Becker and Richardson, 1999). Examining cells preincubated with DFO or PIH for 30 min or 12 h, the addition of H_2O_2 led to a marked decrease in the viability of fibroblasts from control subjects or patients with FA. Although both chelators were not markedly effective at preventing H_2O_2-mediated cytotoxicity, PIH was significantly ($p < 0.05$) more active than DFO at inhibiting the H_2O_2-mediated reduction of viability after a 12-h preincubation (Fig. 2, C and D). This may be related to the greater lipophilicity and membrane permeability of PIH relative to DFO (Richardson and Baker, 1991).

When cells were preincubated with PCTH for 30 min (Fig. 2, A and B) or 12 h (Fig. 2, C and D), the subsequent cytotoxic effects of H_2O_2 were markedly and significantly ($p < 0.0025$) less pronounced than when cells were exposed to H_2O_2 alone or when cells were preincubated with DFO or PIH. The preincubation of cells with PCTH for 12 h (Fig. 2, C and D) was significantly ($p < 0.0025$) more effective than that for 30 min (Fig. 2, A and B) at preventing the decrease in viability of fibroblasts from both control subjects and patients with FA. Considering this, a 12-h preincubation was used in all other experiments examining the effects of chelators on H_2O_2-mediated cytotoxicity. Although PCTH was effective at rescuing the cytotoxicity imparted by H_2O_2, the protective effect was less marked in fibroblasts from patients with FA than control subjects. For example, after a 12-h preincubation of control or FA fibroblasts with PCTH, the viability after a 24-h incubation with H_2O_2 was equal to 86 ± 5% (n = 3) and 71 ± 1% (n = 3), respectively (Fig. 2, C and D).

To determine whether the effect of PCTH at rescuing H_2O_2-mediated cytotoxicity was not just a property of fibroblasts, similar experiments were repeated with age- and sex-matched lymphoblasts from control subjects and patients with FA after a preincubation of 12 h with the chelators (Fig. 3, A and B). In these studies, similar results were obtained to those shown with fibroblasts (Fig. 2, C and D), with PCTH being significantly more effective than DFO and PIH at preventing H_2O_2-mediated toxicity in lymphoblasts (Fig. 3, A and B). Again, the chelators were less effective at preventing

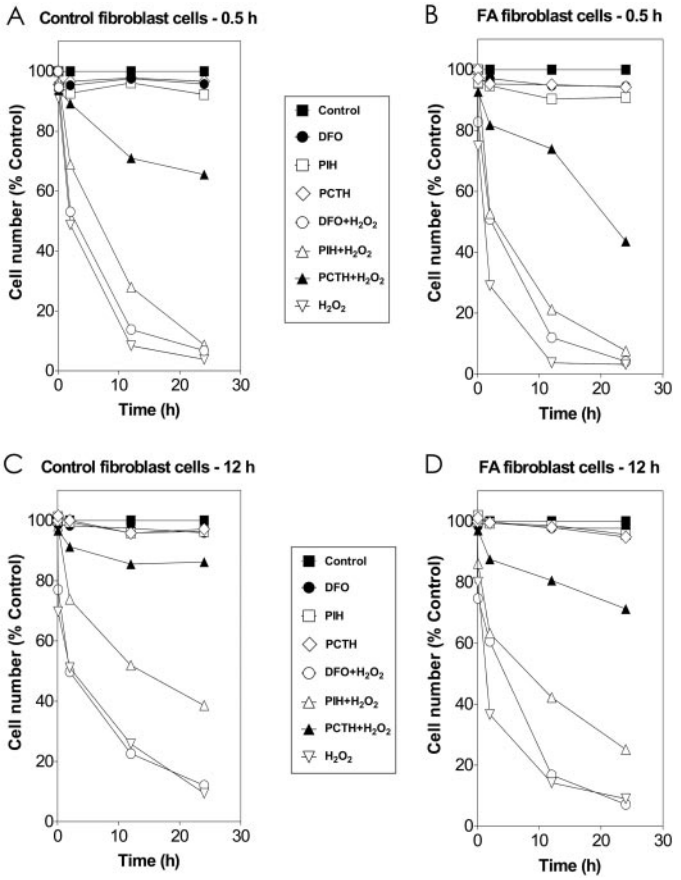

Fig. 2. PCTH, but not DFO or PIH, effectively rescues control and FA fibroblasts from H_2O_2-mediated cytotoxicity. Fibroblasts were preincubated with control medium or medium containing the chelator (50 μM) for 0.5 or 12 h at 37°C. Then, 50 μM H_2O_2 or media alone were added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

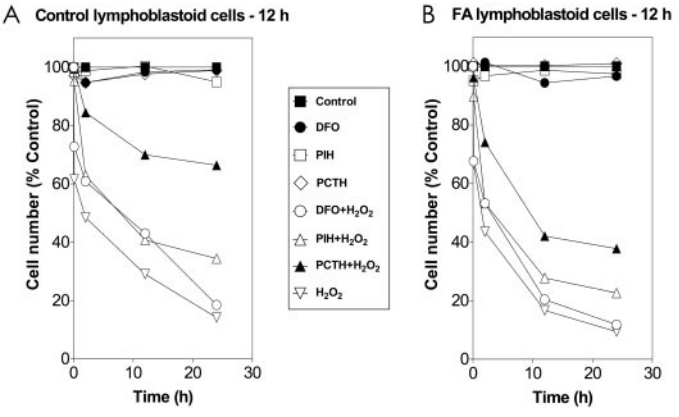

Fig. 3. PCTH, but not DFO or PIH, effectively rescues control and FA lymphoblasts from H_2O_2-mediated cytotoxicity. Control and FA lymphoblasts were preincubated with control medium or medium containing the chelator (50 μM) for 12 h at 37°C. H_2O_2 (50 μM) or media alone were then added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

the decrease in viability of lymphoblasts from FA than control patients (Fig. 3, A and B). PIH or PCTH added in the absence of H_2O_2 showed no significant effect on the viability of control FA lymphoblasts. PCTH also showed similar efficacy at preventing the detrimental effects of H_2O_2 on the viability of human SK-N-MC neuroepithelioma cells as that found in fibroblasts and lymphoblastoid cells from control subjects and patients with FA (data not shown).

In summary, these experiments demonstrated that PCTH was markedly more effective than either DFO or PIH at preventing H_2O_2-mediated cytotoxicity of fibroblasts and lymphoblasts from control subjects and patients with FA.

PCTH, but not Other Closely Related PCIH Analogs, Effectively Rescues H_2O_2-Mediated Cytotoxicity. To further investigate the effects of iron chelators on H_2O_2-mediated cytotoxicity, we examined the effect of five other chelators of the PCIH class that show high structural similarity to PCTH (Fig. 1). Examining control fibroblasts, PCIH and PCBH showed limited activity at rescuing H_2O_2-mediated cytotoxicity, having efficacy that was not significantly ($p > 0.05$) different from PIH and significantly ($p < 0.001$) less marked than PCTH (Fig. 4A). In agreement with the results in Fig. 2, the ability of the chelators to rescue H_2O_2-mediated toxicity of FA fibroblasts was less pronounced, with only PCTH leading to a marked rescue (Fig. 4B). The other PCIH analogs, namely, PCBBH, pyridylcarboxaldehyde *p*-aminobenzoyl hydrazone (PCAH), and 2-pyridylcarboxaldehyde *p*-hydroxybenzoyl hydrazone (PCHH) (Fig. 1), demonstrated no effective activity at preventing H_2O_2-mediated cytotoxicity in either control or FA fibroblasts, again being significantly ($p < 0.001$) less effective than PCTH (Fig. 4, C and D). None of the PCIH analogs when added in the absence of H_2O_2 showed any effect on the cellular viability of fibroblasts from control subjects or patients with FA (data not shown), in good agreement with previous studies using tumor cells (Becker and Richardson, 1999).

The Permeable Chelators L1 and Dipyridyl Show High Activity at Preventing H_2O_2-Mediated Cytotoxicity, whereas Poorly Permeable Chelators Show Much Less Effect. To understand the significance of chelator permeability in preventing H_2O_2-mediated toxicity in fibroblasts, the effect of PCTH was compared with two permeable chelators, namely, DP (Fig. 1) and L1; two chelators that are known to be impermeable, namely, DTPA and EDTA (Fig. 1); and a partially permeable ligand, BPS (Richardson and Baker, 1994; Richardson et al., 1994; Kicic et al., 2001) (Fig. 1).

Both L1 and DP showed activity that was slightly greater than PCTH in both control and FA fibroblasts (Fig. 5, A and B). For example, control fibroblast cell viability was equal to 92, 83, and 78% of the control in the presence of H_2O_2 and either L1, DP, or PCTH, respectively after 24 h, whereas H_2O_2 alone reduced viability to 12% of the control (Fig. 5A). Both DTPA and EDTA showed significantly ($p < 0.02$) less activity than all of the above-mentioned chelators in both control and FA fibroblasts (Fig. 5, A and B). For example, in control fibroblasts after a 24-h incubation, cellular viability was equal to 25 and 31% of the control in the presence of H_2O_2 and EDTA or DTPA, respectively (Fig. 5A). It is noteworthy that despite the latter two compounds being largely impermeable ligands (Richardson and Baker, 1994; Kicic et al., 2001), some rescuing effect against H_2O_2-induced toxicity was observed, suggesting that extracellular iron chelation plays some role in preventing cytotoxicity (Fig. 5). In accordance with its intermediate permeability, in control fibro-

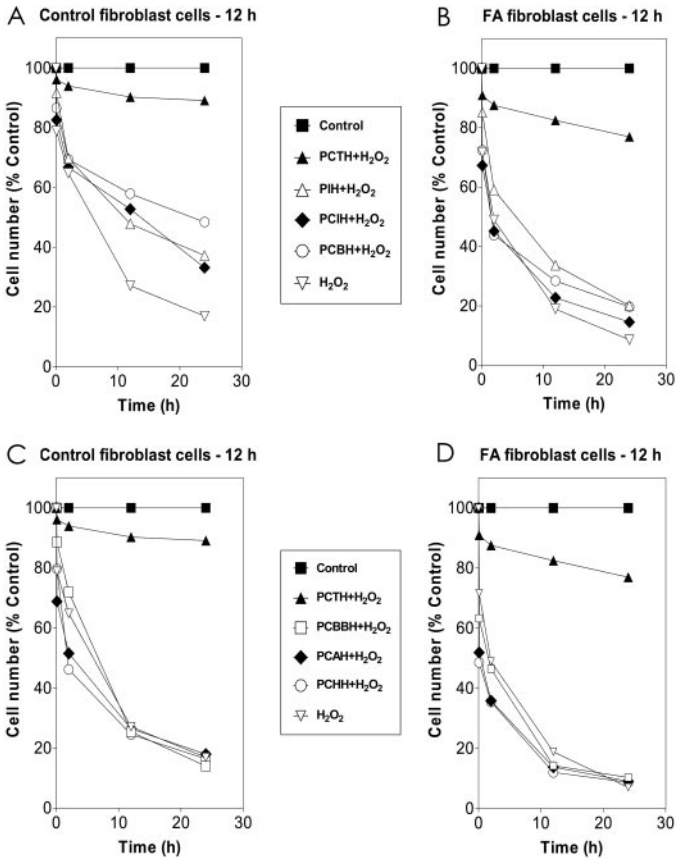

Fig. 4. PCTH, but not its closely related analogs PCIH, PCBH, PCBBH, PCAH, or PCHH, effectively rescues control and FA fibroblasts from H_2O_2-mediated cytotoxicity. Fibroblasts were preincubated with control medium or medium containing the chelator (50 μM) for 12 h at 37°C. H_2O_2 (50 μM) or media alone were then added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

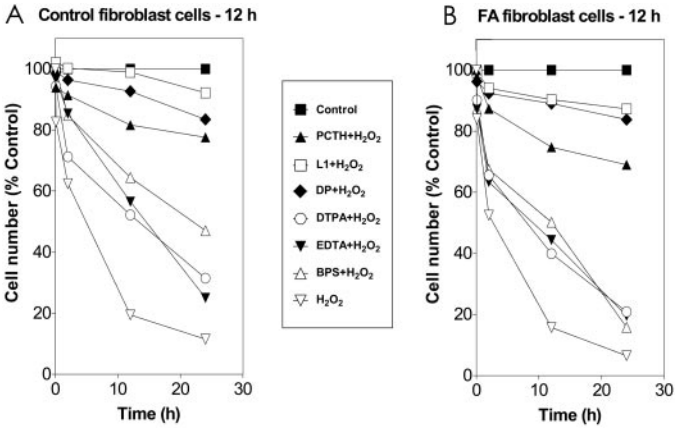

Fig. 5. PCTH, L1, and dipyridyl, but not BPS, DTPA, or EDTA, effectively rescues control and FA fibroblasts from H_2O_2-mediated cytotoxicity. Fibroblasts were preincubated with control medium or medium containing the chelator (50 μM) for 12 h at 37°C. H_2O_2 (50 μM) or media alone were then added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

blasts, BPS was slightly more effective than DTPA and EDTA at preventing the decrease in viability in the presence of H_2O_2 (Fig. 5A). However, in FA fibroblasts, BPS showed similar activity to DTPA and EDTA, there being little rescue against H_2O_2-induced cytotoxicity (Fig. 5B).

In summary, membrane permeability of chelators played an important role in effective rescue against the H_2O_2-induced decrease in cellular viability of control and FA fibroblasts.

Iron Chelation Efficacy of the Ligands in Control and FA Fibroblasts. To interpret the results mentioned above examining the ability of the various ligands to prevent the H_2O_2-induced cytotoxicity of control and FA fibroblasts, we characterized the ability of the chelators to increase ^{59}Fe mobilization from cells. For relevant comparison, the incubation conditions were analogous to those used in the studies examining the effect of chelators and H_2O_2 on fibroblast viability (Figs. 2–5).

Iron efflux experiments (Fig. 6A) were performed by incubating control and FA fibroblasts with the physiological iron transport protein Tf labeled with ^{59}Fe (0.75 μM ^{59}Fe-Tf). Cells were prelabeled with ^{59}Fe-Tf for 30 h at 37°C and then washed and reincubated with either control medium or medium containing the chelator (50 μM) for 12 h at 37°C. After this, 50 μM H_2O_2 or media alone (control) was then added in the presence and absence of the chelator for 24 h at 37°C. Subsequently, the overlying media containing released ^{59}Fe was separated from the cells. In all experiments, the addition of H_2O_2 did not have any effect on the ability of the chelator to induce cellular ^{59}Fe release compared with chelator alone (Fig. 6A). Thus, subsequent experiments examined the ability of control medium or chelators (50 μM) to mobilize ^{59}Fe from the prelabeled control or FA fibroblasts without incubation with H_2O_2 (Fig. 6B).

Normal or FA fibroblasts control medium resulted in the release of 5 to 6% of cellular ^{59}Fe (Fig. 6B). For all the chelators examined, there was little difference in cellular ^{59}Fe efflux between control and FA fibroblasts. It is noteworthy that despite the limited effect of DFO at preventing H_2O_2-induced cytotoxicity in both control and FA fibroblasts, this chelator showed considerable activity at mobilizing cellular ^{59}Fe. Indeed, DFO led to the efflux of 45 ± 1 and 39 ± 1% (n = 3 determinations) of cellular ^{59}Fe from control and FA fibroblasts, respectively (Fig. 6B). Moreover, the efficacy of DFO was greater than that of PIH, which released 24 ± 4 and 28 ± 1% of cellular ^{59}Fe in control and FA fibroblasts (Fig. 6B). These results did not seem to correlate to the ability of the chelators to prevent H_2O_2-induced cytotoxicity, whereas PIH showed significantly ($p < 0.05$) higher efficacy than DFO at preventing the reduction in viability (Fig. 2, C and D).

Studies using the PCIH analogs demonstrated that PCTH, PCBBH, and PCBH showed similar and high activity at mobilizing cellular ^{59}Fe in control and FA fibroblasts, resulting in the release of 33 to 46% of intracellular ^{59}Fe (Fig. 6B). Thus, the chelator PCTH, which showed greatest efficacy of the PCIH analogs at preventing H_2O_2-induced cytotoxicity (Fig. 4), was also very effective at mobilizing cellular ^{59}Fe from both control and FA fibroblasts (Fig. 6B). However, it is of interest that although PCBH and PCBBH showed high iron chelation efficacy that was similar to PCTH (Fig. 6B), these ligands did not markedly rescue H_2O_2-induced cytotoxicity (Fig. 4, C and D). At present, we do not understand the reason for this discrepancy in the relationship between iron chelation efficacy and prevention of H_2O_2-induced cytotoxicity. However, we cannot rule out potential cytotoxic effects that occur between the iron complexes of PCBH and PCBBH and H_2O_2.

As shown in previous studies in neoplastic cells (Becker and Richardson, 1999), the relatively hydrophilic PCIH analogs PCHH and PCAH (Bernhardt et al., 2007) were ineffective at mobilizing ^{59}Fe, with there being no significant ($p >$ 0.05) difference compared with the control medium alone

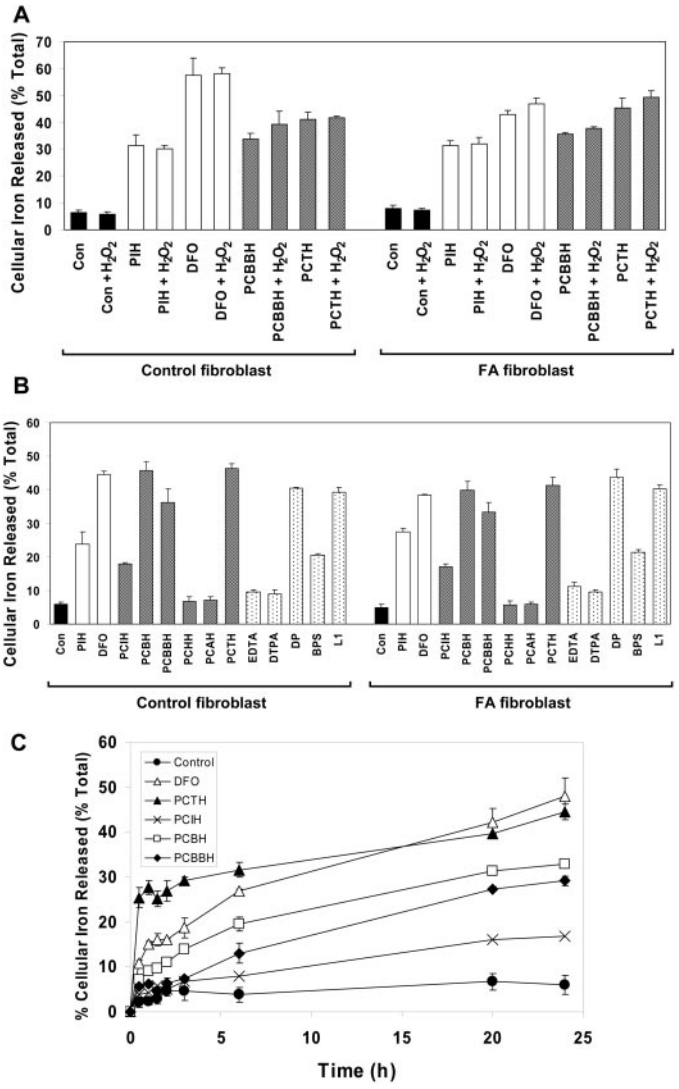

Fig. 6. Effect of chelators at mediating ^{59}Fe mobilization from prelabeled control and FA fibroblasts. A, effect of PIH, DFO, PCBBH, and PCTH in the presence and absence of H_2O_2. Fibroblasts were prelabeled with 0.75 μM ^{59}Fe-Tf for 30 h at 37°C and washed. The cells were then incubated with either control medium or medium containing the chelator (50 μM) for 12 h at 37°C. H_2O_2 (50 μM) or media alone were then added to each of these conditions and incubated for 24 h at 37°C. B, effect of DFO, PIH, and the PCIH analogs on cellular ^{59}Fe mobilization. Fibroblasts were prelabeled with 0.75 μM ^{59}Fe-Tf for 30 h at 37°C and washed. The cells were then incubated with either control medium or medium containing the chelator (50 μM) for 36 h at 37°C. C, effect of control medium, PCTH, DFO, PCIH, PCBH, and PCBBH on ^{59}Fe mobilization from fibroblasts as a function of time. Fibroblasts were prelabeled with 0.75 μM ^{59}Fe-Tf for 30 h at 37°C, washed, and then reincubated with control medium, PCTH, DFO, PCIH, PCBH, or PCBBH at 50 μM for up to 24 h at 37°C. The results are a typical experiment from three performed.

(Fig. 6B). These results indicate that both these ligands are inefficient at permeating fibroblasts and releasing ^{59}Fe, probably because of their higher hydrophilicity compared with other PCIH analogs (Bernhardt et al., 2007). This low iron chelation efficacy could explain their lack of activity at preventing the cytotoxic effects of H_2O_2 (Fig. 4, C and D).

Studies then examined the effect of EDTA, DTPA, DP, BPS, and L1 at mobilizing ^{59}Fe from control and FA fibroblasts (Fig. 6B) to assess their iron chelation efficacy relative to their ability to prevent H_2O_2-induced cytotoxicity (Fig. 5). Control and FA fibroblasts were prelabeled with 0.75 μM ^{59}Fe-Tf for 30 h at 37°C, the cells were washed and then reincubated with either control medium or medium containing the chelator (50 μM) for 12 h at 37°C. After this, 50 μM H_2O_2 or media alone (control) was added in the presence and absence of the chelator for 24 h at 37°C. Again, H_2O_2 had no effect on cellular ^{59}Fe mobilization (data not shown) and the results presented are in the absence of this agent (Fig. 6B).

As expected, the membrane-impermeable chelators EDTA and DTPA showed limited ability to mobilize ^{59}Fe in control and FA fibroblasts, having similar efficacy to that of the control (Fig. 6B). This correlated to their poor ability to rescue both control and FA fibroblasts against the toxic effects of H_2O_2 (Fig. 5) and again indicates that intracellular iron chelation is important for preventing H_2O_2 cytotoxicity. The partially membrane-permeable ligand BPS showed moderate ^{59}Fe-mobilizing activity and was able to release 20 ± 1 and 21 ± 1% of intracellular ^{59}Fe from control and FA fibroblasts, respectively (Fig. 6B). This was in agreement with its intermediate ability to rescue control fibroblasts from H_2O_2 cytotoxicity (Fig. 5A). Conversely, the membrane-permeable chelators DP and L1 showed high ^{59}Fe-mobilizing ability in control and FA fibroblasts, mediating the release of 39 to 44% of ^{59}Fe (Fig. 6B). Again, this result suggested that their high iron-mobilizing efficacy played an integral role in their ability to rescue control and FA fibroblasts from the toxic effects of H_2O_2 (Fig. 5).

Both DFO and PCTH markedly increased ^{59}Fe mobilization from fibroblasts (Fig. 6B), but under the same conditions, DFO did not rescue H_2O_2-induced cytotoxicity, whereas PCTH did (Fig. 2). This led to the hypothesis that it could be the rate at which these chelators penetrate cells that could be important in terms of their protective effect. To assess this, FA fibroblasts were prelabeled with 0.75 μM ^{59}Fe-Tf for 30 h at 37°C, washed and then reincubated for 30 min to 24 h at 37°C with control medium or the chelators PCTH, DFO, PCIH, PCBH, or PCBBH at 50 μM. It is noteworthy that PCTH rapidly penetrated cells to induce ^{59}Fe efflux, whereas DFO was significantly ($p < 0.05$) slower (Fig. 6C). The remaining chelators, PCBH, PCBBH, and PCIH, also demonstrated an initial slow rate of ^{59}Fe efflux. After 30 min, PCTH was rapidly able to mediate the release of 25 ± 2% of cellular ^{59}Fe, whereas DFO, PCBH, PCBBH, and PCIH were significantly ($p < 0.001$) less effective, leading to the efflux of 4 to 11% of cellular ^{59}Fe (Fig. 6C). Only after 6 h did PCTH and DFO start to show similar ^{59}Fe-mobilizing efficacy. In addition, PCIH, PCBBH, and PCBH showed reduced levels of cellular ^{59}Fe release compared with DFO and especially PCTH (Fig. 6C). These results suggest that the increased ability of PCTH in preventing H_2O_2-induced cytotoxicity compared with these other ligands could be explained by its rapid rate of penetrating cells to induce efficient ^{59}Fe mobilization from H_2O_2-sensitive pools. Hence, the ability of the ligand to permeate the cell membrane, and particularly the rate at which it does so, were crucial factors in preventing H_2O_2-induced cytotoxicity. This would be particularly the case when the preincubation time with PCTH is short (e.g., 2 h), relative to much longer preincubation periods (e.g., 12 h).

Combination of DFO and PCTH Increases the Efficacy of Rescue against H_2O_2-Induced Cytotoxicity in Control and FA Fibroblasts. Previous studies using several ligands, including L1 and PIH, have demonstrated that combination of a poorly permeable chelator (i.e., DFO) and a permeable ligand (i.e., L1 or PIH) can markedly increase iron chelation efficacy (Link et al., 2001, 2003). This is probably due to the shuttle principle, whereby a permeable chelator enters cells, binds iron, and then delivers it to a largely extracellular ligand (Link et al., 2003). This chelator outside the cell removes iron from the complex, allowing the permeable ligand to enter the cell to chelate more iron. Considering this, we assessed the combination of the highly permeable PCTH ligand with DFO. In these experiments the concentration of DFO was kept constant at 50 μM, whereas the concentration of PCTH was increased from 5 to 50 μM (Fig. 7, A

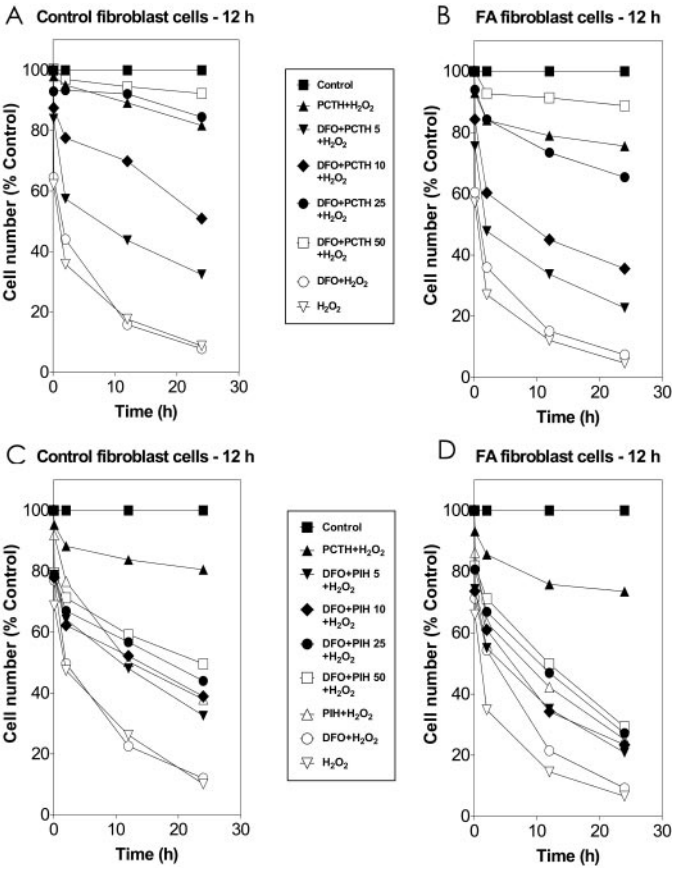

Fig. 7. Combination of DFO with PCTH (A and B) or DFO with PIH (C and D) increases the efficacy of PCTH alone, but not PIH alone, at rescuing control and FA fibroblasts from H_2O_2-mediated cytotoxicity. Control and FA fibroblasts were preincubated with control medium or medium containing 50 μM DFO in the presence of a range of concentrations of 5 to 50 μM PCTH in control (A) or FA fibroblasts (B); or 5 to 50 μM PIH in control (C) and FA fibroblasts (D) for 12 h at 37°C. Then, 50 μM H_2O_2 or media alone were added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

and B).

As shown previously (Fig. 2), DFO had no significant effect on rescuing cells from H_2O_2-induced toxicity. However, upon the addition of increasing concentrations of PCTH to DFO, cellular viability in the presence of H_2O_2 markedly increased (Fig. 7, A and B). In fact, the combination of the highest PCTH concentration (50 μM) with DFO resulted in greater ability to prevent H_2O_2-induced cytotoxicity in control (Fig. 7A) and FA fibroblasts (Fig. 7B) than 50 μM PCTH alone. However, only for FA fibroblasts was the activity of the combination of DFO with PCTH significantly ($p < 0.006$) more effective than PCTH alone (Fig. 7B). This may indicate a shuttle effect between PCTH and DFO that led to greater activity than PCTH alone. Further studies then examined whether the combination of increasing concentrations of PIH (5–50 μM) with 50 μM DFO also led to some improved ability of the former to prevent the H_2O_2-mediated decrease in fibroblast viability (Fig. 7, C and D). However, this combination did not lead to any significant alteration in cellular viability compared with PIH alone (Fig. 7, C and D). This difference in the protective effect of the combination of DFO with PIH or PCTH is intriguing. However, it could be because, in contrast to PCTH that binds Fe(II) alone, PIH is a Fe(III) chelator that may less readily donate its iron to DFO (Bernhardt et al., 2007). The pM (-log of the free metal concentration at physiological pH in the presence of a ligand) values that allow comparison of the relative ability of different ligands to bind a metal ion under comparable physiological conditions have been reported for PCTH and PIH (Bernhardt et al., 2007) and support this concept.

PCTH Is More Effective at Preventing the H_2O_2-Mediated Decrease in Viability of Fibroblasts than Free Radical Scavengers. The efficacy of PCTH at preventing the H_2O_2-induced decrease in viability of control and FA fibroblasts was compared with a variety of well known radical scavengers alone or in combination (Fig. 8, A and B). These scavengers were used at concentrations that have been shown to be effective in previous investigations (Konorev et al., 1999; Kwok and Richardson, 2002; Chaston et al., 2004) and included 1000 U/ml SOD, which eliminates superoxide; 1000 U/ml Cat, which degrades H_2O_2; and the permeable glutathione peroxidase mimetic MnTBAP at 200 μM, which also decreases H_2O_2 levels at the expense of oxidizing glutathione. All scavengers either alone or in combination were found to effectively prevent the H_2O_2-mediated decrease in viability of control and FA fibroblasts. However, PCTH showed slightly greater activity than all of the above-mentioned scavengers, with there being a significant ($p < 0.015$) difference between all of the scavengers and PCTH after 24 h when examining control fibroblasts (Fig. 8A). Examining FA fibroblasts, a significant ($p < 0.05$) difference in the efficacy of preventing H_2O_2-induced cytotoxicity was only found between PCTH and SOD. None of the radical scavengers alone or in combination had any effect on cellular viability when added in the absence of H_2O_2 (data not shown), all being well tolerated.

Considering the efficacy of the radical scavengers at preventing the decreased viability observed in the presence of H_2O_2, studies were designed to assess the effect of combining these agents with PCTH (Fig. 9, A and B). The combination of Cat and SOD markedly prevented the decreased viability of control and FA fibroblasts in the presence of H_2O_2 (Fig. 9, A and B). It is noteworthy that the combination of Cat and SOD with PCTH did not significantly improve cellular viability over that observed with PCTH alone (Fig. 9, A and B). A similar effect was observed with L1, in which the combination of Cat and SOD with L1 again did not significantly improve cellular viability over that seen with L1 alone (Fig. 9, A and B).

Idebenone Is Less Effective than PCTH at Rescuing the H_2O_2-Induced Decrease in Fibroblast Viability. Idebenone has shown some beneficial effects in the treatment of patients with FA in clinical trials as a result of its antioxidant properties (Rustin et al., 2002; Di Prospero et al., 2007). Hence, it was an appropriate positive control to compare the effects of PCTH in the current model. In contrast to 50 μM PCTH, which did not reduce fibroblast viability when it was incubated with cells in the absence of H_2O_2 (Fig. 2, A–D),

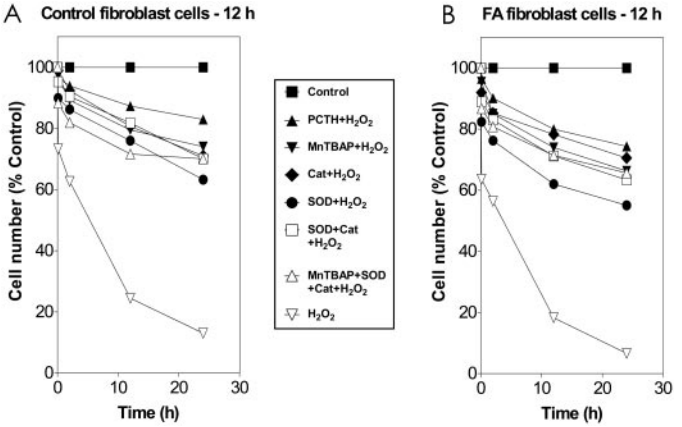

Fig. 8. PCTH is similar to, or more effective, than free radical scavengers at rescuing control (A) and FA fibroblasts (B) from H_2O_2-mediated cytotoxicity. Fibroblasts were preincubated with control medium or medium containing either PCTH (50 μM), MnTBAP (200 μM), Cat (1000 U/ml), SOD (1000 U/ml), SOD (1000 U/ml) + Cat (1000 U/ml), or MnTBAP (200 μM) + SOD (1000 U/ml) + Cat (1000 U/ml) for 12 h at 37°C. Then, 50 μM H_2O_2 or media alone were added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h or 24 h at 37°C. The results are a typical experiment from three performed.

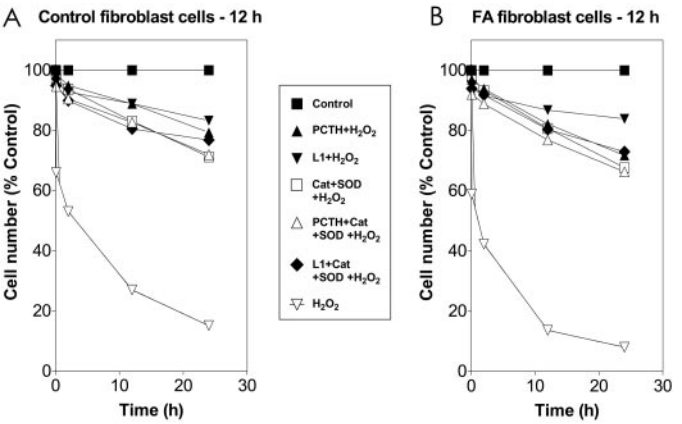

Fig. 9. The combination of PCTH with free radical scavengers does not lead to increased efficacy at rescuing control (A) or FA fibroblasts (B) from H_2O_2-mediated cytotoxicity. Fibroblasts were preincubated with control medium or medium containing either PCTH (50 μM), L1 (50 μM), Cat (1000 U/ml) + SOD (1000 U/ml), PCTH (50 μM) + Cat (1000 U/ml) + SOD (1000 U/ml), or L1 (50 μM) + Cat (1000 U/ml) + SOD (1000 U/ml) for 12 h at 37°C. H_2O_2 (50 μM) or media alone were then added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

idebenone alone markedly reduced cellular viability at 25 and 50 μM (Fig. 10, A and B). In the presence of H_2O_2, there was increased fibroblast viability from both control subjects and patients with FA compared with H_2O_2 alone as the idebenone concentration increased to 25 μM (Fig. 10, C and D). However, at an idebenone concentration of 50 μM, the efficacy at preventing H_2O_2-induced cytotoxicity was similar to that found at 5 μM in control and FA fibroblasts. This decreased efficacy at the highest idebenone concentration in the presence of H_2O_2 (Fig. 10, C and D) is probably due to toxicity observed with idebenone alone (Fig. 10, A and B). Collectively, these studies demonstrate that PCTH was better tolerated and more effective under the current conditions at preventing the H_2O_2-induced cytotoxicity of fibroblasts from control subjects and patients with FA.

Discussion

The mitochondrial iron-loading and oxidative stress in FA may play a role in this disease (Pandolfo, 2006). Thus, we investigated whether our novel PCIH iron chelators, compared with other ligands, could prevent H_2O_2-mediated cytotoxicity. We showed that one of the most biologically active ligands of the PCIH class, PCTH (Becker and Richardson, 1999; Wong et al., 2004), markedly prevented H_2O_2-mediated cytotoxicity in normal and FA fibroblasts.

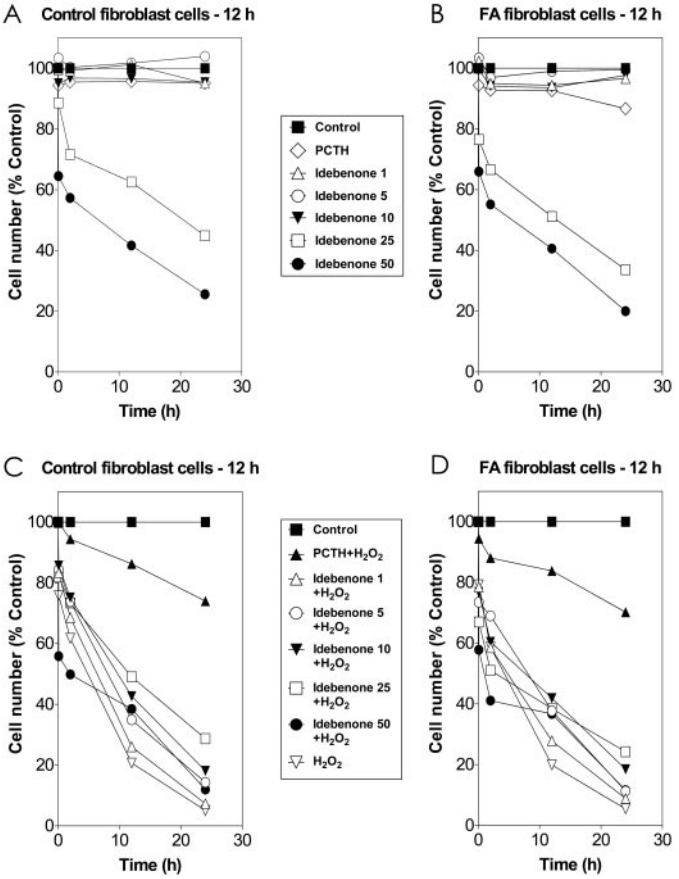

Fig. 10. Idebenone in contrast to PCTH decreases the cell number of control (A) and FA fibroblasts (B) as the concentration is increased to 25 and 50 μM. Idebenone is not as effective as PCTH at rescuing control (C) or FA fibroblasts (D) from H_2O_2-mediated cytotoxicity. Control or FA fibroblasts were preincubated with control medium or medium containing either 50 μM PCTH or 1 to 50 μM idebenone. Then, either medium alone (A and B) or 50 μM H_2O_2 (C and D) was added to each of these conditions, and the incubation continued for 1 min, 2 h, 12 h, or 24 h at 37°C. The results are a typical experiment from three performed.

In this investigation, a range of iron chelators with varying properties were assessed for their ability to prevent the decreased viability induced by H_2O_2. This was important considering that several chelators have shown protective effects in control and FA fibroblasts (Wong et al., 1999; Sturm et al., 2005). In our investigation, DFO had no significant effect on rescuing H_2O_2-mediated cytotoxicity. This could be explained by its slow permeation rate, which was due to its relatively high hydrophilicity (Richardson et al., 1994, 2001). However, under the same experimental conditions, ^{59}Fe efflux studies demonstrated that DFO effectively permeated the cell and resulted in pronounced iron efflux from control and FA fibroblasts (Fig. 6, A and B). In addition, the effect of DFO at inducing ^{59}Fe mobilization was more marked than the lipophilic ligand PIH. This could be important considering the relatively long 36-h incubation period with the chelators, which would facilitate the slow access of DFO to ^{59}Fe pools (Fig. 6, A and B).

Although PIH was significantly more active than DFO at preventing H_2O_2-mediated cytotoxicity, it was less efficient than other closely related aroylhydrazones, such as PCTH (Fig. 1). Indeed, of all the PCIH analogs, PCTH was the most effective, markedly preventing the effect of H_2O_2 at inducing decreased fibroblast viability. Previous studies showed PCTH to be highly efficient at mobilizing iron from neoplastic cells, being particularly effective at low concentrations (Becker and Richardson, 1999). Furthermore, PCTH was also efficient at chelating iron from iron-loaded mitochondria of reticulocytes (Richardson et al., 2001). From the work comparing the ability of PIH and DFO to prevent H_2O_2-mediated cytotoxicity, it could be suggested the ability of chelators to penetrate membranes and bind iron pools is important to prevent the effects of H_2O_2. Significantly, this was clearly demonstrated by kinetic studies examining the ability of the chelators to enter cells, bind intracellular iron pools, and induce iron efflux as a function of time. These experiments showed that PCTH could rapidly mobilize intracellular ^{59}Fe within only 30 min of incubation and was the most effective chelator tested (Fig. 6C). In marked contrast, DFO only showed similar ^{59}Fe-mobilizing efficacy to PCTH after 6 h. Furthermore, PCBH, PCIH, and PCBBH were shown to induce cellular iron depletion at a slower rate than DFO and particularly PCTH. These results suggest the rapid rate of penetration by PCTH into fibroblasts compared with DFO, PCIH, PCBH, and PCBBH and its ability to efficiently mobilize ^{59}Fe may be crucial in preventing H_2O_2-induced cytotoxicity. Hence, these data indicate that it is the rate at which the ligand permeates the cell, which dictates its ability to prevent H_2O_2-mediated cytotoxicity. In view of this, it is notable that H_2O_2 can, within seconds, rapidly partition across cell membranes (Antunes and Cadenas, 2000) and thus its cytotoxic effects via interaction with iron occur very quickly. Although the ability of chelators to permeate cells has been shown to be important for chelation of intracellular iron (Porter et al., 1988; Lipinski et al., 2001; Ma et al., 2006), this study demonstrates that the rapid access to intracellular iron is vital for protection against H_2O_2-mediated cytotoxicity.

The low activity of the hydrophilic PCHH and PCAH li-

gands at preventing H_2O_2-induced cytotoxicity is probably explained by their inability to act as efficient iron chelators to mobilize ^{59}Fe from fibroblasts (Fig. 6B), as observed in iron chelation studies in tumor cells (Becker and Richardson, 1999). Hence, lipophilicity plays an important role in the effect observed, and it is notable that PCTH and its iron complex display high hydrophobicity (Bernhardt et al., 2007). However, PCBBH and its iron complex are also very lipophilic (Bernhardt et al., 2007), but far less active than PCTH in preventing H_2O_2-induced toxicity, indicating other factors are also important.

Considering other beneficial properties of these compounds, in contrast to PIH, we showed that in the presence of H_2O_2 and Fe(II), PCIH analogs did not induce hydroxyl radical generation (Chaston and Richardson, 2003). In fact, the PCIH analogs form Fe(II) complexes with a high potential Fe(III/II) redox couple (>500 mV versus normal hydrogen electrode), and thus, the generation of reactive oxygen species would not be facile (Bernhardt et al., 2007). Hence, the lack of redox activity of the formed PCTH-iron complex may explain, in part, its low toxicity and high efficacy in preventing the H_2O_2-mediated decrease in fibroblast viability (Fig. 2). Another potentially important chemical property of the PCIH ligands, relative to PIH, which has strong affinity for Fe(III) (Richardson and Ponka, 1998), is that the PCIH chelators are well characterized Fe(II) ligands (Bernhardt et al., 2007). The chelation of Fe(II) pools that are known to exist within cells (St Pierre et al., 1992) are probably crucial, because these interact with H_2O_2, leading to toxic radicals. It is noteworthy that because of dynamic equilibria, an Fe(III) chelator also has indirect access to chelatable Fe(II) and thus can prevent H_2O_2 toxicity.

Although the latter factors are significant, further evidence of the importance of membrane permeability and iron chelation to the protective effects of a compound was demonstrated using a range of ligands with different membrane permeability characteristics that ranged from highly permeable (i.e., DP, L1) to limited permeability (BPS), to not permeable (EDTA and DTPA) (Richardson and Baker, 1994; Kicic et al., 2001). The ability of DP to effectively prevent H_2O_2-mediated cellular toxicity in control and FA fibroblasts agreed with previous studies (Sturm et al., 2005). The orally effective chelator L1 (Richardson, 2001) also showed very high activity, probably because of its efficacy to permeate cells and chelate iron pools (Fredenburg et al., 1996), as illustrated by our iron mobilization experiments (Fig. 6B). It is noteworthy that in a small, poorly controlled clinical trial, L1 given in combination with idebenone improved neuropathy and ataxic gait in patients with FA (Boddaert et al., 2007). However, because idebenone has beneficial effects on neurological symptoms of patients with FA (Di Prospero et al., 2007), further studies are essential to confirm these preliminary findings with L1.

Both DTPA and EDTA showed little activity to prevent H_2O_2-mediated cytotoxicity, probably because of their extracellular location and inability to permeate cells (Richardson and Baker, 1994; Kicic et al., 2001). The Fe(II) ligand BPS showed activity that was slightly greater than DTPA and EDTA and much less than DP, L1, or PCTH. The structure of BPS includes two sulfonate groups (Fig. 1) that are negatively charged at physiological pH, and the molecule has been suggested to be impermeable. However, our studies using fibroblasts (Fig. 6B) and previous investigations implementing neoplastic cells (Richardson and Baker, 1994) indicate that BPS is capable of limited cellular iron mobilization, being more effective than DTPA and EDTA but less active than DP.

The ability of PCTH to prevent H_2O_2-mediated cytotoxicity of fibroblasts was also compared with conventional antioxidants. It is noteworthy that PCTH was similarly or more effective than SOD, Cat, MnTBAP, or idebenone as single agents or when used in combination. The antioxidant idebenone is of relevance because it is beneficial for treating the cardiomyopathy (Rustin et al., 2002) and neurological deficits (Di Prospero et al., 2007) of patients with FA. It was surprising that, in this study, this agent alone was shown to be cytotoxic at 50 μM. At lower idebenone concentrations, its activity at preventing H_2O_2-mediated cytotoxicity was far less than PCTH.

An interesting observation was that although control and FA fibroblasts could be rescued by chelators, the FA fibroblasts were more sensitive to H_2O_2. Chelators and antioxidants were less capable of preventing the decreased viability in FA fibroblasts. The higher sensitivity of FA fibroblasts to H_2O_2 was reported previously (Sturm et al., 2005), but their decreased response to iron chelators and antioxidants was not described. The reason for their higher sensitivity could relate to the known alterations in mitochondrial iron metabolism in patients with FA (Napier et al., 2005; Pandolfo, 2006). In fact, mitochondrial iron deposits are known in FA fibroblasts (Delatycki et al., 1999), which could react with H_2O_2. However, both control and FA fibroblasts were rescued from the H_2O_2-mediated toxicity by iron chelation, suggesting common physiologically relevant iron pools were targeted by this oxidant. There is oxidative stress in FA (Wong et al., 1999; Pandolfo, 2006) and the sensitivity of FA fibroblasts to H_2O_2 could be due to alterations in mitochondrial iron metabolism and/or processes important in redox management.

In summary, the novel orally effective chelator PCTH shows high activity at preventing the cytotoxic effects of H_2O_2 in fibroblasts being as effective as or more effective than conventional antioxidants. Our results suggest that it is the rate at which permeable chelators penetrate the cell that dictates their effectiveness at rescuing H_2O_2-mediated toxicity. These data support the use of PCTH as an agent suitable to treat iron-loading conditions.

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