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Inhibition of peroxynitrite-mediated tyrosine nitration by a novel pyrrolopyrimidine antioxidant

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Abstract

Peroxynitrite is a cytotoxic, free radical species that is formed by the combination of superoxide and nitric oxide. The goal of the present study was to examine the ability of a novel antioxidant, U-101033E, to prevent peroxynitrite-mediated oxidative damage of red blood cell membrane proteins. Treatment of red blood cell membranes with peroxynitrite resulted in oxidative damage as evidenced by the presence of both membrane protein cross-linking and nitration of tyrosine residues. Membrane protein cross-linking was the result of oxidation of sulfhydryl groups and was completely blocked by the addition of dithiothreitol. Dithiothreitol also prevented peroxynitrite-mediated nitration of tyrosine red blood cell proteins. U-101033E prevented nitrotyrosine formation in peroxynitrite-treated red blood cell membrane proteins in a concentration-dependent manner, with maximal protection observed at 100 μM U-101033E. However, at a similar concentration where U-101033E prevented tyrosine nitration, it had little or no effect on membrane protein cross-linking. Our results suggest that U-101033E may be intercepting a peroxynitrite-derived reactive nitrogen species that is capable of nitrating tyrosine residues. The ability of U-101033E to prevent tyrosine nitration by peroxynitrite represents a new role for this class of antioxidants and suggests that the pyrrolopyrimidines may be useful in the treatment of diseases where peroxynitrite-mediated injury is implicated.

Keywords: Antioxidant; Peroxynitrite; Tyrosine nitration; Pyrrolopyrimidine

1. Introduction

Peroxynitrite is a potent oxidant produced following the reaction of superoxide with nitric oxide (Beckman et al., 1990) and has been demonstrated to be a cytotoxic agent at physiological pH (Zhu et al., 1992). The cytotoxic potential of peroxynitrite may be related to its ability to initiate lipid peroxidation (Radi et al., 1991a; Rubbo et al., 1994), inhibit mitochondrial respiration (Hausladen and Fridovich, 1994), inactivate membrane ion pump ATPases (Hu et al., 1994), and promote cellular energy depletion (Szabó et al., 1996). Along with its ability to act as a powerful oxidant, a more subtle action of peroxynitrite is its ability to nitrate tyrosine residues on of a variety of proteins (Beckman and Koppenol, 1996; Ye et al., 1996; Beckman, 1996). Consequently, tyrosine nitration can lead to the inactivation of enzymes and receptors that depend on tyrosine residues for their activity (e.g., by blocking phosphorylation of key tyrosine residues important for signal transduction, etc.) (Ischiropoulos et al., 1992; Kong et al., 1996; Martin et al., 1990).

Nitrotyrosine is a stable product that can also serve as indirect evidence that peroxynitrite is involved in certain pathological conditions. In this regard, nitrotyrosine has served as a fingerprint for peroxynitrite-mediated damage of cellular proteins in a variety of pathological conditions, including amyotrophic lateral sclerosis (Beckman et al., 1993; Abe et al., 1997), multiple sclerosis (Hooper et al., 1997), Alzheimer's disease (Smith et al., 1997a), atherosclerosis (Kooy et al., 1995; Beckman et al., 1994), myocardial inflammation (Kooy et al., 1997), ischemia-reperfusion injury (Wang and Zweier, 1996), endotoxic shock (Wizemann et al., 1994), transplant rejection (MacMillan-Crow et al., 1996), and rheumatoid arthritis (Kaur and Halliwell, 1994).

Because of the important role peroxynitrite plays in the etiology of a number of diseases, the identification and characterization of effective antioxidants that can prevent peroxynitrite-mediated oxidative damage is of key impor-

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tance. The goal of the present study was to test the ability of a novel antioxidant, U-101033E, to prevent peroxynitrite-induced oxidative damage to membrane proteins. U-101033E belongs to a novel group of antioxidant compounds, the pyrrolopyrimidines, which possess neuroprotective activity in brain injury and ischemic models, presumably by their ability to inhibit lipid peroxidation (Hall et al., 1997; Bundy et al., 1995). The present report details results demonstrating that U-101033E is a specific inhibitor of tyrosine nitration caused by peroxynitrite. Furthermore, our studies suggest that U-101033E does not react directly with peroxynitrite itself, but instead scavenges a reactive intermediate species that is capable of nitrating tyrosine residues on membrane proteins.

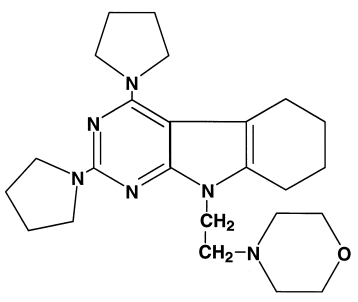
2. Materials and methods

2.1. Materials

All chemicals used were of the highest grade available. Imidazole, histidine, bovine serum albumin, and rabbit anti-human spectrin monoclonal antibody were all purchased from Sigma (St. Louis, MO). Alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G and alkaline phosphate substrate kit were from BioRad (Hercules, CA). Protran (nitrocellulose transfer and immobilization membrane) was from Schleicher and Schuell (Keene, NH). Dulbecco's phosphate-buffered saline was purchased from GibcoBRL (Grand Island, NY). Lipid peroxidation assay kits (# 437634) were purchased from Calbiochem (San Diego, CA). Sodium peroxynitrite (> 90% pure with the balance being nitrate/nitrite) was purchased from Cayman Chemical (Ann Arbor, MI). Anti-nitrotyrosine rabbit polyclonal antibody was a generous gift from Dr. Joe Beckman (University of Alabama, Birmingham, AL). U-101033E was a kind gift from Dr. Edward Hall (Pharmacia/Upjohn, Kalamazoo, MI). For reference, the structure of U-101033E is shown in Fig. 1.

2.2. Preparation of red blood cell membranes

Plasma membranes from human red blood cells were prepared as previously described (Rohn et al., 1993).



U-101033E

Fig. 1. Chemical structure of the pyrrolopyrimidine, U-101033E.

Membrane protein content was determined by the BCA method (Smith et al., 1985) using bovine serum albumin as a standard. The membranes were stored on ice in the refrigerator until used.

2.3. Treatment of red blood cell membranes with peroxynitrite

Red blood cell membranes (1 mg/ml) were incubated with peroxynitrite in phosphate-buffered saline, pH 7.4 at 37°C. All control samples (i.e., samples not containing peroxynitrite) had an equal volume of 0.3 M NaOH to serve as solvent controls. Due to the instability of peroxynitrite, stock aliquots of peroxynitrite were stored at −80°C in a solution of 0.3 M NaOH. On the days of the experiments, the concentration of the stock solution was determined by measurement of the absorbance at 302 nm with cold 0.3 M NaOH as a blank and then calculated using the extinction coefficient for peroxynitrite (1670 M^{-1} cm^{-1}). The stock peroxynitrite was then diluted to the appropriate concentration in 0.3 M NaOH prior to use.

U-101033E was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and stored at −20°C. On the day of the experiment, the stock solution was serially diluted in DMSO to achieve the desired drug concentration. For control samples in experiments where U-101033E was tested, an equal volume of DMSO was added to serve as a vehicle control (i.e., samples labeled 0 μM U-101033E).

2.4. Measurement of lipid peroxidation

The aldehydes, malondialdehyde and 4-hydroxy-2-nonenal were quantified by the spectrophotometric procedure of Smith et al. (1997b) using a kit from Calbiochem. For this assay, the lower limit of measurable malondialdehyde or 4-hydroxy-2-nonenal in a sample is ~0.5 μM. All sample measurements were compared to standard curves obtained using authentic malondialdehyde and 4-hydroxy-2-nonenal.

2.5. Electrophoresis and western blotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was performed as previously described (Rohn et al., 1993) using a 7.5% polyacrylamide separating gel and a 4% polyacrylamide stacking gel for resolving proteins. Gels were stained with Coomassie brilliant blue. Under certain conditions where indicated, samples were diluted in sample buffer either in the presence (reducing conditions) or in the absence (non-reducing conditions) of β-mercaptoethanol before separation by SDS–PAGE.

Western blot analysis was performed as previously described (Quinn et al., 1989). Transfers were incubated with 2 μg/ml primary antibody for 1 h, followed by

alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G secondary antibody (1:1000 dilution) for 1 h. All incubations were at room temperature and blots were developed using a Bio-Rad alkaline phosphatase development kit. Pre-stained molecular weight standards were used on all gels (BRL, Gaithersburg, MD).

3. Results

To examine the effects of U-101033E on peroxynitrite-induced oxidative damage to membrane proteins, we utilized a model system consisting of peroxynitrite-treated red blood cell membranes. In initial experiments, we determined optimal conditions by which peroxynitrite resulted in oxidative damage and/or tyrosine nitration in red blood cell membranes. As shown in Fig. 2, treatment of red blood cell membranes with peroxynitrite resulted in extensive oxidative damage as well as tyrosine nitration of red blood cell membrane proteins. Peroxynitrite treatment caused little or no effect at a final concentration of 10 μM; however, when red blood cell membranes were treated with ≥100 μM peroxynitrite, both membrane protein oxidative damage (Fig. 2A) and nitration of membrane protein tyrosines (Fig. 2B) was observed. The most noticeable effect of this oxidative damage was protein cross-linking, as demonstrated by the appearance of several high molecular weight bands in the stacking gel, which were absent in the control lane (Fig. 2A, see arrow). Aside from the presence of these high molecular weight bands in the stacking gel, the protein-banding pattern was very similar for samples treated with 100 μM or greater peroxynitrite when compared to controls. One exception, however, were the bands corresponding to spectrin (α and β chains) running at about 216 kDa. These bands appeared to decrease in intensity after peroxynitrite treatment, and this effect was most evident at ≥200 μM peroxynitrite (Fig. 2A). These results suggest that spectrin may be a protein particularly sensitive to oxidative damage by peroxynitrite.

As stated previously, peroxynitrite also resulted in a concentration-dependent nitration of tyrosine residues on red blood cell membrane proteins (Fig. 2B). The action of peroxynitrite was relatively non-selective as indicated by the numerous bands present following Western blot analysis with an antibody recognizing nitrotyrosine (Fig. 2B). This result suggests that peroxynitrite, or some reactive species derived from it, is able to nitrate tyrosine residues on most red blood cell membrane proteins. In addition, there was a strong signal corresponding to nitrotyrosine immunoreactivity observed near the top of the stacking gel at both 200 and 300 μM peroxynitrite, indicating the presence of a significant amount of nitrotyrosine in the cross-linked protein species (Fig. 2B, see arrow). The action of peroxynitrite was extremely rapid, and significant protein oxidative damage and tyrosine nitration was observed even when red blood cell membrane proteins were sampled immediately after the addition of peroxynitrite (nominal zero time point). We analyzed samples treated for as long as 1 h, but found that protein oxidative damage and tyrosine nitration was maximal by ~30 min (data not shown). Therefore, a standard treatment protocol of 200 μM peroxynitrite for a time period of 30 min was used for all further experiments.

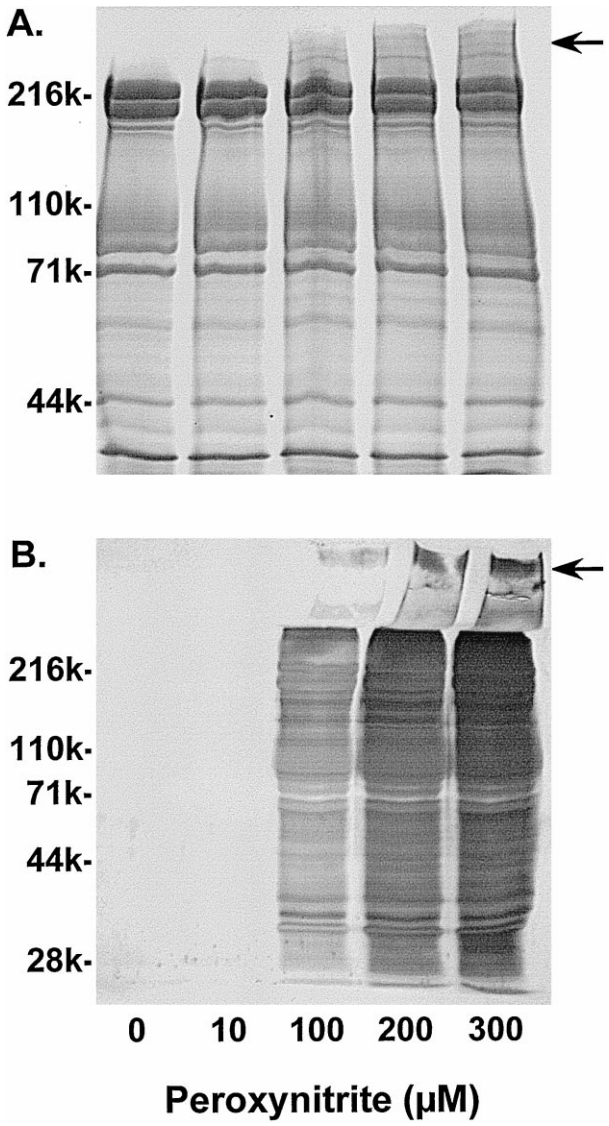


Fig. 2. Concentration-dependent oxidative damage and tyrosine nitration of red blood cell membrane proteins by peroxynitrite. Red blood cell membranes were treated for 1 h with various concentrations of peroxynitrite as shown. Membrane proteins were analyzed by SDS–PAGE under reducing conditions (Coomassie blue-stained gel shown in Panel A) and by Western blotting with an anti-nitrotyrosine antibody (Panel B) described under Section 2. Sample lanes are identical for both Panels A and B, and molecular weight markers are shown on the left, and the data are representative of three separate, independent experiments. Arrows to the right of both Panels A and B indicate the presence of a high molecular weight proteinaceous material in the stacking gel.

As mentioned above, spectrin appeared to be one of the membrane proteins particularly susceptible to oxidative

damage by peroxynitrite (see Fig. 2A). Thus, we examined in further detail the actions of peroxynitrite on spectrin by Western blot analysis with an antibody specific for both the α and β chains of human spectrin. Peroxynitrite resulted in a significant reduction in the intensities of the two bands corresponding to the α- and β-chains of spectrin (Fig. 3). This decrease in immunoreactivity to native spectrin (lower arrow) can be explained by the increase in immunoreactivity now present in the stacking gel (upper arrow). Thus, we hypothesized that the oxidative damage to spectrin, as well as other red blood cell membrane proteins, may be a result of peroxynitrite-mediated oxidation of sulfhydryl groups and subsequent inter- or intramolecular disulfide cross-linking between red blood cell membrane proteins. To directly test this hypothesis, red blood cell membranes were incubated with peroxynitrite in the presence or absence of dithiothreitol. It is known that sulfhydryl reducing agents, such as dithiothreitol, can scavenge peroxynitrite by directly interacting with peroxynitrite via a simple second order reaction (Radi et al., 1991b). As shown in Fig. 4, when red blood cell membranes were treated with peroxynitrite in the presence of dithiothreitol, membrane protein cross-linking and the appearance of high molecular weight complexes was completely prevented. In addition, dithiothreitol also prevented peroxynitrite-mediated nitration of tyrosine residues (Fig. 4B).

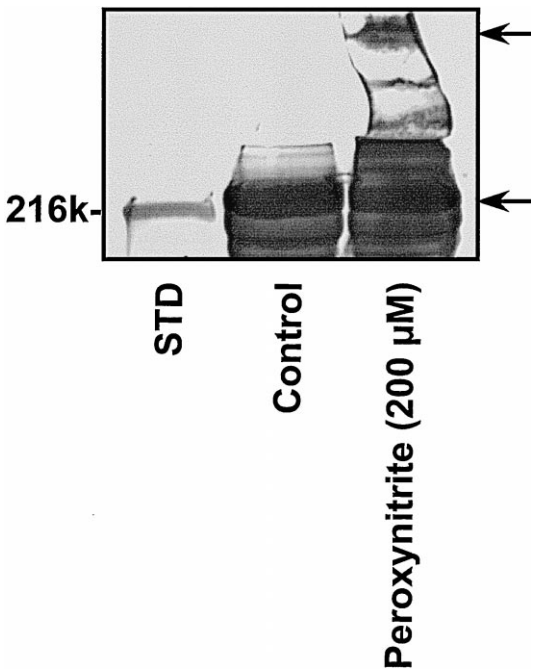


Fig. 3. Peroxynitrite-mediated loss in red blood cell spectrin and formation of a high molecular weight complex. Red blood cell membranes were treated with 200 μM peroxynitrite for 30 min, separated by SDS–PAGE under reducing conditions, and immunoblotted with a monoclonal antibody that recognizes both the α and β chains of human spectrin. A molecular weight marker corresponding to 216 kDa is shown to the left (STD). Representative of two separate, independent experiments.

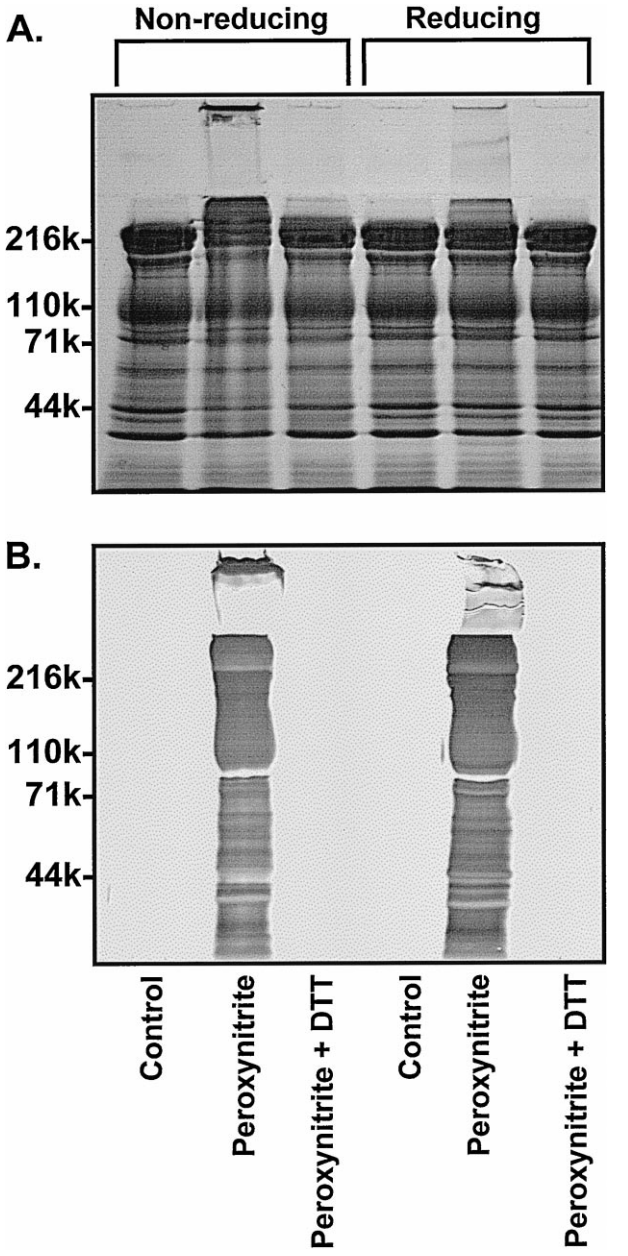


Fig. 4. Peroxynitrite-mediated oxidation of sulfhydryls and tyrosine nitration are prevented by the reducing agent, dithiothreitol. Red blood cell membranes were treated for 30 min with 200 μM peroxynitrite alone (Peroxynitrite) or with 200 μM peroxynitrite + 2 mM dithiothreitol (Peroxynitrite + DTT). Control, untreated membranes (solvent only) and the treated membranes were then analyzed by SDS–PAGE (Coomassie blue-stained gel shown in Panel A) and Western blot analysis using an anti-nitrotyrosine antibody (Panel B) described under Section 2. Samples were separated by SDS–PAGE either under non-reducing conditions or under reducing conditions (see Section 2) as indicated. Sample lanes for both Panels A and B are identical, and molecular weight markers are shown on the left. The data are representative of at least two separate, independent experiments.

To further evaluate the role of thiol oxidation as the mechanism of oxidative damage and protein cross-linking by peroxynitrite, we also analyzed the peroxynitrite-treated red blood cell membrane samples by SDS–PAGE under

both reducing and non-reducing conditions (i.e., in the presence or absence of β-mercaptoethanol in the sample buffer). Under reducing conditions, the amount cross-linked of membrane protein was significantly decreased as compared to similar samples run under non-reducing conditions (Fig. 4), again suggesting a role for thiol oxidation by peroxynitrite as a major cause for the observed membrane protein cross-linking.

We also analyzed whether peroxynitrite treatment resulted in peroxidation of red blood cell membranes, leading to the generation of reactive aldehydes that could mediate part of the observed protein cross-linking. However, treatment of red blood cell membranes with peroxynitrite did not result in any measurable lipid peroxidation, as evidenced by the lack of production of either of the major aldehyde by-products of lipid peroxidation, 4-hydroxy-2-nonenal or malondialdehyde (detection limit ~0.5 μM; data not shown). Thus, these results further support the conclusion that the oxidative damage of red blood cell membrane proteins can be largely attributed to thiol oxidation, which mediates subsequent membrane protein cross-linking.

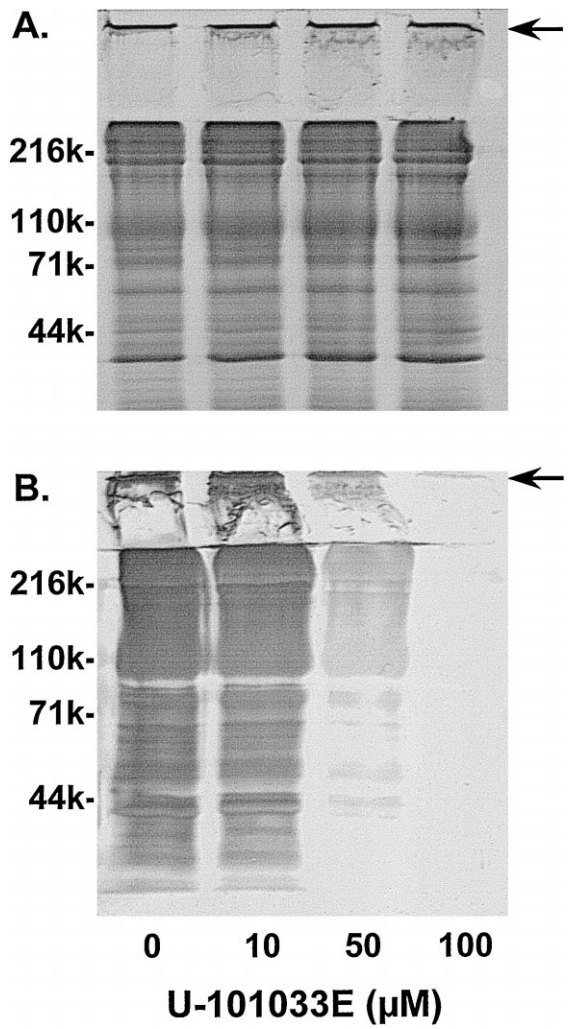


Fig. 5. Inhibition of peroxynitrite-induced tyrosine nitration by U-101033E. Red blood cell membranes were incubated for 30 min in the presence of 200 μM peroxynitrite and increasing concentrations of U-101033E as described under Section 2. The lane marked 0 μM U-101033E contained an equal volume of DMSO alone, which served as the solvent control. Following treatment, membrane proteins were analyzed by SDS–PAGE under non-reducing conditions (Coomassie blue-stained gel shown in Panel A) and Western blotting with an anti-nitrotyrosine antibody (Panel B). Sample lanes for both panels A and B are identical, and the data are representative of at least three separate, independent experiments.

Following characterization of the action of peroxynitrite on red blood cell membrane proteins, we next tested the ability of the novel antioxidant, U-101033E, to prevent peroxynitrite-induced membrane protein cross-linking and/or tyrosine nitration. Fig. 5 shows the results of such an experiment where red blood cell membranes were treated with peroxynitrite and increasing concentrations of U-101033E. In contrast to dithiothreitol, U-101033E, even at the highest concentration used (100 μM), had little or no effect on membrane protein cross-linking (see arrow, Fig. 5A). However, though unable to block membrane protein cross-linking, U-101033E was very effective in preventing peroxynitrite-mediated tyrosine nitration of red blood cell membrane proteins (Fig. 5B). In this regard, a slight reduction in the intensity of nitrotyrosine immunoreactivity was observed at 10 μM U-101033E, while complete inhibition of tyrosine nitration was observed at 100 μM U-101033E (Fig. 5B). DMSO, the solvent used for these experiments, had no effect on the level of peroxynitrite-mediated tyrosine nitration (Fig. 5B, first lane).

4. Discussion

The goal of the present study was to examine the ability of the pyrrolopyrimidine, U-101033E, to prevent oxidative damage to membrane proteins by peroxynitrite. The pyrrolopyrimidines represent a new class of antioxidants, whose structure bears close resemblance to the 21-aminosteroids (Bundy et al., 1995) (see Fig. 1). The 21-aminosteroids have shown excellent activity in experimental models of central nervous system ischemia-reperfusion injury (Hall et al., 1994). The most extensively characterized 21-aminosteroid, tirilazad mesylate, is currently in phase III clinical trials for head and spinal cord injury, stroke, as well as for subarachnoid hemorrhage (Kassel et al., 1996). Interestingly, the pyrrolopyrimidines have been shown to possess significantly increased efficacy over the 21-aminosteroids in protecting cultured neurons against free radical-mediated injury (Hall et al., 1997; Bundy et al., 1995). This increase efficacy of the pyrrolopyrimidines may be related to their enhanced antioxidant activity over the 21-aminosteroids (Hall et al., 1997; Bundy et al., 1995). In support of this point, U-101033E has been shown to be a potent inhibitor of lipid peroxidation with

half-maximal protection observed between 0.5–1.1 μM (Hall et al., 1997; Bundy et al., 1995).

Ideally, a functional antioxidant should be one that is broad spectrum, and therefore, able to scavenge a variety of toxic free radical species. In this regard, an effective antioxidant should be one that is able to not only prevent lipid peroxidation, but also other types of oxidative damage including, for example, direct protein modification by reactive oxygen species. It was for this reason that we examined the ability of U-101033E to prevent potential oxidative damage by peroxynitrite.

Peroxynitrite is a unique reactive oxidant that is produced following the reaction of nitric oxide with superoxide (Beckman and Koppenol, 1996). Because peroxynitrite is a weak initiator of lipid peroxidation (Radi et al., 1991a; Rubbo et al., 1994), its role in tissue injury (Halliwell, 1997) has been suggested to be due to its propensity to oxidize thiol groups and cause nitration of protein tyrosine residues (Beckman, 1996). In support of this hypothesis, we demonstrate in the present study that peroxynitrite treatment of membranes caused significant thiol oxidation, membrane protein cross-linking, and extensive tyrosine nitration of red blood cell membrane proteins; however, these changes occurred in the absence of any measurable amount of lipid peroxidation.

With regards to thiol oxidation and membrane protein cross-linking, we found that spectrin was a red blood cell membrane protein particularly susceptible to this type of peroxynitrite-mediated oxidative damage. Previous studies have shown that sulfhydryl-oxidizing agents exclusively cross-link spectrin via disulfide bonds in intact human erythrocytes (Haest et al., 1977). The reason for this is unknown, but may reflect the fact that spectrin is an extrinsic protein which possesses exposed SH groups that are not localized within the hydrophobic core of the membrane, and therefore, are very susceptible to thiol oxidation (Haest et al., 1981).

In the present studies, we found that the novel antioxidant, U-101033E, was able to inhibit peroxynitrite-induced tyrosine nitration. This is the first demonstration that the pyrrolopyrimidines are able to prevent peroxynitrite-mediated tyrosine nitration, and was a somewhat surprising result based upon a previous study which reported that tyrosine nitration following cerebellar granular cell exposure to peroxynitrite was not prevented by the 21-aminosteroid, U-74006F (Fici et al., 1996). Interestingly, these same authors recently found that post-treatment with U-101033E prevented cell toxicity following exposure of cerebellar granular cells to peroxynitrite (Fici et al., 1997). In addition, treatment of U-101033E together with certain sulfhydryl-containing compounds resulted in additive protection from peroxynitrite-mediated cellular toxicity compared to either treatment alone (Fici et al., 1997); however, the mechanism by which U-101033E provided protection was not investigated. One possible explanation, which is supported by our present studies, is that protection by U-101033E in their model system resulted from the ability of U-101033E to prevent peroxynitrite-mediated nitration of tyrosine residues on cellular proteins.

While U-101033E inhibited tyrosine nitration in peroxynitrite-treated red blood cell membranes, it did not prevent thiol oxidation and subsequent cross-linking of membrane proteins (see Fig. 5). In contrast, the reducing agent, dithiothreitol, also blocked thiol oxidation and membrane protein cross-linking in this model system. These results can be explained by the propensity of peroxynitrite to be a relatively selective oxidant of cellular sulfhydryls. Indeed, it has been demonstrated that peroxynitrite oxidizes sulfhydryls about 1000 times faster than hydrogen peroxide (Radi et al., 1991b). Thus, sulfhydryl agents, such as dithiothreitol, will react directly with peroxynitrite resulting in its decomposition in direct proportion to their sulfhydryl concentration. Therefore, in the present model system dithiothreitol may be acting as a sink for peroxynitrite, and when used in great excess compared to the concentration of peroxynitrite, dithiothreitol may be able to fully inactivate peroxynitrite before it can attack cellular sulfhydryl groups. In contrast to dithiothreitol, U-101033E does not contain any sulfhydryl groups (Fig. 1), and, thus, is unable to react directly with peroxynitrite in a similar manner as dithiothreitol. Based on these observations, it is not surprising that U-101033E is unable to prevent peroxynitrite-mediated thiol oxidation and membrane protein cross-linking. Thus, we propose that U-101033E may prevent tyrosine nitration by intercepting a reactive nitrating species derived from peroxynitrite, and not by reacting directly with peroxynitrite itself. The identity of this reactive species is currently unknown, but may be a nitronium-like species (NO_2^+) derived from the spontaneous decomposition of peroxynitrite or through iron-catalyzed formation of a nitronium ion-like species from heterolytic cleavage of peroxynitrite (Crow and Beckman, 1996; Beckman et al., 1996; Beckman, 1996). Because the membranes used in the present model system were from red blood cells, it is possible that trace amounts of iron were present and were capable of catalyzing such a reaction. However, irrespective of the specific mechanism involved, U-101033E was able to partially protect membrane proteins from oxidative damage by inhibiting peroxynitrite-mediated tyrosine nitration.

Peroxynitrite-mediated tyrosine nitration may alter the structure and function of proteins, and it is known that tyrosine nitration leads to decreased surfactant protein A function (Haddad et al., 1994), inhibits cytochrome *P*-450 (Janig et al., 1987), and inhibits protein phosphorylation (Martin et al., 1990). Recently, it has been demonstrated that peroxynitrite-mediated tyrosine nitration also inhibits protein phosphorylation (Mondoro et al., 1997; Kong et al., 1996) and targets proteins for degradation (Gow et al., 1996). Finally, the existence of nitrotyrosine is important indirect evidence that peroxynitrite is involved in pathophysiological processes as recently demonstrated for nu-

merous pathological conditions (Beckman et al., 1993; Abe et al., 1997; Hooper et al., 1997; Smith et al., 1997a; Kooy et al., 1995; Beckman et al., 1994; Kooy et al., 1997; Wang and Zweier, 1996). Therefore, in addition to their role as potent inhibitors of lipid peroxidation (Hall et al., 1997; Bundy et al., 1995), the pyrrolopyrimidines, such as U-101033E, may be of additional use in the prevention and treatment of certain diseases by preventing tyrosine nitration in cases where peroxynitrite-mediated tyrosine nitration plays a role in the etiology of the disease.

As discussed above, U-101033E was not able to inhibit protein oxidation, while it effectively blocked protein tyrosine nitration. Thus, this feature may limit the therapeutic utility of this pyrrolopyrimidine in conditions involving high levels of protein oxidation. In such cases, a combination of protective compounds may be the treatment of choice. For example, Szabó et al. (1997) recently found that a cell-permeable superoxide dismutase mimetic (Mn(III)tetrakis(4-benzoic acid)porphyrin) was able to reduce oxidation of mitochondrial and nuclear proteins caused by endogenously generated peroxynitrite in immunostimulated macrophages. Thus, a combination of U-101033E and a superoxide dismutase mimetic (for example) could effectively block both tyrosine nitration and protein oxidation, resulting in an enhanced therapeutic effect in treating inflammatory diseases.

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