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Role of Oxidative Stress in Nickel Chloride-Induced Cell Injury in Rat Renal Cortical Slices

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ABSTRACT. Nickel chloride ($NiCl_2$) induced lactate dehydrogenase (LDH) release and lipid peroxidation (LPO) in rat renal cortical slices *in vitro* in a concentration- (0–2 mM) and time- (0–4 hr) dependent manner, with initial significant LDH release occurring as early as 1 hr, whereas significant increase in LPO started 3 hr after exposure, suggesting that LPO results from renal cell injury. Both $NiCl_2$-induced LDH release and LPO were prevented significantly by glutathione and dithiothreitol, suggesting that $NiCl_2$-induced renal cell injury is dependent on thiols. However, such injury is not dependent solely on thiols, because (a) these thiols failed to inhibit completely the uptake of Ni^{2+} by the renal cortex, and (b) diethylmaleate pretreatment failed to increase $NiCl_2$-induced cell injury further. Superoxide dismutase partially reduced the $NiCl_2$-induced LDH release without affecting LPO and glutathione, whereas catalase did not affect such LDH release and LPO. Dimethylthiourea and DMSO completely prevented $NiCl_2$-induced LPO, but only partially reduced LDH release. Deferoxamine prevented $NiCl_2$-induced renal cell injury without affecting LPO and without significantly reducing Ni^{2+} uptake by the renal cortex, suggesting that nickel chelation is not important in such prevention of injury. $NiCl_2$-induced inhibition of *para*-aminohippurate uptake was prevented significantly by thiols, deferoxamine, and dimethylthiourea. $NiCl_2$-induced loss of cellular glutathione content was prevented significantly by thiols and deferoxamine, but not by superoxide dismutase and dimethylthiourea. These results suggest that LPO was not related to $NiCl_2$-induced lethal renal cell injury, whereas such injury may be caused by the induction of the Fenton reaction, generating hydroxyl radicals. BIOCHEM PHARMACOL **58**;9:1501–1510, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. nickel chloride; renal cortical slices; oxidative stress; renal cell injury; lipid peroxidation; PAH uptake; nickel uptake

Nickel is used extensively in electroplating, in the manufacture of steel and other alloys, and in the manufacture of batteries and electronic devices. More and more cooking utensils made of steel are used. Many nickel compounds are released into the atmosphere during mining, smelting, and refining operations [1]. Consequently, nickel salts are considered an industrial health hazard.

Nickel and its compounds have been reported to be potent carcinogenic and/or toxic agents in humans and experimental animals [2, 3]. The kidney plays a principal role in the toxicokinetics of nickel, since it serves as a major organ of nickel excretion [4] and as a site of accumulation [4], as well as a target organ for nickel toxicity [4]. Studies of the distribution of nickel following acute i.p. injection of $^{63}NiCl_2$ in the rat have shown the highest accumulation of nickel in the kidney [5]. With regard to nephrotoxicity of nickel compounds, toxic nephropathy with proteinuria, aminoaciduria, and reduced renal clearance has been demonstrated in rats given i.p. injections of 65–85 μmol $NiCl_2$/kg body weight [6]. Histopathological examination of renal tissue revealed alterations in the glomerular epithelial cells of these rats. Concurrent administration of triethylenetetramine and Ni(II) compounds prevented nickel-induced toxicity in rats [7]. However, the exact mechanism of their action still remains unclear. Recent studies have shown that nickel compounds can induce LPO† and modify the antioxidant system. Thus, increased LPO has been observed in the livers and kidneys of rats treated s.c. with $NiCl_2$ (0.12 to 0.75 mmol/kg body weight) [8]. The administration of $NiCl_2$ to rats produced enhanced levels of lipid peroxide and GSH with a concomitant decrease in glutathione peroxidase activity in the liver, and this enhanced LPO was inhibited by treatment with hydroxyl radical scavengers, such as benzoate and ethanol, but not by SOD and catalase [9]. In another study, the treatment of rats with Ni(II) compounds caused a decrease of the GSH level, followed by a rebound in GSH levels in the liver but not in the kidney, as well as a decrease in

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† *Abbreviations*: LPO, lipid peroxidation; DFX, deferoxamine; DEM, diethylmaleate; DMTU, dimethylthiourea; DTT, 1,4-dithiothreitol; LDH, lactate dehydrogenase; MDA, malondialdehyde PAH, *para*-aminohippurate; and SOD, superoxide dismutase.

glucose-6-phosphate dehydrogenase and glutathione reductase activity [10]. The mortality and hepatic LPO caused by $NiCl_2$ were found to be correlated with the decreased level of GSH [11]. Administration of Ni(II) inhibited catalase activity *in vitro*, possibly through its direct interaction with the catalase protein moiety. Administration of Ni(II) to rats decreased the activity of catalase in the liver and kidney, but there was no direct relationship between the enzyme inhibition and Ni(II) concentration in these tissues, suggesting that the enzyme inhibition by Ni(II) is more complex *in vivo* than *in vitro* [12]. Administration (i.p.) of 107 μmol nickel acetate/kg body weight increased LPO in the kidneys and livers of rats with concomitant decreases in GSH levels and antioxidant enzyme activity, such as catalase, glutathione peroxidase, and glutathione reductase, but did not modify SOD activity in the liver and kidney, although there was a decrease of activity of glutathione *S*-transferases in the liver and an increase of activity of glutathione *S*-transferases in the kidney [13]. Furthermore, whereas renal iron was increased, liver iron remained unchanged by such nickel treatment [13]. Inoue and Kawanishi [14] reported possible production of superoxide, hydroxyl radical, and singlet oxygen from H_2O_2 reacting with the Ni(II) complex of glycylglycyl-L-histidine.

The time pattern of the resulting renal and hepatic LPO has been shown to be due to a possible increased concentration of nickel and concurrent inhibition of catalase, glutathione peroxidase, and glutathione reductase, but not to increased iron or copper levels [13]. On the other hand, another study reported a direct contribution of iron to LPO [15]. However, all these studies did not examine whether there is any relationship between renal toxicity due to $NiCl_2$ and LPO or oxidative stress. Hence, the present study was undertaken to examine the role of oxidative stress in $NiCl_2$-induced cell injury in rat renal cortical slices.

MATERIALS AND METHODS

Preparation of Renal Cortical Slices

Adult male Sprague–Dawley rats (280–300 g body weight) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the kidneys were removed rapidly. The kidneys were perfused immediately through the renal artery with an ice-cold isotonic solution containing 140 mM NaCl, 10 mM KCl, and 1.5 mM $CaCl_2$ to remove as much blood as possible. Thin (0.4- to 0.5-mm thick) slices of renal cortex were prepared using a Stadie–Riggs microtome and were stored in an ice-cold modified Cross–Taggart medium [16] containing 130 mM NaCl, 10 mM KCl, 1.5 mM $CaCl_2$, 5 mM glucose, and 20 mM Tris–HCl (pH 7.4).

Measurement of Biochemical Parameters Using Renal Cortical Slices

Renal cortical slices were treated with $NiCl_2$ (0–2 mM) in a modified Cross–Taggart medium [16] for 180 min at 37° under a 100% oxygen atmosphere. Following incubation, LDH release, LPO, PAH uptake, and GSH concentration were measured.

For measurement of LDH release, renal cortical slices were homogenized in 2 mL of demineralized distilled water, and the tissue homogenate was centrifuged at 2000 *g* for 5 min. The pellet was discarded, and the supernatant was saved. LDH activity was determined in the supernatant and incubation medium using a colorimetric method described by Bergmeyer *et al.* [17]. The amount of LDH released was calculated from the total LDH measured minus the LDH remaining in the tissue. LDH, measured in micromoles per minute per gram of wet tissue, i.e. U/g wet weight, was finally expressed as the fraction of LDH released from the total LDH multiplied by 100.

LPO was determined indirectly by measuring the production of MDA in the renal tissue following the method of Uchiyama and Mihara [18]. Briefly, renal cortical slices were homogenized in ice-cold 1.15% KCl. A 0.5-mL aliquot of the homogenate was mixed with 3 mL of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated for 30 min in a boiling water bath. Following the addition of 4 mL of *n*-butanol, the contents were vortexed vigorously and centrifuged at 2000 *g* for 20 min. Then the absorbance of the organic layer was measured at 535 nm and compared with freshly prepared MDA tetraethylacetal standard. Protein content was estimated by the method of Bradford [19], using bovine serum albumin as a standard.

PAH uptake by cortical slices was determined as previously described [20]. Briefly, 75- to 100-mg cortical slices (wet weight) were incubated with 2.7 mL of 20 mM Tris–HCl buffer (pH 7.4) in a medium containing 96 mM NaCl, 40 mM KCl, 0.75 mM $CaCl_2$, 10 mM lactate, and 7.4×10^{-5} M PAH (sodium salt). After incubation for 90 min at 25° in a Dubnoff metabolic shaker under a 100% oxygen atmosphere, tissue slices were removed from the medium, blotted dry, and weighed. Both the tissue slices and a 2-mL aliquot of the medium were homogenized in 3 mL of 10% trichloroacetic acid and brought to a final volume of 10 mL. After centrifugation, aliquots of supernatants thus obtained from the tissue and the medium were assayed for PAH spectrophotometrically by the method of Smith *et al.* [21]. Accumulation of PAH in the renal cortical slices then was expressed as the slice-to-medium (S/M) concentration ratio, where S equals milligrams of PAH per gram of tissue slice (wet weight) and M equals milligrams of PAH per milliliter of medium.

The GSH content of the renal cortical slices was estimated by the method of Griffith [22]. Briefly, GSH was assayed by an enzymatic recycling procedure in which it was oxidized sequentially by 5,5′-dithiobis-2-nitrobenzoic acid and reduced by NADPH in the presence of glutathione reductase and 2-vinylpyridine. The extent of 2-nitro-5-thiobenzoic acid formation was monitored at 412 nm, and the GSH content was quantitated by comparison of the results with a standard curve.

Uptake of Nickel Ion by Renal Cortical Slices

Following the incubation of renal cortical slices with 0 and 1 mM $NiCl_2$ as described above for 180 min, the slices were blotted dry and weighed. Then tissue slices were digested with ultrapure nitric acid and homogenized. After centrifugation, the protein-free supernatant was neutralized and extracted with a mixture of ammonium pyrrolidine-dithiocarbamate and 4-methylpentane-2-one [23, 24]. For studying the effect of GSH, DTT, DFX, and DMTU on the uptake of nickel, the uptake was initiated by the addition of 1 mM $NiCl_2$ and either GSH (5 mM), DTT (2 mM), DFX (0.1 mM), or DMTU (20 mM) to the incubation medium and incubating for 3 hr at 37° as before. Then the uptake of nickel (Ni^{2+}) was measured in each case, following the protocol as described above. Finally, all samples were analyzed by electrothermal atomic absorption spectrometry. A Perkin-Elmer Atomic Absorption Spectrometer model 703 equipped with an HGA 500 graphite furnace was used for nickel analyses.

Chemicals

Nickel chloride ($NiCl_2 \cdot 6H_2O$) (99.99% pure) and DMTU were purchased from the Aldrich Chemical Co. GSH, DTT, catalase, SOD, DFX, DEM, and MDA tetraethylacetal were purchased from the Sigma Chemical Co. All other chemicals were of the highest commercial grade available.

Statistics

Data are expressed as the means ± SEM for five or six separate experiments. Data were analyzed by one-way analysis of variance. The difference between treatment means was tested by the Newman–Keuls multiple comparison test. The level of significance was set at $P < 0.05$.

RESULTS

Effects of $NiCl_2$ on LDH Release and LPO

The overt toxicity of $NiCl_2$ was determined using rat renal cortical slices. Using LDH as a marker of plasma membrane damage and cell death, the concentration-dependent effects of $NiCl_2$ on the lethal cell injury and peroxidation of membrane lipids were examined over a concentration range of 0–2 mM in renal cortical slices incubated for 180 min at 37°. As seen in Fig. 1, $NiCl_2$ caused a significant increase of LDH release in a concentration-dependent manner, with an initial significant increase occurring at 0.5 mM. Likewise, the LPO increased in a concentration-dependent manner when renal cortical slices were exposed to $NiCl_2$ (Fig. 1).

The effects of time of incubation on LDH release and LPO from rat renal cortical slices exposed to 2 mM $NiCl_2$ also were studied, and the results are presented in Fig. 2. A significant amount of LDH leakage (cell death) started to occur only after 1 hr of incubation, whereas a significant amount of LPO (as measured by MDA production) started

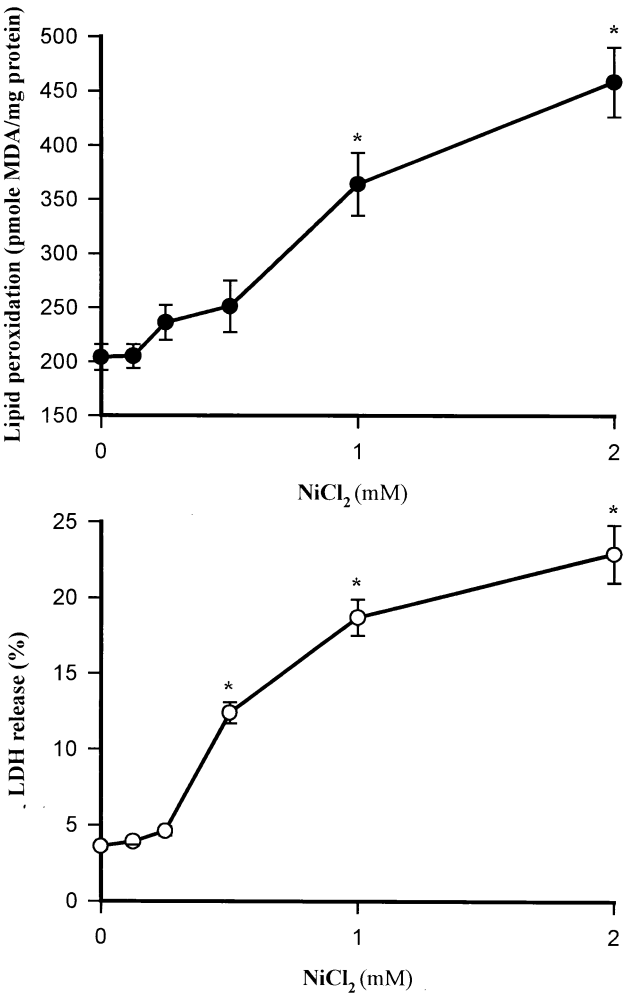

FIG. 1. Effects of various concentrations of $NiCl_2$ on LDH release and LPO in rat renal cortical slices. Slices were treated with different concentrations of $NiCl_2$ at 37° for 2 hr for measurement of LDH release and for 3 hr for measurement of LPO. The results are means ± SEM of four experiments. Key: (*) $P < 0.05$ compared with the control. The amount of LDH released was calculated from the total LDH (tissue LDH plus LDH in the medium) minus LDH remaining in the tissue and expressed as a percent of total LDH. The control value of LDH was 4.21 ± 0.22 (U/g wet wt). For details, see Materials and Methods.

to occur only after 3 hr of incubation. Furthermore, while this LPO continued to increase with time, the amount of LDH leakage did not increase significantly further with time (Fig. 2).

Effects of Thiol Reagents on $NiCl_2$-Induced LDH Release and LPO

Since depletion of protein thiols or GSH has been reported as an early event of nickel-induced nephrotoxicity [10, 11], we examined whether GSH and DTT could exert a protective effect against $NiCl_2$-induced cytotoxicity in rat renal cortical slices. When renal cortical slices were exposed to 1 mM $NiCl_2$ in the presence of 5 mM GSH or 2 mM DTT, the $NiCl_2$-induced LDH release and LPO were

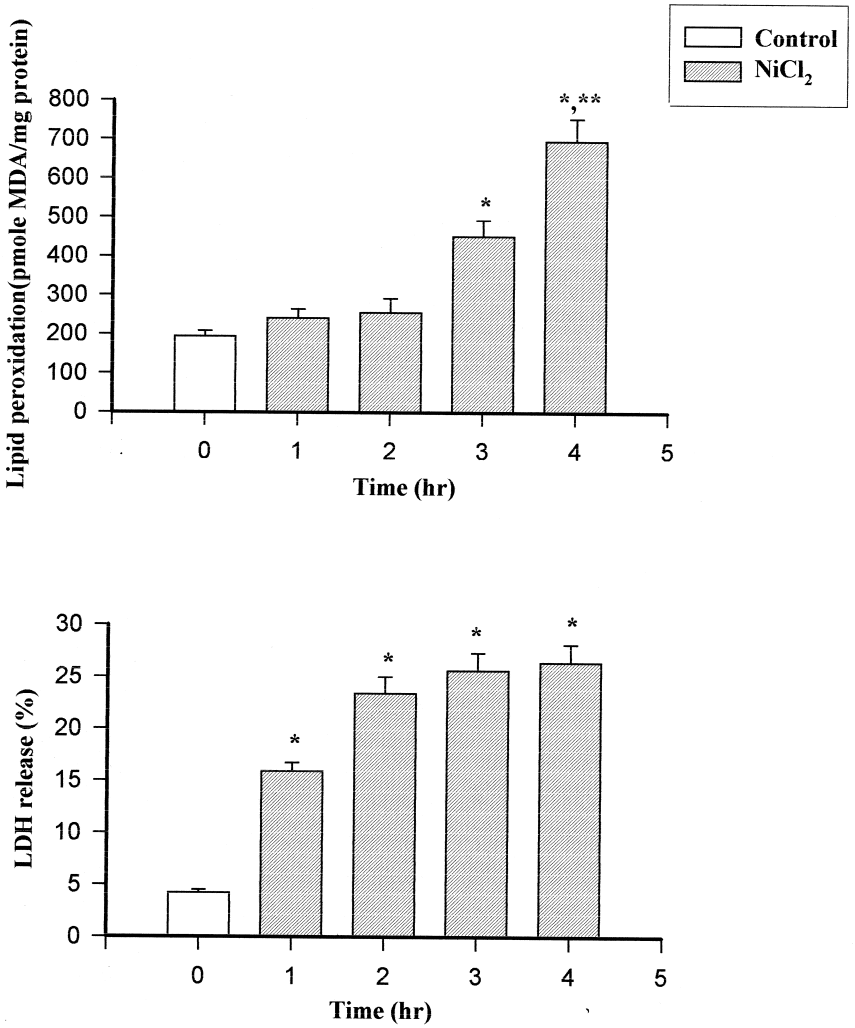

FIG. 2. Effect of time of incubation on LDH release and LPO from rat renal cortical slices exposed to 2 mM $NiCl_2$. The results are means ± SEM of four experiments. Key: (*) $P < 0.05$ compared with the control; and (**) $P < 0.05$ compared with 3-hr-treated $NiCl_2$. The amount of LDH released was calculated from the total LDH (tissue LDH plus LDH in the medium) minus LDH remaining in the tissue and is expressed as a percent of total LDH. The control value of LDH was 4.19 ± 0.18 (U/g wet wt).

prevented almost completely (Fig. 3). On the other hand, pretreatment (25 min) of renal cortical slices with DEM to deplete intracellular GSH failed to further increase the renal cell injury induced by $NiCl_2$ as well as $NiCl_2$-induced LPO (Fig. 3).

Effects of Reactive Oxygen Species (ROS) Scavengers and Antioxidants on $NiCl_2$-Induced LDH Release and LPO

To determine whether the cell injury and the LPO induced by $NiCl_2$ were associated with the production of ROS, the effects of various scavengers of ROS were examined, and the results are presented in Figs. 4 and 5. SOD, a superoxide scavenger, attenuated LDH release significantly, whereas it failed to modify the $NiCl_2$-induced LPO (Fig. 4). Catalase, a H_2O_2 scavenger, did not produce any changes in these two parameters induced by $NiCl_2$ (Fig. 4). Hydroxyl radical scavengers, such as DMTU and DMSO, prevented $NiCl_2$-induced LPO completely, whereas significant but only partial reduction of LDH release was obtained due to treatment with these hydroxyl radical scavengers (Fig. 5). On the other hand, an iron chelator, DFX, almost completely prevented $NiCl_2$-induced LDH release but failed to reduce LPO (Fig. 6). The concentrations of scavengers used in this study were similar to those that effectively prevented the generation of ROS or oxidant-induced cell injury in other reported studies [25–27].

Effects of Various Drugs on $NiCl_2$-Induced Depletion of Cellular GSH

GSH is the most important controlling factor for maintaining cellular defense against chemical-induced cell injury [28]. Acute treatment with $NiCl_2$ has been found to deplete cellular GSH, which may be an early event leading to irreversible renal cell injury [10, 11]. However, it is not known whether GSH depletion is associated with $NiCl_2$-induced LPO. Therefore, we examined the effects of $NiCl_2$ on GSH depletion in the presence of various drugs that modulate LPO, and the results are summarized in Table 1. The results indicate that thiols (GSH and DTT) and DFX significantly restored the $NiCl_2$-induced loss of GSH content. On the other hand, DMTU and SOD failed to reduce the loss of GSH content due to $NiCl_2$.

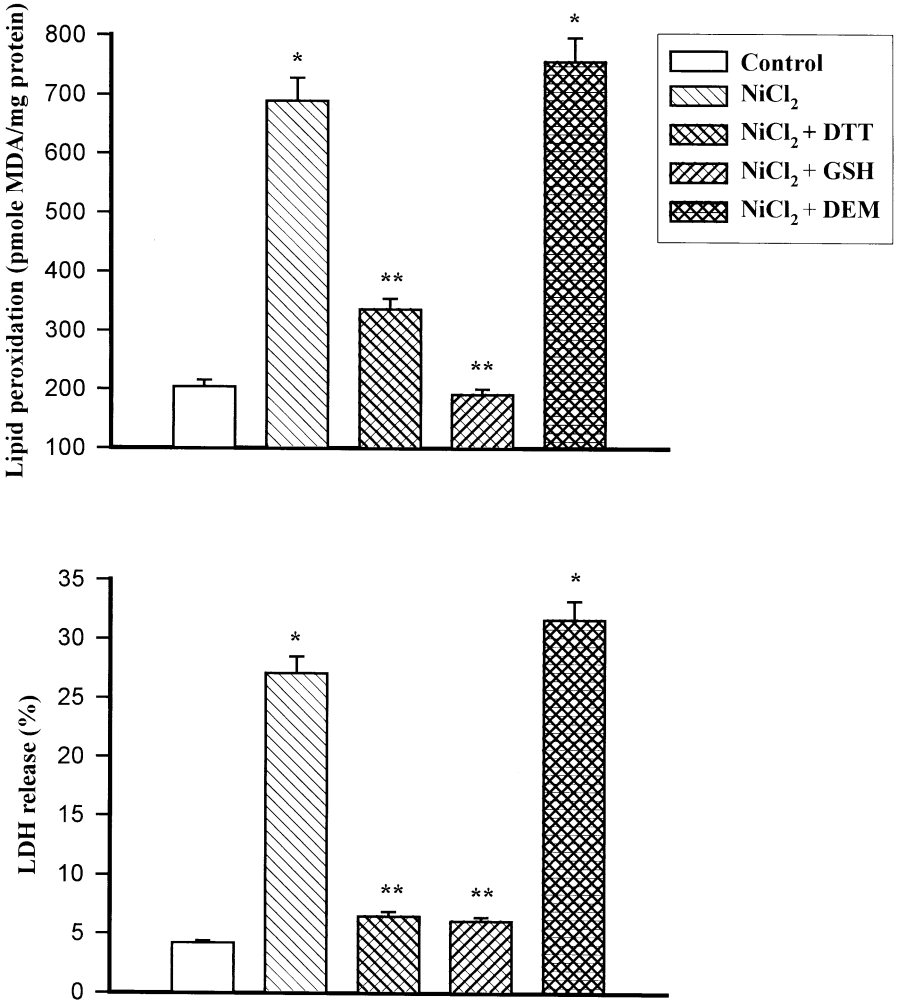

FIG. 3. Effects of GSH, DTT, and DEM on $NiCl_2$-induced LDH release and LPO in rat renal cortical slices. Slices were treated with 1 mM $NiCl_2$ in the presence or absence of either 5 mM GSH or 5 mM DEM or 2 mM DTT for 3 hr at 37°. For other details, see Materials and Methods. The results are means ± SEM of four experiments. Key: (*) $P < 0.05$ compared with the control; and (**) $P < 0.05$ compared with $NiCl_2$ alone. The amount of LDH released was calculated from the total LDH (tissue LDH plus LDH in the medium) minus LDH remaining in the tissue and is expressed as a percent of total LDH. The control value of LDH was 3.92 ± 0.15 (U/g wet wt).

Effects of Various Drugs on $NiCl_2$-Induced Inhibition of PAH Uptake

We have measured the concentration-dependent effects of $NiCl_2$ on PAH uptake by rat renal cortical slices. $NiCl_2$ inhibited PAH uptake in a concentration-dependent manner over the concentration range of 0.25 to 2.0 mM, showing 50% inhibition at approximately 0.65 mM $NiCl_2$ (data not shown). We further examined whether the mechanism of inhibition of PAH uptake due to $NiCl_2$ was different from that involving $NiCl_2$-induced LDH release. The results are summarized in Table 2. $NiCl_2$ at a 1 mM concentration reduced the PAH uptake by renal cortical slices significantly. This inhibition of PAH uptake was prevented by thiol reagents, such as GSH and DTT, and by DFX and DMTU (Table 2).

Effect of Various Drugs on the Uptake of Nickel (Ni^{2+}) from $NiCl_2$ by Rat Renal Cortical Slices

It is seen from Table 3 that a moderate amount of ionic nickel (Ni^{2+}) remained associated with renal cortical slices following a 3-hr incubation at 37° in buffered saline, pH 7.4, in the presence of 1 mM $NiCl_2$. Coincubation of either 5 mM GSH or 2 mM DTT with 1 mM $NiCl_2$ in the same buffer markedly decreased the accumulation of Ni^{2+} in the rat renal cortex. In contrast to these effects due to GSH and DTT, coincubation of 1 mM $NiCl_2$ with 0.1 mM DFX or with 20 mM DMTU, under similar conditions as above, failed to inhibit significantly the uptake of nickel ion from 1 mM $NiCl_2$ by renal cortical slices (Table 3).

DISCUSSION

In cultured cells and animal studies, nickel compounds have been shown to modify the cellular antioxidant system differentially, depending upon the animal species and the organ as well as the dose of nickel administration [29]. *In vivo* studies have shown that the induction of LPO by nickel compounds is associated with decreased cellular levels of antioxidants, in particular GSH and glutathione peroxidase activity [13]. In spite of this evidence, studies examining the effect of antioxidants on nickel-induced lethality in mammalian cells and animal studies are very limited at present, and hence, the underlying mechanisms have not been elucidated yet. Our present studies have shown that $NiCl_2$ treatment *in vitro* caused LDH release

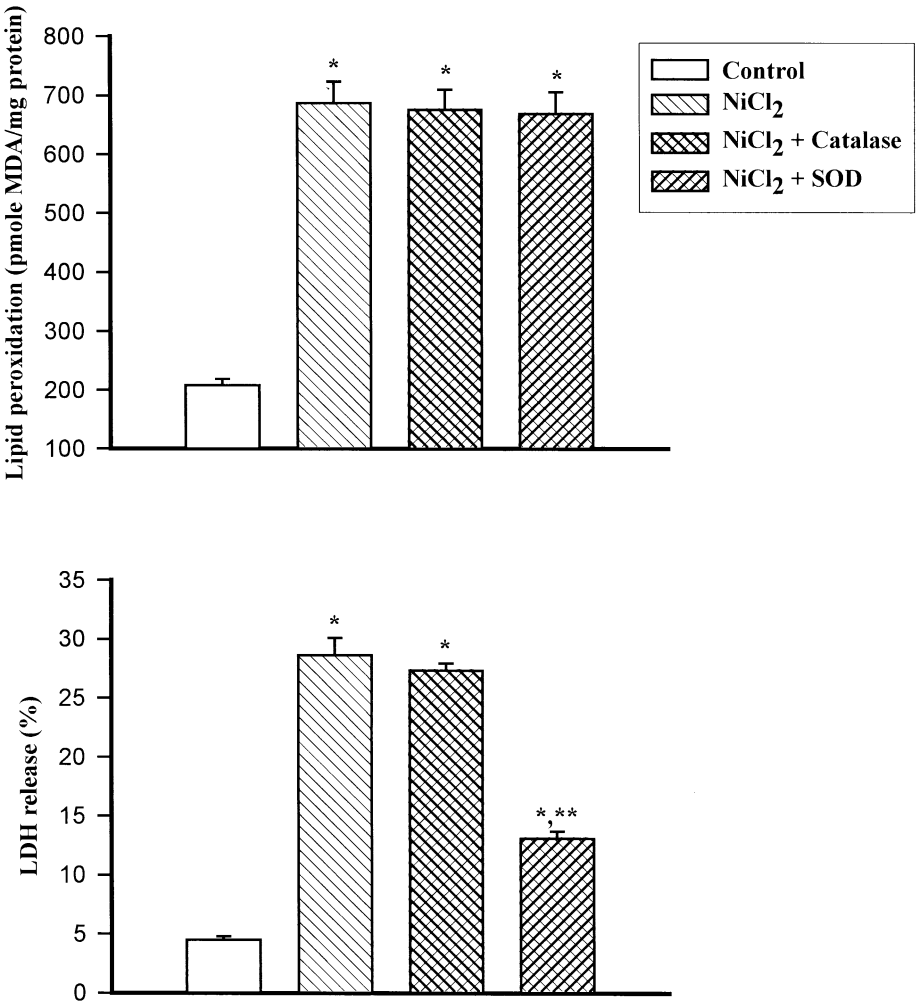

FIG. 4. Effects of SOD and catalase on $NiCl_2$-induced LDH release and LPO in rat renal cortical slices. Slices were treated with 1 mM $NiCl_2$ in the presence or absence of SOD or catalase for 3 hr at 37°. For other details, see Materials and Methods. The results are means ± SEM of five experiments. Key: (*) $P < 0.05$ compared with the control; and (**) $P < 0.05$ compared with $NiCl_2$ alone. The amount of LDH released was calculated from the total LDH (tissue LDH plus LDH in the medium) minus LDH remaining in the tissue and is expressed as a percent of total LDH. The control value of LDH was 3.85 ± 0.16 (U/g wet wt).

and LPO in a concentration-dependent manner and that these effects could be prevented by thiols (GSH and DTT) in rat renal cortical slices. The decrease of Ni(II)-induced renal cell injury and LPO by exogenous GSH and DTT may be ascribed to binding of Ni(II) with GSH and DTT. Consequently, such a mechanism would result in reduction of cellular uptake of nickel ion (Ni^{2+}), as observed in the present study (Table 3). Thus, these data suggest that the depletion of cellular thiols may contribute to the development of $NiCl_2$-induced nephrotoxicity. Similarly, thiols significantly prevented the inhibition of PAH uptake and the GSH depletion induced by $NiCl_2$ in isolated rat renal cortical slices. Therefore, these results again suggest that thiols may play an important role in mediating $NiCl_2$-induced renal cell injury. However, such renal cell injury cannot be considered to be dependent solely upon endogenous nonprotein thiols, as supported by the following arguments: (a) the depletion (~66%) of intracellular GSH by DEM did not result in further significant increase in $NiCl_2$-induced renal cell injury (Fig. 3), and (b) exogenous addition of both GSH and DTT failed to inhibit the uptake of Ni^{2+} by the renal cortex completely (Table 3).

Although DFX already is known to chelate iron and abolish its redox activity at physiological pH, there is a possibility that it may also be able to chelate other transition metals, including nickel. Therefore, DFX also can modify the cellular uptake and redox activity of nickel(II) as well. However, DFX treatment actually failed to inhibit the uptake of nickel(II) ion by renal cortical slices significantly (Table 3). Thus, it is evident that chelation of Ni(II) by DFX is not important under the experimental conditions used in this study. However, DFX may also act as a free radical scavenger. Therefore, the protective effects of DFX observed in this study were due to DFX acting as both an iron chelator and a free radical scavenger. Thus, the results of this study have illustrated two principal characteristic features of $NiCl_2$-induced renal cytotoxicity in rats. First, LPO and its degradation products are not major contributors to renal cell death, as evidenced by the detection of significant amounts of LDH leakage 2 hr prior to LPO and by the observation that once LPO occurred, the amount of LDH leakage was not affected by it (Fig. 2). Second, the generation of hydroxyl radicals by the Fenton reaction is largely responsible for $NiCl_2$-induced cytotoxic damage to the rat kidney, as evidenced by the complete inhibition of LDH leakage from the renal cortical slices by the iron-specific chelator DFX. Similar phenomena have been observed in another toxicity study. Thus, in

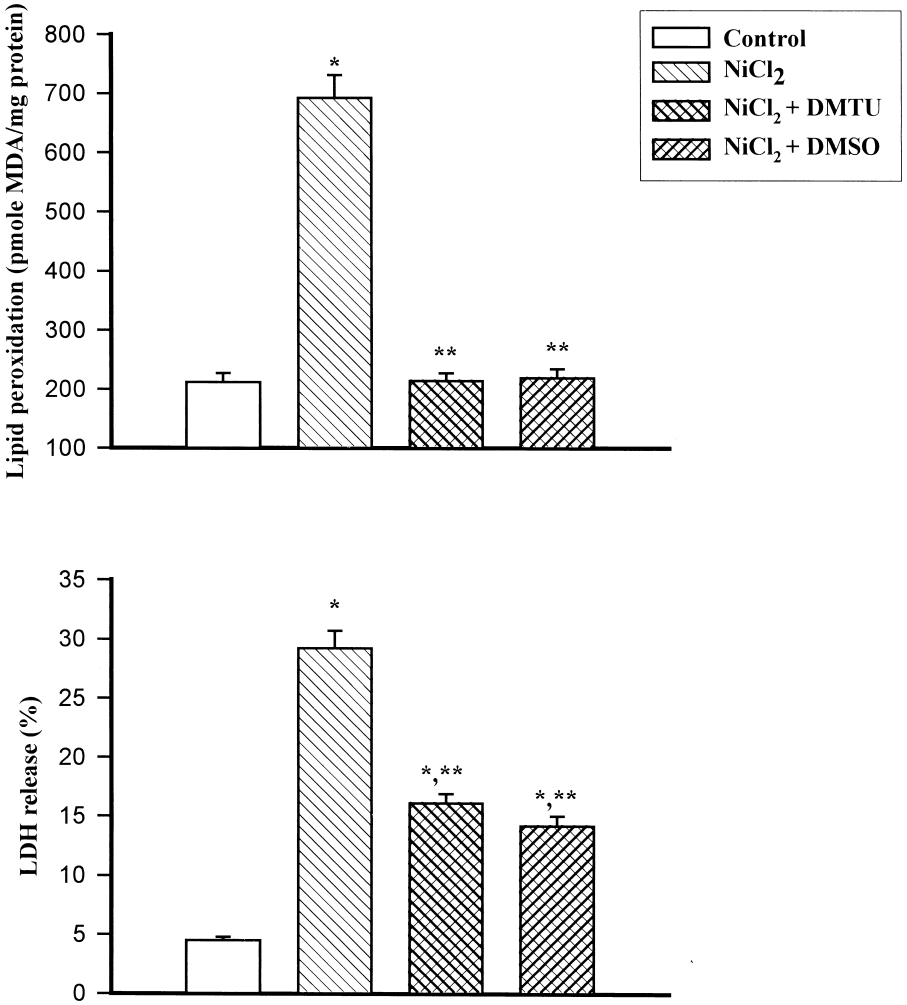

FIG. 5. Effects of hydroxyl radical scavengers on $NiCl_2$-induced LDH release and LPO in rat renal cortical slices. Slices were treated with 1 mM $NiCl_2$ in the presence or absence of 20 mM DMTU or 30 mM DMSO for 3 hr at 37°. For other details, see Materials and Methods. The results are means ± SEM of five experiments. Key: (*) $P < 0.05$ compared with the control; and (**) $P < 0.05$ compared with $NiCl_2$ alone. The amount of LDH released was calculated from the total LDH (tissue LDH plus LDH in the medium) minus LDH remaining in the tissue and is expressed as a percent of total LDH. The control value of LDH was 4.05 ± 0.19 (U/g wet wt).

an *in vivo* study involving $NiCl_2$ it has been observed that DFX treatment inhibits DNA strand breakage completely but has no effect on LPO in rat liver [30]. This suggests that LPO is not related to such DNA strand breakage, but may be caused by hydroxyl radicals generated by the induction of the Fenton reaction [30].

A partial reduction of LDH release by SOD but not by catalase also was observed in the present study, suggesting that superoxide anion and not H_2O_2 may be partly associated with the $NiCl_2$-induced lethal cell injury in rat kidney. Alternatively, bulky catalase, unlike nickel, failed to enter the renal cells, and hence could not prevent internalized nickel from reacting with metabolic H_2O_2. But the results of the present study do not support the hypothesis that superoxide anion and H_2O_2 are involved in $NiCl_2$-induced lipid peroxidation in rat kidney (Fig. 4). A similar phenomenon also was observed in $NiCl_2$-induced LPO in rat liver, that is to say, such enhanced LPO in rat liver is not inhibited by catalase and SOD [9].

The failure of DFX to inhibit LPO was not due to its failure to reach the target site in the kidney, because DFX was found to be quite effective in protecting against $NiCl_2$-induced lethal renal cell injury (as measured by LDH leakage). Thus, it is possible that $NiCl_2$-induced LPO is not iron-dependent. The reactive species responsible for initiating nickel-dependent LPO have not been identified clearly in the target organs. In the presence of trace quantities of iron, it is known that hydroxyl radicals are formed through the Haber–Weiss and/or Fenton reactions [31]. Since DFX reduced $NiCl_2$-induced renal cell death effectively, it does indicate that iron-dependent formation of hydroxyl radical is involved in $NiCl_2$-induced renal cell injury. Since DFX was found to be ineffective in $NiCl_2$-induced LPO, it suggests that a mechanism other than the iron-dependent Fenton reaction was responsible for this LPO. In principle, Ni(II)/Ni(III) may undergo redox cycling and maintain this reaction, especially when bound to protein [32, 33]. While such a mechanism might be hypothesized to explain nickel(II)-induced LPO, another possibility may include direct metal attack to initiate LPO.

A somewhat similar type of mechanism has been reported in $NiCl_2$-induced DNA adducts. $NiCl_2$ in the presence of H_2O_2 caused two major DNA adducts *in vitro* that were inhibited by hydroxyl radical scavengers such as sodium formate and *p*-nitrosodimethylamine and by the $^1O_2$ scavenger sodium azide [32]. Thus, the DNA adducts may be formed via hydroxyl radicals and $^1O_2$. Furthermore, these adducts looked similar to those induced by $FeSO_4$ and

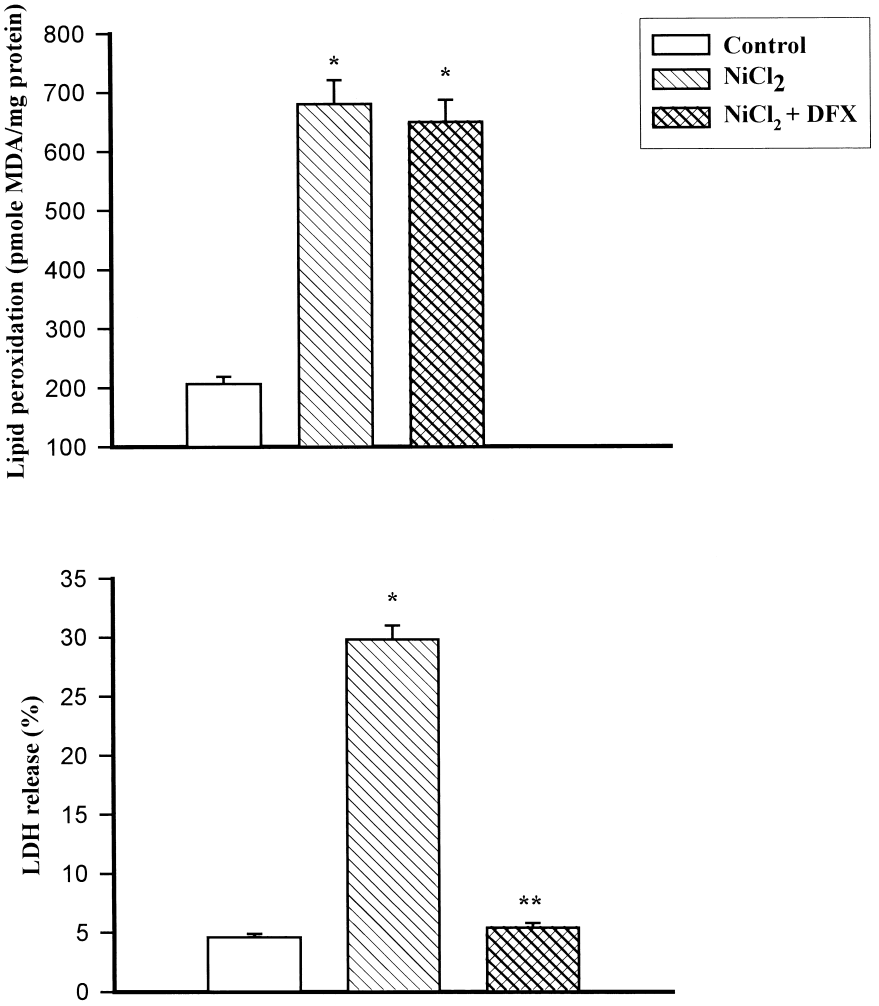

FIG. 6. Effects of the iron chelator DFX on $NiCl_2$-induced LDH release and LPO in rat renal cortical slices. Slices were treated with 1 mM $NiCl_2$ in the presence or absence of 0.1 mM DFX for 3 hr at 37°. For other details, see Materials and Methods. The results are means ± SEM of five experiments. Key: (*) $P < 0.05$ compared with the control; and (**) $P < 0.05$ compared with $NiCl_2$ alone. The amount of LDH released was calculated from the total LDH (tissue LDH plus LDH in the medium) minus LDH remaining in the tissue and is expressed as a percent of total LDH. The control value of LDH was 3.87 ± 0.18 (U/g wet wt).

H_2O_2, showing that a Fenton-type reaction was involved in the induction of DNA adducts by $NiCl_2$ and H_2O_2.

Thus, the prevention of $NiCl_2$-induced renal cell death by DFX suggests that the mechanism of such cell death involves a cytotoxic induction of the Fenton reaction, with the subsequent formation of hydroxyl radical, thereby causing direct cell damage. Cellular damage caused by exposure to $NiCl_2$ could result in production of hydroxyl radicals by lysis of iron storage vacuoles, causing the release of iron from ferritin, or by causing a high uptake and accumulation of iron [9]. Interestingly, however, hydroxyl radical scavengers also partially attenuated the LDH release, and the inhibition of PAH uptake, induced by $NiCl_2$, while they produced a significant but complete reduction of LPO. This suggests that hydroxyl radicals may be related to the initiation of $NiCl_2$-induced LPO, as well as the cell

TABLE 1. Effects of $NiCl_2$ on cellular GSH concentration in the presence of various drugs

Treatment	GSH (μmol/g)
Control	1.85 ± 0.13
$NiCl_2$ (1 mM)	0.83 ± 0.07*
+ GSH (2 mM)	1.61 ± 0.15†
+ DTT (2 mM)	1.53 ± 0.06†
+ DFX (0.1 mM)	1.45 ± 0.11†
+ SOD (500 U/mL)	0.86 ± 0.05*
+ DMTU (20 mM)	0.89 ± 0.07*

Renal cortical slices were treated with 1 mM $NiCl_2$ in the presence or absence of various drugs for 120 min at 37°, and GSH concentration was estimated. Data are the means ± SEM of five experiments.

*$P < 0.05$ compared with the control.

†$P < 0.05$ compared with $NiCl_2$ alone.

TABLE 2. Effects of $NiCl_2$ on the renal cortical slice accumulation of PAH in the presence of various drugs

Treatment	PAH uptake (S/M ratio)
Control	11.8 ± 0.56
$NiCl_2$ (1 mM)	3.4 ± 0.22*
+ GSH (5 mM)	7.9 ± 0.52†
+ DTT (2 mM)	7.1 ± 0.48*†
+ DFX (0.1 mM)	7.6 ± 0.68†
+ DMTU (20 mM)	8.7 ± 0.64†

Renal cortical slices were treated with 1 mM $NiCl_2$ in the presence or absence of various drugs for 120 min at 37°. PAH uptake was measured as described in Materials and Methods. Data are means ± SEM of five experiments.

*$P < 0.05$ compared with the control.

†$P < 0.05$ compared with $NiCl_2$ alone.

TABLE 3. Effects of various drugs on the uptake of nickel (Ni^{2+}) by rat renal cortical slices simultaneously treated with 1 mM $NiCl_2$ for 3 hr at 37°

Incubation conditions	Uptake of Ni^{2+}* (μg/mL tissue supernatant)
1 mM $NiCl_2$ in Tris-HCl buffer, pH 7.4	32.5 ± 2.4
1 mM $NiCl_2$ plus 5 mM GSH	12.6 ± 1.0† (61)
1 mM $NiCl_2$ plus 2 mM DTT	13.9 ± 1.2† (57)
1 mM $NiCl_2$ plus 0.1 mM DFX	23.1 ± 2.2 (29)
1 mM $NiCl_2$ plus 20 mM DMTU	29.9 ± 2.4 (8)

*Results are means ± SEM of four different experiments. The values in parentheses represent the percentage of reduction from the corresponding value of 1 mM $NiCl_2$ alone. For details, see Materials and Methods.

†Significantly different from the $NiCl_2$-alone-treated group, $P < 0.05$.

injury induced by $NiCl_2$. However, since early cell death, as signified by release of LDH from renal cortical slices, preceded the onset of LPO by 2 hr (Fig. 2), it indicates that LPO was a result, and not the cause, of cell damage by Ni^{2+}. In other words, the $NiCl_2$-induced nephrotoxicity could result from an LPO-independent mechanism.

Mortality and hepatic LPO in $NiCl_2$-treated mice were found to be correlated with the decreased GSH content [33]. Although $NiCl_2$ has been reported to decrease intracellular GSH concentration of renal cells *in vivo* and *in vitro* [29], the interrelationship between GSH depletion and LPO due to $NiCl_2$ remains to be defined. In this study, $NiCl_2$ caused a marked reduction in cellular GSH content, which was reversed significantly by antioxidants such as GSH, DTT, and DFX (Table 1). This was due to chelation of Ni(II) with these antioxidants, GSH and DTT. These results could be interpreted to indicate that GSH depletion of renal cells may be related to the $NiCl_2$-induced LPO. However, a hydroxyl radical scavenger, DMTU, did not prevent $NiCl_2$-induced GSH depletion, although it inhibited LPO significantly. Since SOD and DMTU do not sequester Ni^{2+}, they had no effect on cellular GSH content. Therefore, the effects of antioxidants on cellular GSH may result from increased biosynthesis of GSH or Ni(II) chelation with these antioxidants rather than inhibition of LPO. Furthermore, since DFX prevented $NiCl_2$-induced depletion of cellular GSH content, it emphasizes again the involvement of an iron-dependent free radical mechanism in the nephrotoxicity of $NiCl_2$.

In summary, $NiCl_2$ induced LDH release and LPO, inhibited PAH uptake, and reduced cellular GSH content in rat renal cortical slices. These changes were prevented by GSH, DTT, antioxidants, and DFX. LPO was not related causally to renal cell death. $NiCl_2$-induced cytotoxicity to renal cells may be caused by the induction of the Fenton reaction, generating hydroxyl radicals.

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