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Nephrotoxicity Induced by the R- and S-Enantiomers of N-(3,5-Dichlorophenyl)-2-hydroxysuccinimide (NDHS) and Their Sulfate Conjugates in Male Fischer 344 Rats

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Abstract

The agricultural fungicide N-(3,5-dichlorophenyl)succinimide (NDPS) induces nephrotoxicity characterized as polyuric renal failure and mediated via metabolites arising from oxidation of the succinimide ring. Recent findings have suggested that the stereochemical nature of NDPS metabolites may be an important factor in NDPS metabolite-induced nephrotoxicity. The purpose of the present study was to determine the role of stereochemistry in the *in vivo* nephrotoxicity induced by R-(+)- and S-(-)-N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (R- and S-NDHS) and the *in vitro* nephrotoxicity induced by their enantiomeric sulfate conjugates, R-(-)- and S-(+)-N-(3,5-dichlorophenyl)-2-hydroxysuccinimide-O-sulfate (R- and S-NSC). Male Fischer 344 rats (4 rats/group) were administered intraperitoneally (ip) an enantiomer of NDHS (0.05, 0.1 or 0.2 mmol/kg) or vehicle, and renal function monitored for 48 h. R-NDHS (0.1 or 0.2 mmol/kg) had little effect on renal function. In contrast, S-NDHS (0.1 mmol/kg) induced marked nephrotoxicity. The nephrotoxic potential of R- and S-NSC (0.5, 0.75 or 1.0 mM) was determined using freshly isolated rat renal cortical cells (IRCC, $3-4 \times 10^6$ cells/ml). Cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) at the end of a 1 h incubation period. The LDH release observed in these studies was similar between R- and S-NSC. These results indicate that stereochemistry is an important factor for NDPS metabolite nephrotoxicity and that the role of stereochemistry, at least for NSC, occurs at extra-renal sites.

Keywords

Nephrotoxicity; Fungicides; N-(3,5-dichlorophenyl)-2-hydroxysuccinimide; Sulfate Conjugation; Rats

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1. Introduction

N-(3,5-Dichlorophenyl)succinimide (NDPS) was developed as an agricultural fungicide in Japan during the early 1970s (Fujinami et al., 1971,1972). Although NDPS was a highly efficacious agent, potential health concerns associated with NDPS exposure have limited its usefulness in agriculture (Heaton, 1980). Nonetheless, NDPS is still available in several countries, including the United States. The primary toxicity induced by exposure to NDPS is nephrotoxicity. Administration of NDPS to male Sprague-Dawley or Fischer 344 rats at intraperitoneal (ip) doses of 0.4 mmol/kg or greater results in acute polyuric renal failure and proximal tubular necrosis (Rankin, 1982; Rankin et al., 1984,1985). Chronic exposure to NDPS in food (5000 ppm) results in the development of chronic interstitial nephritis in rodents (Sugihara et al., 1975;Barrett et al., 1983).

NDPS is extensively biotransformed in the liver through hydrolysis (ring opening) and oxidation pathways (Fig. 1), and less than 1% of NDPS is excreted unchanged (Ohkawa et al., 1974;Nyarko and Harvison, 1995; Griffin and Harvison, 1998). The NDPS metabolite that results from hydrolysis, N-(3,5-dichlorophenyl)succinamic acid (NDPSA), is a weakly nephrotoxic metabolite at ip doses up to 1.0 mmol/kg, but accounts for ~40% of urinary metabolites (Yang et al., 1985;Griffin and Harvison, 1998). Oxidation of the succinimide ring results in the formation of N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS), which can be hydrolyzed in vivo to form N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (2-NDHSA) and N-(3,5-dichlorophenyl)-3-hydroxysuccinamic acid (3-NDHSA). Although NDHS is not detected in urine, 2- and 3-NDHSA constitute ~30 and 20% of urinary NDPS metabolites, respectively (Griffin and Harvison, 1998). Both NDHS and 2-NDHSA are at least four times more potent than NDPS as a nephrotoxicant in rats with 3-NDHSA being approximately equipotent to NDPS in inducing nephrotoxicity (Hong et al., 1998; Rankin et al., 1989; 1991a,b;1994) When 2-NDHSA undergoes decarboxylation, N-(3,5-dichlorophenyl) malonic acid (DMA), a non-nephrotoxic metabolite that constitutes ~10% of urinary NDPS metabolites is formed (Rankin et al., 1988;Griffin and Harvison, 1998). Oxidation of the phenyl ring of NDPS also results in minor non-nephrotoxic metabolites (Harvison et al., 1992). Thus, the primary route of bioactivation of NDPS to a nephrotoxic metabolite(s) initially involves oxidation of the succinimide ring to form NDHS and 2-NDHSA.

Previous studies have determined that Phase II conjugates (glucuronide and sulfate conjugates) of NDPS metabolites contribute to the primary mechanism of NDPS nephrotoxicity (Hong et al., 1999a-d;Rankin et al. 1997). Recently, Cui et al. (2005) reported the detection of small amounts of the O-glucuronide and O-sulfate conjugates of 2-/3-NDHSA in the urine of rats treated with NDPS. It was determined that these conjugates were formed by conjugation of NDHS followed by hydrolysis to the NDHSA conjugates. While earlier studies had demonstrated that N-(3,5-dichlorophenyl)-2-hydroxysuccinimide-O-sulfate (NSC, Fig. 2) was a nephrotoxicant in vitro (Rankin et al., 2001a), Cui et al. (2005) also found that the NDHS conjugates are highly reactive and readily form glutathione-derived conjugates. Thus, the mechanism of NDPS nephrotoxicity appears to be linked to the formation of these reactive metabolites.

An asymmetric carbon atom is present at the 2-position of the succinimide ring in NDHS and in the ethylene bridge of 2-NDHSA. Therefore, two stereoisomers, the R- and S-enantiomers, for NDHS and 2-NDHSA are possible. In a previous study, it was noted that the two enantiomers of 2-NDHSA exhibited very different nephrotoxic potentials (Rankin et al., 2001b). The S-(-)-2-NDHSA enantiomer was a potent nephrotoxicant, while the R-(+)-2-NDHSA enantiomer was a weak nephrotoxicant at the doses tested. Thus, for at least one nephrotoxicant metabolite of NDPS, there is enantiomer selective nephrotoxicity. However, it

is not known if this stereoselectivity for nephrotoxic potential applies to NDHS or if the mechanism for this enantiomeric selectivity has a renal or extra-renal origin.

The purpose of this study was to examine the nephrotoxic potential of the two enantiomers of NDHS (Fig. 2) *in vivo* using male Fischer 344 rats as the animal model. The two enantiomers of NSC (Fig. 2) were examined *in vitro* using freshly isolated renal cortical cells from male Fischer 344 rats to determine if, at least for NSC, the stereoselectivity in the nephrotoxic response derived from a renal or extra-renal mechanism.

2. Materials and methods

2.1 Experimental animals

Male (200-250 g) Fischer 344 rats were obtained from Hilltop Lab Animals Inc. (Scottsdale, PA) and housed in standard plastic animal cages (four rats/cage) prior to use. Animal holding and experimental rooms had a controlled light period (on 06.00 h, off 18.00 h), humidity (40-55%) and temperature (21-23°C). At least one week was allowed for acclimation to the animal facilities prior to initiation of experiments. All animals were maintained and handled in agreement with the Institutional Guide for the Care and Use of Laboratory Animals and all procedures were approved by the Marshall University Institutional Animal Care and Use Committee.

2.2 Chemicals

R-(+)- and S-(-)-NDHS were synthesized, purified and characterized as previously described for racemic NDHS starting with R-(+)- and S-(-)-malic acid respectively (Shih and Rankin, 1989; Rankin et al., 2002). R-(-)- and S-(+)-NSC were synthesized as previously described by Rankin et al. (2002) from the pure corresponding enantiomer of NDHS and chlorosulfonic acid. Purity and specific rotations of the NDHS and NSC enantiomers were determined as previously reported (Rankin et al., 2002). Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific Co. (Pittsburgh, PA, USA) and was certified A.C.S. grade. Mazola corn oil (100% pure) was obtained from a local vendor. Blood urea nitrogen (BUN) concentration was determined using Sigma Chemical Co. (St. Louis, MO, USA) Kit No. 640 utilizing the Berthelot reaction, while urinary glucose was measured using Sigma Kit No. 510 using the glucose oxidase assay. Coomassie brilliant blue G-250 dye was purchased from Calbiochem Corp. (La Jolla, CA, USA). All other chemicals were obtained from Sigma Chemical Co. or Aldrich Chemical Co. (St. Louis, MO, USA) and were of the highest purity available.

2.3 *In vivo* experiments

In vivo experiments were conducted as previously described for racemic NDHS (Hong et al., 1998). After the acclimation period, rats (four rats/group) were placed singly in stainless steel metabolism cages to provide for the separation of urine from feces. Rats were allowed one acclimation day in the metabolism cages, followed by a control day (day 0) to obtain baseline values. On the following day, rats were administered either R- or S-NDHS (0.05, 0.1 or 0.2 mmol/kg, 2.5 ml/kg, *ip*) or vehicle (12.5% DMSO in corn oil; 2.5 ml/kg, *ip*) and renal function monitored through 48 h. These doses were selected since 0.1 mmol/kg is the minimal nephrotoxic dose for racemic NDHS in male Fischer 344 rats (Hong et al., 1998). If nephrotoxicity was observed at this dose, the dose was reduced in a second group of rats to 0.05 mmol/kg. If minimal or no nephrotoxicity was observed at 0.1 mmol/kg, the dose was increased to 0.2 mmol/kg. Urine volume, food and water intake, and body weight were measured on day 1 and day 2. Control groups were pair-fed to the appropriate treated group to assure that any changes in renal function observed was due to chemical-induced toxicity rather than altered food intake. Each day food was withheld for a 6-h period to allow for the collection of a urine sample uncontaminated by food. The 6-hr urine sample was collected in an ice cold

container for the analysis of urinary protein and glucose. Urinary protein concentration was quantitated spectrophotometrically at 595 nm using Coomassie Blue with bovine albumin (fraction V) serving as the protein standard (Bradford, 1976).

At 48 h post-treatment with an NDHS enantiomer or vehicle, rats were anesthetized with diethyl ether and laparotomized. Blood was withdrawn from the dorsal aorta into heparinized syringes and plasma was obtained following centrifugation ($3,000 \times g$, 5 min). Plasma was stored at -20°C for BUN concentration determination. A sample of blood was also obtained from the tail of each rat prior to placement in a metabolism cage to allow for the determination of the day 0 BUN concentration. After obtaining the aortic blood sample, the kidneys from each rat were rapidly removed and weighed. Kidneys were quartered and placed in 10% neutral buffered formalin. Tissues from all treated and control rats were examined using light microscopy for evidence of chemically induced morphological changes.

In all experiments, control rats were pair-fed to the appropriate treated group to insure that any observed effects were chemically induced and not the result of altered food intake.

2.4 *In vitro* experiments

Untreated rats were anesthetized with pentobarbital sodium (75 mg/kg, ip) and isolated renal cortical cells (IRCC) were obtained using the collagenase perfusion method of Jones et al. (1979). Initial cell viability was typically 85-95% as judged by the exclusion of trypan blue (2% w/v) and initial lactate dehydrogenase (LDH) release ($\sim 5 - 10\%$). The yield of cortical cells for these experiments normally ranged between 35 - 45 million cells/two kidneys. Isolated cells were resuspended at a concentration of 3 - 4 million cells/ml in Krebs-Henseleit buffer pH 7.37 containing 25 mM Hepes and 2% (w/v) bovine serum albumin. Toxicity experiments were conducted as previously reported (Rankin et al., 2001a) and begun by placing 3 ml of the IRCC resuspension in a 25 ml polycarbonate Erlenmeyer flask. The flask was placed in a shaking incubator (37°C water temperature) and sealed with a serum bottle stopper containing an inlet and outlet for gas flow. The atmosphere within the flask was equilibrated with 95% $\text{O}_2/5\%$ CO_2 and shaking for 5 min. An NSC enantiomer (0.5, 0.75 or 1.0 mM) or vehicle (distilled water, 30 μl) was then added, and the incubations continued for 1 h. The concentrations of NSC tested were selected based on our previous findings for cytotoxic concentrations of racemic NSC (Rankin et al., 2001a). At the end of the 1 h incubation period, flasks were removed, and a 0.5 ml aliquot was taken from each flask for determination of LDH release. Each aliquot was centrifuged ($3,000 \times g$, 5 min), and the supernatant decanted and saved. Pelleted cells were resuspended in 1 ml of Triton X-100 (10% solution) to release the remaining cellular LDH activity. LDH activity was then determined (Sigma Kit No. LDL-20) in each fraction and LDH release was expressed as % of total.

2.5 Statistical analysis

Values are expressed as the mean $\pm$ S.E. for $N=4$ rats per in vivo group and for four experiments per in vitro group. Values from each rat used in the in vitro experiments represented an $N = 1$, such that each in vitro experiment was repeated four times. Data were analyzed using a one- or two-way analysis of variance (ANOVA) followed by a Dunnett's or Newman-Keuls analysis. All statistical tests were run at a 95% confidence interval and significance noted at $P < 0.05$.

3. Results

3.1 *R*- and *S*-NDHS nephrotoxicity in vivo

Rats treated with *R*-NDHS (0.1 mmol/kg) exhibited little evidence of nephrotoxicity. Food and water intake (Table 1), urine volume (Fig. 3), urinary protein (Fig. 4) and urinary glucose

(Table 2) excretion, and kidney weight (Fig. 5) were not altered in the R-NDHS (0.1 mmol/kg) treatment group. BUN concentration decreased slightly on day 2 relative to day 0 value for the treated group and the day 2 value for the control group (Table 2), but this change is not considered to be toxicologically relevant. Increasing the dose of R-NDHS to 0.2 mmol/kg also had no effect on food intake (Table 1) or urine volume (Fig. 3A) on days 1 and 2 or kidney weight on day 2 (Fig. 5). A small, but significant, increase in water intake on day 2 (Table 1), urinary protein excretion on day 1 (Fig. 4A), urinary glucose excretion on days 1 and 2 (Table 2) and BUN concentration on day 2 (Table 2) suggest that a weak nephrotoxic response was observed at this dose level. However, it is unlikely that the very small change in BUN concentration observed in the treated group is toxicologically important, since it is almost identical to the control group value for that day's measurement.

R-NDHS administration did not induce marked renal morphological changes. In the R-NDHS 0.1 mmol/kg treatment group, findings ranged from normal to mild scattered hydropic changes in some proximal tubular epithelial cells (S1 and S2 segments). In the 0.2 mmol/kg treatment group, morphological changes were mostly minor, but more pronounced than in the lower dose group. Foci of mild hydropic changes with cytoplasmic vacuoles were noted in proximal tubular cells (S1 and S2 segments) with small protein casts focally seen in distal tubules. Occasional dilation of both proximal and distal tubules was noted. These morphological changes are in line with previous findings with racemic NDHS (Hong et al., 1999d; Rankin et al., 1988) and support a weak nephrotoxic response to even the high dose of R-NDHS used in this study.

In contrast to the results observed in the R-NDHS treated animals, rats treated with S-NDHS exhibited greater evidence of nephrotoxicity. In the S-NDHS (0.05 mmol/kg) treated group, there was no significant effect on food or water intake (Table 1) or urinary protein excretion (Fig. 4B) on days 1 and 2 or kidney weight (Fig. 5) on day 2. On day 2, the BUN concentration was slightly, but significantly, increased relative to the day 0 value within the group and from the day 2 control group value (Table 2). However, urinary volume (Fig. 3B) was elevated relative to the control group value on both post-treatment days and urinary glucose excretion (Table 2) was markedly elevated on day 1 compared to the control group value. Increasing the S-NDHS dose to 0.1 mmol/kg resulted in a marked decrease in food intake on days 1 and 2 (Table 1) and increased urine volume within the treatment group on day 1 and above control group values on days 1 and 2 (Fig. 3B). Increased proteinuria (Fig. 4B) was observed on days 1 and 2 and elevated glucosuria was seen on day 1 relative to the day 0 and control group value (Table 2). Kidney weight was significantly increased on day 2 compared to the control group value (Fig. 5). Although BUN concentration was not significantly increased (Table 2), two of the four rats have BUN concentration levels at 48 h of 52 and 81 mg%. Thus, S-NDHS is a more potent nephrotoxicant than R-NDHS *in vivo*.

Morphological changes observed in kidneys from the S-NDHS 0.05 mmol/kg treatment group included mild to moderate hydropic changes in the cytoplasm of proximal tubular cells (S1 and S2), dilation of distal tubules and focal protein casts within some distal tubules. In the S-NDHS 0.1 mmol/kg treatment group, these changes were more dramatic with one rat exhibiting severe proximal tubular necrosis, prominent protein casts and severe dilation of distal tubules. These morphological changes are consistent with previous findings induced by nephrotoxic doses of racemic NDHS (Hong et al., 1999a,d; Rankin et al., 1988).

3.2 R- and S-NSC nephrotoxicity *in vitro*

R- and S-NSC were evaluated for nephrotoxic potential *in vitro* using IRCC. R-NSC increased LDH release at 1 h with all concentrations tested (0.5 - 1.0 mM) (Fig. 6). S-NSC increased LDH release at the 0.75 and 1.0 mM concentrations after a 1 h incubation (Fig. 6). While R-NSC increased LDH release in the 0.5 mM group and S-NSC did not, there were no differences

between the corresponding group values. Thus, there do not appear to be any significant differences in the renal response to the two enantiomers of NSC.

4. Discussion

Biological activity or pharmacodynamic properties of drugs and toxicants can be stereoselective in nature (Mathison et al., 1995). One example of this effect is that while the anti-inflammatory activity of the non-steroidal anti-inflammatory drugs resides mainly in the S-(+)-enantiomers (de la Lastra et al., 2000), R-ibuprofen stereoselectively inhibits beta-oxidation of lipids (Browne et al., 1999). An example where toxicants exhibit stereoselective properties can be found with 6-amino-3-(chloromethyl)-1-[5,6,7-trimethoxyindol-2-yl]carbonyl]indoline where the S-(+)-enantiomer is three times more cytotoxic than the R-(-)-enantiomer in AA8 cells (Tercel et al., 1999). Additionally, the (-)-enantiomer of difluoromethylornithine induces deafness in guinea pigs, while the (+)-enantiomer is not ototoxic (McWilliams et al., 2000). Therefore, enantiomers of a compound can exhibit differences in the magnitude or nature of the biological effects the compound induces. However, there are few examples of stereoselective nephrotoxics. For example, L-serine is a beneficial, naturally occurring amino acid, but D-serine is a nephrotoxicant (Carone and Ganote, 1975; Willimas and Lock, 2005). The S-enantiomer of ganciclovir is a nephrotoxicant in mice at 50 mg/kg/day for 15 days, while the R-enantiomer is not a nephrotoxicant at twice the dose (Smee et al., 1994). We also previously reported the stereoselective nephrotoxicity induced by 2-NDHSA (Rankin et al., 2001b). Thus, our present findings with the selective nephrotoxicity induced by the S-enantiomer of NDHS represent one of only a small number of examples of stereoselective nephrotoxicity for a compound.

In an earlier study, we reported enantiomer selective nephrotoxicity for the NDPS metabolite 2-NDHSA (Rankin et al., 2001b). In that study, the S-enantiomer of 2-NDHSA was four times more potent as a nephrotoxicant than the R-enantiomer. In the present study, it was determined that NDHS also exhibits a similar stereoselective nephrotoxicity with S-NDHS being a more potent nephrotoxicant than R-NDHS in vivo. As with R-2-NDHSA (Rankin et al., 2001b), R-NDHS was at best a weak nephrotoxicant at doses up to 0.2 mmol/kg. S-2-NDHSA (0.1 mmol/kg) and S-NDHS (0.1 mmol/kg) both induced marked nephrotoxicity, although at 0.1 mmol/kg S-2-NDHSA induced changes in protein excretion and BUN concentration (Rankin et al., 2001b) were more pronounced than observed in this study with S-NDHS. Part of this difference may be due to hydrolysis of S-NDHS to 3-NDHSA, a weaker nephrotoxicant species than 2-NDHSA (Rankin, 2004). However, the exact mechanisms for these modest differences in the magnitude of nephrotoxicity induced by S-NDHS and S-2-NDHSA remain to be determined with certainty. Nonetheless, these results confirm that enantiomeric differences in nephrotoxic potential exist for a second NDPS metabolite.

Another important finding from this work was the lack of a significant difference in cytotoxicity induced by R-NSC and S-NSC in IRCC. Many previous reports support NSC as being a penultimate or ultimate nephrotoxicant metabolite of NDPS (Cui et al., 2005; Rankin, 2004). Since the comparison of nephrotoxic potential for R- and S-NSC was made in an in vitro renal system, the lack of stereoselective nephrotoxicity indicates that, at least one mechanism to explain the stereoselective nephrotoxicity observed in vivo with NDPS metabolites is extra-renal in origin.

The exact mechanism to explain why stereoselective nephrotoxicity exists with NDPS metabolites remains to be determined. However, the most likely mechanisms for the stereoselective nephrotoxicity observed with NDPS metabolites are related to renal accumulation of the nephrotoxicant species, bioactivation of NDPS to nephrotoxicant metabolites, different rates of detoxification or stereochemical requirements for the ultimate

target in kidney cells. The transport of substrates can be stereoselective at proximal tubular cells (Williams and Huang, 1981; Somogyi et al., 1996; Higaki et al., 1994, 1998). However, stereoselective renal transport of nephrotoxicant NDPS metabolites into renal proximal tubular cells is an unlikely explanation, based on the current findings with R- and S-NSC. These results also lessen the likelihood that there are stereochemical requirements for the ultimate target in kidney cells.

On the other hand, bioactivation of NDPS metabolites to the ultimate nephrotoxicant species may have a stereoselective component. Although NDHS and 2-NDHSA are nephrotoxics in vivo, neither metabolite is a nephrotoxicant in vitro (Aleo et al., 1991; Rankin et al., 1988). Thus, the kidney does not appear to be able to form nephrotoxic sulfate conjugates. However, reactive sulfate metabolites are formed from NDHS by rat liver (Cui et al., 2005) and NSC is a nephrotoxicant in vitro as shown previously (Rankin et al., 2001a) and in the present study. Studies by Banoglu and Duffel (1997; 1999) determined that enantiomers of secondary alcohols can demonstrate stereoselectivity for sulfation by hydroxysteroid (alcohol) sulfotransferases. Thus, enantiomers of the secondary alcohol NDHS might be sulfated at different rates to produce predominately one toxic sulfate conjugate (e.g. S-NSC) in vivo. However, additional studies are needed to determine whether R- and S-NDHS are sulfated at different rates.

Enantiomeric selectivity in NDHS hydrolysis is another possible explanation for the stereoselective nephrotoxicity observed with NDHS. Hydrolytic enzymes such as carboxylesterases and amidases can exhibit stereoselective hydrolysis of R- and S-enantiomers (Huang et al., 2005; Polhuijs et al., 1993). Thus, it is possible that R-NDHS is hydrolyzed faster than S-NDHS to limit availability of R-NDHS to form R-NSC. However, this potential mechanism needs to be explored in more detail.

The possibility does exist that the stereoselective nephrotoxicity observed with NDHS results from nonselective hydrolysis of the enantiomers of NDHS to 2-NDHSA followed by differential rates of sulfation of the R- and S-enantiomers of 2-NDHSA to form a nephrotoxic sulfate conjugate. Cui et al. (2005) demonstrated that sulfate conjugates of 2-/3-NDHSA are formed in vivo following administration of NDPS to male Fischer 344 rats. However, unlike NDHS, 2- or 3-NDHSA do not form sulfate conjugates in vitro when incubated with rat liver cytosol (Cui et al., 2005). Thus, this mechanism is not likely to be responsible for the stereoselective nephrotoxicity observed with NDHS.

In summary, the current study has demonstrated that S-NDHS is a more potent nephrotoxicant in vivo than R-NDHS, a finding that is consistent with another nephrotoxicant NDPS metabolite, 2-NDHSA. In addition, the finding that R- and S-NSC do not exhibit stereoselective nephrotoxicity in vitro suggests that the mechanism(s) involved in the stereoselective nephrotoxic nature of NDPS metabolites is an extra-renal mechanism. Additional studies are needed to determine the exact nature of this mechanism.

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Figure 1.
Biotransformation pathway for NDPS.

Figure 2.

Stereochemical configuration for R-(+)- and S-(-)-NDHS and R-(-)- and S-(+)-NSC.

Figure 3.

The effect of R- or S-NDHS administration on urine volume. Rats (4 per group) were administered R-NDHS (A) or S-NDHS (B) or vehicle and urine volume monitored for 48 h. Values are means $\pm$ S.E. An * symbol indicates significantly different from the Day 0 value within a group, $P < 0.05$. A # symbol indicates significantly different from the control group value for that day's measurement, $P < 0.05$.

Figure 4.

The effect of R- or S-NDHS administration on urinary protein excretion. Urine was collected between 0900h and 1500h each day (Day 0, 1 and 2) for these measurements. Rats (4 per group) were administered R-NDHS (A) or S-NDHS (B) or vehicle. Values are means $\pm$ S.E. An * symbol indicates significantly different from the Day 0 value within a group, $P < 0.05$. A # symbol indicates significantly different from the control group value for that day's measurement, $P < 0.05$.

Figure 5.

The effect of R- or S-NDHS on kidney weight. Rats (4 per group) were administered an NDHS enantiomer (treated) or vehicle (control). Kidneys were obtained at 48 post-treatment. Values are means $\pm$ S.E. A # symbol indicates significantly different from the appropriate control group value, $P < 0.05$.

Figure 6.

The effect of concentration on R- and S-NSC cytotoxicity in IRCC. IRCC used in these experiments were isolated from male Fischer 344 rats. Data are presented as the mean $\pm$ S.E. for four separate experiments. Experiments examining NSC concentration (treated) or vehicle (distilled water; control) effects were conducted using a 1 h exposure time. An * symbol indicates significantly different from the appropriate control group value, $P < 0.05$. A # symbol indicates significantly different from the opposite enantiomer group value for that concentration, $P < 0.05$.

Table 1

Effect of R- or S-NDHS Administration on Food and Water Intake^a

Compound	Dose mmol/kg	Food Intake (g)			Water Intake (ml)		
		Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
R-NDHS	0.1	16 ± 1	17 ± 1	18 ± 1	22 ± 2	25 ± 2	26 ± 1
S-NDHS	0.2	16 ± 1	13 ± 1	15 ± 1	21 ± 1	21 ± 1	27 ± 1 ^b
	0.05	18 ± 1	12 ± 3	15 ± 2	25 ± 3	36 ± 6	39 ± 5
	0.1	17 ± 1	4 ± 3 ^b	3 ± 2 ^b	23 ± 1	37 ± 9	34 ± 10

^a Values are means ± S.E. for N=4 rats per group.

^b Significantly different from the appropriate Day 0 value, P<0.05.

Table 2

Effect of R- or S-NDHS on Urinary Glucose and BUN concentrations^a

Compound	Dose mmol/kg	Group ^b	Urinary Glucose (µg/6 h)		BUN (mg%)	
			Day 0	Day 1	Day 0	Day 2
R-NDHS	0.1	C	78 ± 23	77 ± 13	12 ± 1	18 ± 1 ^c
		T	62 ± 17	95 ± 16	14 ± 1 ^d	10 ± 1 ^{cd}
S-NDHS	0.2	C	78 ± 23	77 ± 13	12 ± 1	18 ± 1 ^c
		T	57 ± 8	242 ± 23 ^{cd}	15 ± 1	19 ± 1 ^{cd}
	0.05	C	78 ± 9	104 ± 14	13 ± 1	13 ± 1
		T	85 ± 16	1,991 ± 657 ^d	14 ± 1	17 ± 1 ^{cd}
	0.1	C	37 ± 16	22 ± 14	13 ± 1	15 ± 1 ^c
		T	143 ± 34	4,996 ± 786 ^{cd}	13 ± 1	41 ± 16

^aValues are means ± S.E. for N=4 rats per group.

^bC = Control group; T = Treated group.

^cSignificantly different from the appropriate Day 0 value, P < 0.05.

^dSignificantly different from appropriate Control group value, P < 0.05.

^eN.D. = Not detected.