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D-Serine-induced nephrotoxicity: a HPLC–TOF/MS-based metabonomics approach

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Abstract

HPLC–MS-based metabonomic analysis was used to investigate urinary metabolic perturbations associated with D-serine-induced nephrotoxicity. D-Serine causes selective necrosis of the proximal straight tubules in the rat kidney accompanied by aminoaciduria, proteinuria and glucosuria. Alderely Park (Wistar-derived) rats were dosed with either D-serine (250 mg/kg ip) or vehicle (deionised water) and urine was collected at 0–12, 12–24, 24–36 and 36–48 h post-dosing. Samples were analysed using a Waters Alliance® HT 2795 HPLC system coupled to a Waters Micromass Q-ToF-micro™ equipped with an electrospray source operating in either positive or negative ion mode. Changes to the urinary profile were detected at all time points compared to control. In negative ion mode, increases were observed in serine ($m/z = 103.0077$), $m/z = 104.0376$ (proposed to be hydroxypyruvate) and glycerate ($m/z = 105.0215$), the latter being metabolites of D-serine. Furthermore, an increase in tryptophan, phenylalanine and lactate and decreases in methylsuccinic acid and sebacic acid were observed. Positive ion analysis revealed a decrease in xanthurenic acid, which has previously been assigned and reported using HPLC–MS following exposure to mercuric chloride and cyclosporine A. A general aminoaciduria, including proline, methionine, leucine, tyrosine and valine was also observed as well as an increase in acetyl carnitine. Investigation of additional metabolites altered as a result of exposure to D-serine is on-going. Thus, HPLC–MS-based metabonomic analysis has provided information concerning the mechanism of D-serine-induced renal injury.
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Keywords: D-Serine; Proximal tubule; Kidney; HPLC–MS; Metabonomics

1. Introduction

Metabonomic analysis using ^{1}H NMR spectroscopy is widely used to profile urinary endogenous proton-containing metabolites and has been used to identify

Abbreviations: D-AAO, D-amino acid oxidase; PCA, principal component analysis

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biomarkers associated with toxic perturbations and strain and diurnal variation in rodents (Nicholson et al., 1999; Gartland et al., 1989; Holmes et al., 1992; Beckwith-Hall et al., 1998; Gavaghan et al., 2000; Bollard et al., 2001). Electrospray mass spectrometry coupled with HPLC has now emerged as an additional technique for generating metabonomic data, often detecting a different, and complementary, range of metabolites to NMR. To date, the technique has been applied to the investigation of toxicity in rats (Plumb et al., 2002; Idborg-Bjorkman et al., 2003; Lafaye et al., 2003; Lenz et al., 2004a, 2004b), to the discrimination of strain, gender and diurnal variation in mice (Plumb et al., 2003) and to disease models in rats (Williams et al., 2004).

Recent studies have shown a clear biological role for D-serine as an endogenous modulator of the glycine site on the *N*-methyl-D-aspartate receptor in the brain of experimental animals and humans (Mothet et al., 2000). Extracellular levels of D-serine in the forebrain are comparable to glycine, whereas in some regions such as the stratium, the concentration of D-serine is 2.6 times that of glycine (Hashimoto et al., 1995). Because D-serine like L-serine can cross the blood–brain barrier, some of the D-serine is thought to be of dietary origin. The distribution of D-serine in rats following intravenous administration has been studies using autoradiography (Imai et al., 1998). The highest concentration of radiolabel from D-serine was found in the pancreas and kidney. D-Serine is known to cause selective damage to renal proximal tubule cells in rats resulting in proteinuria, glucosuria and aminoaciduria, the latter preceding the onset of necrosis (Carone and Ganote, 1975; Carone et al., 1985). The mechanism whereby D-serine, but not L-serine, produces renal injury in the rat is currently not fully understood. D-Serine is reabsorbed in the *pars recta* region of the rat proximal tubule (Shimomura et al., 1988; Silbernagl et al., 1999) where it is metabolised by D-amino acid oxidase (D-AAO), located in the peroxisomes, to produce the corresponding α-keto acid plus ammonia and hydrogen peroxide (Pilone, 2000). Thus, it has been proposed that the localization, concentration and metabolism of D-serine in renal tubule cells in the *pars recta* may account for the selective toxicity. This work is part of a programme aimed at understanding in more detail the toxicity of D-serine.

Perturbations characteristic of proximal tubule cell damage, including increased glucose, lactate, acetate and alanine and depletions in citrate, α-ketoglutarate and succinate have previously been characterised using ^{1}H NMR analysis (Williams et al., 2003). In this study we apply HPLC–MS-based metabonomics in order to further investigate the urinary metabolic profile associated with D-serine-induced nephrotoxicity. The identification of several metabolites, not previously detected by ^{1}H NMR, are discussed in context of the mechanism of toxicity, demonstrating the additional value that HPLC–MS offers.

2. Methods

2.1. Animals and treatments

Male (190–220 g; 7–8 weeks) Alderley Park (Wistar-derived) rats were housed under controlled humidity (30–70%) and temperature ($22 \pm 3\,^{\circ}$C) with a 12 h light/dark cycle. Animals had free access to food (RM-1 diet; Special Diet Services) and water. Animal procedures were performed in accordance with licenses issued under the Animals (Scientific Procedures) Act, 1986.

Animals were housed individually in metabolism cages and dosed with D-serine (250 mg/kg; 4 ml/kg ip; $n = 3$) or vehicle (deionised water; 4 ml/kg ip; $n = 3$). Urine was then collected over dry ice at 12 h intervals for 48 h then the rats were weighed then killed by exposure to halothane followed by exsanguination. Terminal blood samples, taken from the heart into heparinised tubes, were centrifuged ($3000 \times g$, 4 °C, 10 min) and plasma used for clinical chemistry analysis. The right kidney was submitted for pathological examination.

2.2. Clinical chemistry

Urine was analysed for glucose and total protein content. Creatinine and urea concentrations were analysed in plasma. The measurements were made using a Konelab 60i instrument (Labmedics) using standard assay kits supplied by Labmedics (NAG assay kit supplied by PPR Diagnostics Ltd.).

2.2.1. Histopathology

A transverse section of the right kidney was fixed in 10% neutral buffered formalin, embedded in paraffin

wax and 5 μm thick sections were cut and stained with haematoxylin and eosin for histopathological assessment.

2.2.2. HPLC–MS analysis on urine

For analysis by HPLC–MS, urine samples were centrifuged (13,000 rpm; 5 min; room temperature) to remove debris and then analysed neat. Chromatography was performed on a Waters Alliance® 2795 HPLC system (Waters Corporation, Milford, USA) equipped with a column oven. The HPLC system was coupled to a Waters Micromass Q-Tof micro™ (Manchester, UK) equipped with an electrospray source operating in either positive ion or negative ion mode. The source temperature was set at 120 °C with a cone gas flow of 40 l/h, a desolvation gas temperature of 250 °C and a desolvation gas flow of 400 l/h were employed. The capillary voltage was set at 3.2 kV for positive ion mode and 2.6 kV in negative ion mode and the cone voltage to 30 V. A scan time of 0.4 s with an inter-scan delay of 0.1 s was used throughout, with collision energy of 4 eV. A lock-mass of leucine enkephalin at a concentration of 0.2 ng/μl, in 50:50 acetonitrile:water + 0.1% formic acid for positive ion mode ($[M+H]^+ = 556.2771$) and 1.0 ng/μl in 50:50 acetonitrile:water for negative ion mode ($[M-H]^- = 554.2615$), was employed via a lock spray interface. Data was collected in centroid mode, the lock spray frequency was set at 5 s and the lock mass data was averaged over 10 scans for correction.

An aliquot of urine (5 or 10 μl for negative or positive ion mode respectively) was injected onto a 2.1 mm × 10 cm Symmetry® C18 3.5 μm column (Waters Corporation) held at 40 °C. The column was eluted with a linear gradient of 0–20% B over 0.5–4 min, 20–95% B over 4–8 min, the composition was held at 95% B for 1 min then returned to 100% A at 9.1 min at an eluent flow rate of 600 μl/min; where A = 0.1% formic acid (aq) and B = 0.1% formic acid in acetonitrile. A "purge–wash–purge" cycle was employed on the autosampler, with 90% aqueous methanol used for the wash solvent and 0.1% aqueous formic acid used as the purge solvent, this ensured that the carry-over between injections was minimized. The mass spectrometric data was collected in full scan mode from *m/z* 50 to 850 from 0 to 10 min, in positive and negative ion mode. The column eluent was split such that approximately 100 μl/min were directed to the mass spectrometer.

2.2.3. HPLC–MS data analysis

The HPLC–MS data were analysed using the Micromass MarkerLynx Applications Manager Version 1.0 (Waters Corporation). MarkerLynx incorporates a peak deconvolution package, which allows detection and retention time alignment of the peaks eluting in each chromatogram. The data is combined into a single matrix by aligning peaks with the same mass/retention time pair together from each data file in the dataset, along with their associated intensities. The intensity for each mass is normalised to the total intensity for each sample to partially compensate for differences in sample dilution. The processed data list was analysed by PCA using SIMCA-P (Version 10.0.2; Umetrics, Sweden), the data were mean-centered for this analysis.

3. Results

3.1. Clinical chemistry analysis and histopathology

Statistically significant glucosuria and proteinuria were observed at all time points post-dosing in the urine of animals dosed with D-serine (Fig. 1). Additionally, plasma urea (mmol/l) and creatinine (μmol/l) concentrations were significantly increased 48 h after treatment with D-serine (26.8 ± 1.6 and 137.0 ± 12.8, respectively) compared to control (5.4 ± 0.8 and 28.3 ± 1.2, respectively; data expressed as mean ± S.D.; $P < 0.05$; one-way ANOVA).

Marked necrosis of the *pars recta* of the proximal renal tubules as described previously (Williams et al., 2003) was observed in all rats treated with D-serine.

3.2. HPLC–MS analysis of urine

Analysis of urine by HPLC–MS revealed marked metabolic alterations in the urine of animals treated with D-serine compared to control animals at all time points post-dosing, in both positive and negative ion mode.

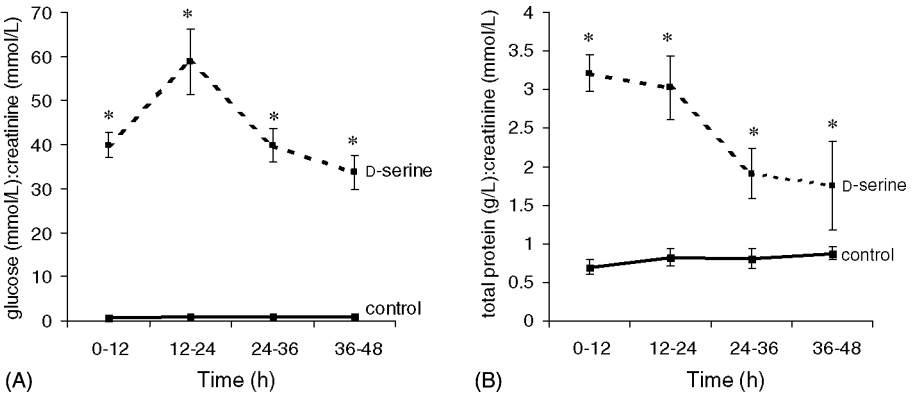

Fig. 1. Clinical chemistry. Urinary glucose (A) and protein (B) content measured at specified time intervals post-dosing (data expressed as mean ± S.D.; $^{*}P < 0.05$ compared to control; one-way ANOVA).

3.3. HPLC–MS analysis in positive ion mode

Examination of the total ion current (TIC) chromatograms of urine acquired in positive ion mode yielded a clear difference in the profile between control and treated animals (Fig. 2). For example, the presence of three peaks in urine of treated animals at 0–12 h post-dosing associated with ions $m/z = 132.1017$, 166.0862 and 205.0973, with retention times of 1.11, 2.17 and 2.73 min, respectively (subsequently identified, see Table 1). Furthermore, a peak at 3.52 min, associated with an ion of $m/z = 206.0464$, appeared to be reduced in intensity compared to control urine. Analysis of the data set following peak deconvolution and principal component analysis (PCA) yielded a clear separation of the treated and control data sets at all time points (Fig. 3). The major changes that contributed to the separation are shown in Table 1, with atomic compositions, accurate masses and metabolite identities. The ion chromatograms associated with each mass were examined to ensure the difference between control and treated data was real, as shown for e.g. $m/z = 204.1236$ identified as acetyl carnitine (Fig. 4A). The retention times and MS/MS spectra of authentic standards were obtained and used to confirm these identifications. An example is an ion of $m/z = 205.0977$ found to be elevated in the urine of D-serine dosed animals where the postulated identification of the compound as tryptophan was confirmed using the standard (Fig. 4B). In the D-serine treated animals a general aminoaciduria was observed including increased proline, valine, methionine, leucine, isoleucine, phenylalanine, tyrosine

Table 1
HPLC–MS analysis; positive ion

Trend	RT (min)	m/z	Postulated atomic composition of $[M+H]^+$ ion (with accurate mass)	Metabolite identity (with RT of standard)
↑ (0–48 h, T)	0.65	116.0697	$C_5H_{10}NO_4$ (116.0712)	Proline (0.60)
↑ (0–48 h, T)	0.69	118.0859	$C_5H_{12}NO_2$ (118.0868)	Betaine (0.63), Valine (0.63)
↑ (0–48 h, T)	0.77	150.0584	$C_5H_{12}NO_2S$ (150.0589)	Methionine (0.74)
↑ (0–48 h, T)	0.86	132.1017	$C_6H_{14}NO_2$ (132.1024)	Leucine (0.75), Isoleucine (0.75)
↑ (0–48 h, T)	2.17	166.0862	$C_9H_{12}NO_2$ (166.0868)	Phenylalanine (2.24)
↑ (0–48 h, T)	0.77	182.0809	$C_9H_{12}NO_3$ (182.0817)	Tyrosine (0.74)
↑ (0–48 h, T)	0.64	204.1210	$C_{11}H_{13}N_2O_2$ (204.1236)	Acetylcarnitine (0.58)
↑ (0–48 h, T)	2.73	205.0973	$C_{11}H_{13}N_2O_2$ (205.0977)	Tryptophan (2.79)
↓ (0–48 h, T)	0.69	114.0632	$C_4H_8N_3O$ (114.0667)	Creatinine (0.52)
↓ (0–48 h, T)	3.52	206.0464	$C_{10}H_8NO_4$ (206.0453)	Xanthurenic Acid (3.59)

T: treated. Metabolites perturbed following exposure to D-serine.

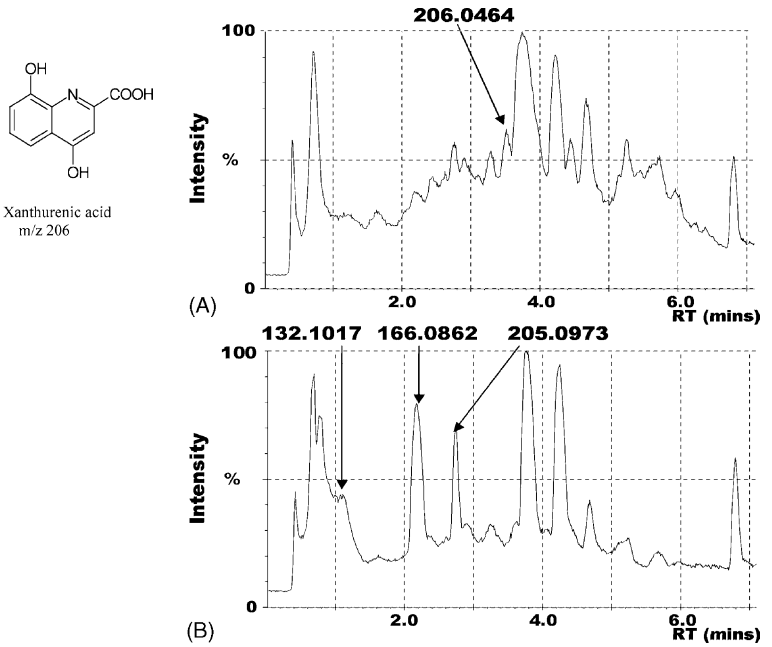

Fig. 2. HPLC–MS analysis; positive ion. Total ion current chromatogram (0–7 min) for urine samples obtained from (A) a control rat and (B) a D-serine treated rat at 0–12 h post-dosing. The chromatogram has been annotated with *m*/*z* values associated with specific peaks. Structure in inset is xanthurenic acid.

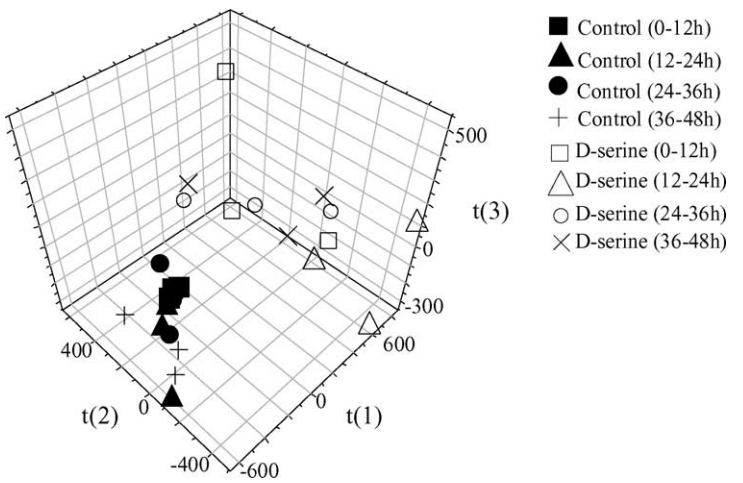

Fig. 3. HPLC–MS analysis; positive ion. Three-dimensional PCA scores plot (*t*(1) vs. *t*(2) vs. *t*(3)) obtained from positive ion HPLC–MS data acquired from rat urine samples collected at various times post-dosing.

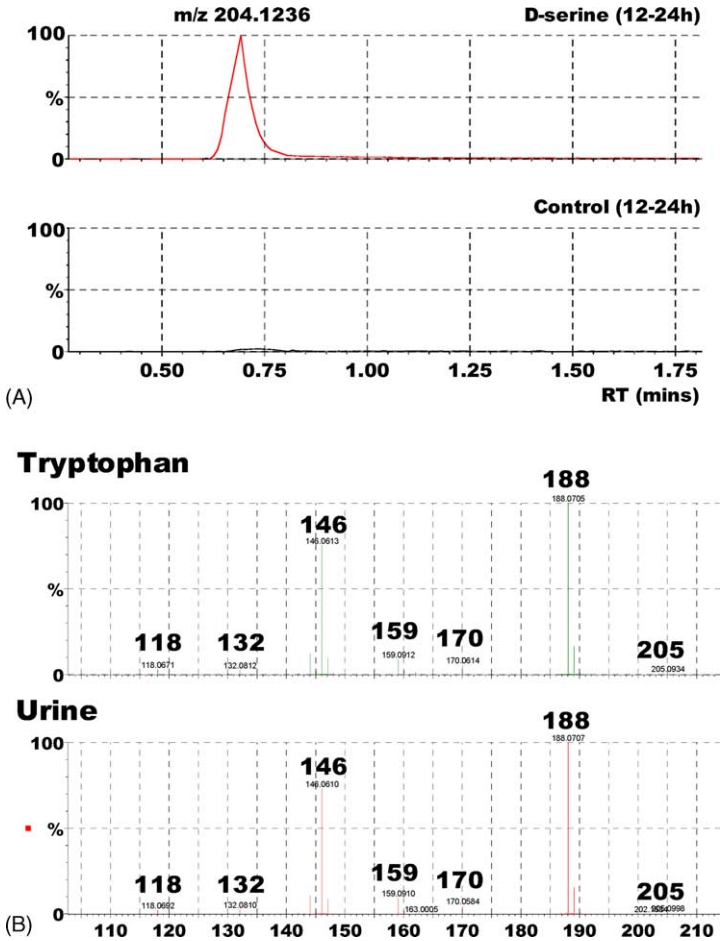

Fig. 4. HPLC–MS analysis; positive ion. (A) Extracted ion chromatogram for $m/z = 204$ (acetyl carnitine) in control and treated urine samples at 12–24 h post-dosing and (B) MS/MS analysis for $m/z = 205$ in urine and for tryptophan.

and tryptophan together with a decrease in xanthurenic acid (4,8-dihydroxy-2-quinolinecarboxylic acid, structure inset to Fig. 2). Furthermore, increases in ions at $m/z = 207.0460$, 424.2119, 448.1420 and 543.3270 were observed together with decreases in ions at $m/z = 146.0932$, 212.1040, 227.0481, 236.1290, 245.0943, 257.1125, 297.1440, 328.1027 and 465.1790, which have not been identified as yet.

3.4. HPLC–MS analysis in negative ion mode

Similar profiling experiments in negative ion mode also revealed changes in the profiles of dosed versus control animals. Typical two-dimensional "maps" showing mass chromatograms of urine (m/z versus retention time) acquired in negative ion mode for control and treated animals are shown in Fig. 5. This alternative type of representation provides a clearer indication of the complexity of the data sets obtained using HPLC–MS compared to the TICs shown in Fig. 2. Whilst appearing complex, and obviously containing many more ions than are discussed and identified here, further studies will eventually lead to the identification of all the components detected. Numerous ions were observed to have increased in the urine of treated animals at 0–12 h post-dosing including those at $m/z = 103.0049$, 104.0350, 105.0205, 89.0244, 164.0712 and 203.0831 (subsequently identified, see

Table 1). Furthermore, the ions at $m/z = 131.0349$, 181.0704 and 201.1118 appeared to be present at a lower concentration than in control urine (Fig. 5; see Table 1 for their identities). Analysis of the data set by PCA yielded a clear separation of the treated and control data sets at all time points (Fig. 6) with the 0–12 h treated data separating from the remaining treated data. The major changes that contribute to the separation are shown in Table 2, with atomic compositions, accurate masses and metabolite identities where confirmed. As was the case for the positive ion analysis, the ion chromatograms associated with each mass were examined for real differences. This is demonstrated for $m/z = 104.035$, identified as serine (Fig. 7A). Standard retention times and MS/MS were used to confirm the identifications, for example, $m/z = 105.0205$ in urine and its confirmed identity, glycerate (Fig. 7B). Increases in hydroxypyruvate (proposed identity as no standard was available), serine, glycerate ($HOCH_2CH(OH)CO_2H$), lactate, phenylalanine and tryptophan were observed together with decreases in methylsuccinic acid and sebacic acid ($HO_2C(CH_2)_8CO_2H$). Furthermore, increases in ions with masses at $m/z = 115.041$, 174.957, 194.946, 215.0265 and 225.063 were observed and decreases in ions at $m/z = 101.023$, 129.018, 145.008, 188.980 and 267.131, which have not been identified as yet.

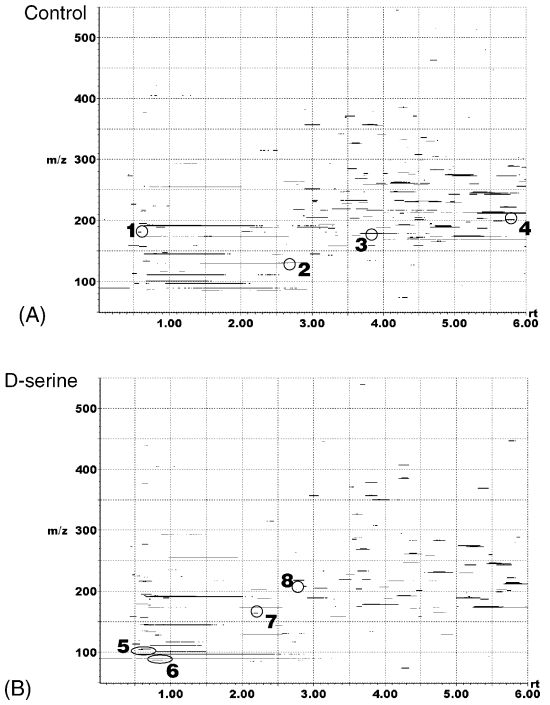

Fig. 5. HPLC–MS analysis; negative ion. Two-dimensional mass ion chromatogram ($m/z = 50$–550 vs. retention time 0–6 min) obtained from (A) a control rat and (B) a D-serine treated rat at 0–12 h post-dosing. The m/z of the annotated ions are (1) 181.0704, (2) 131.0349, (3) 178.0504 (hippurate), (4) 201.1118, (5) 103.0049, 104.035, 105.0205, (6) 89.0244, (7) 164.0712, (8) 203.0831.

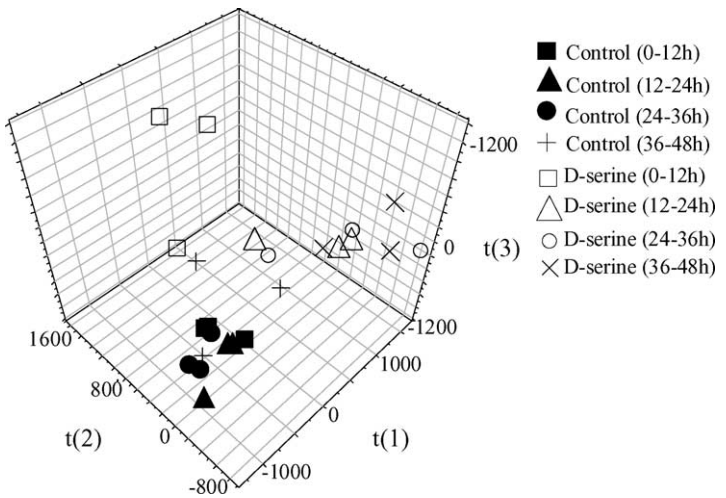

Fig. 6. HPLC–MS analysis; negative ion. Three-dimensional PCA scores plot ($t(1)$ vs. $t(2)$ vs. $t(3)$) obtained from negative ion HPLC–MS data acquired from rat urine samples collected at various times post-dosing.

Table 2
HPLC–MS analysis; negative ion

Trend	RT (min)	*m/z*	Postulated atomic composition of $[M-H]^+$ ion (with accurate mass)	Metabolite identity (with RT of standard)
↑ (0–12 h, T)	0.62	103.0049	$C_3H_3O_4$ (103.0031)	Hydroxypyruvate (not known)[a]
↑ (0–12 h, T)	0.64	105.0205	$C_3H_5O_4$ (105.0188)	Glycerate (0.64)
↑ (0–24 h, T)	0.53	104.035	$C_3H_6NO_3$ (104.0348)	Serine (0.58)
↑ (0–48 h, T)	0.77	89.0244	$C_3H_5O_3$ (89.0239)	Lactate (0.84)
↑ (0–48 h, T)	2.17	164.0712	$C_9H_{10}NO_2$ (164.0712)	Phenylalanine (2.24)
↑ (12–48 h, T)	2.74	203.0831	$C_{11}H_{11}N_2O_2$ (203.0821)	Tryptophan (2.79)
↓ (0–48 h, T)	2.64	131.0349	$C_5H_7O_4$ (131.0344)	Methylsuccinic Acid (2.55)
↓ (0–48 h, T)	5.75	201.1118	$C_{10}H_{17}O_4$ (201.1127)	Sebacic Acid

T: treated. Metabolites perturbed following exposure to D-serine.
[a] Metabolite identity not confirmed by MS/MS as standard not commercially available.

4. Discussion

Clinical chemistry measurements and histopathology clearly demonstrate the presence of damage to the proximal tubules in all animals following exposure to D-serine serine in agreement with that reported previously (Carone and Ganote, 1975; Carone et al., 1985; Williams et al., 2003). The renal injury was observed at all times post-dosing as shown by the urinary measurements.

Both positive and negative ion mode HPLC–MS analysis enabled the identification of metabolic perturbations associated with D-serine-induced renal injury. In agreement with previous studies (Carone and Ganote, 1975; Williams et al., 2003), a general aminoaciduria was observed with a number of amino acids being identified, predominantly in positive ion mode. The identification of specific amino acids by HPLC–MS was of interest as using ^{1}H NMR individual amino acids were more difficult to assign due to signal overlap, particularly for leucine, isoleucine and valine whose chemical shifts are close (personal observation, Wilson and Nicholson, 1995). Furthermore an increase in lactate was observed in negative ion mode, which has previously been observed using NMR (Williams and Lock, 2004). As with NMR the increase in lactate was pronounced and clearly defined the separation of the control and treated data. Aminoaciduria and lactic aciduria are characteristic of damage to the proximal tubules together with glucosuria, which was not detected by HPLC–MS. Glucose is not readily ionised by electrospray ionisation, hence despite a large increase in glucose in the urine it is not a diagnostic marker for nephrotoxicity by HPLC–MS. In ^{1}H NMR spectra, glucose resonances are dominant following exposure to D-serine and cover a large region of the spectrum causing signal overlap that may mask other metabolite changes. Hence, the complementary nature of the two techniques allows detection of different metabolite profiles, thus increasing the quantity of information obtained.

Xanthurenic acid, a metabolite of kynurenine normally excreted in urine (Takeuchi et al., 1989) was decreased in the urine of D-serine treated rats at all time points post-dosing. Xanthenuric acid has been reported to be reduced in other models of renal injury, such as mercuric chloride and cyclosporin A (Lenz et al., 2004a, 2004b), suggesting that xanthurenic acid may be a marker of renal proximal tubule injury. In contrast, in chronic renal insufficiency products of kynurenine metabolism, including xanthurenic acid, accumulate in plasma (Pawlak et al., 2001) suggesting the kidney either plays a role in uptake or metabolism of kynenurine. Concomitant with the decrease in urinary xanthurenic acid a prominent increase in tryptophan was observed. This may just reflect the general aminoaciduria, however the amount appeared to be greater than for the other amino acids. Tryptophan undergores catabolism to kynurenine and then onto glutaric acid via the enzyme ACMSD or it can be catabolised via nicotinamide adenine dinucleotide to xanthurenic acid, with the former being more active in the kidney than the liver (Fukuwatari et al., 2001; Allegri et al., 2003). Both xanthurenic acid and kynurenine are metabolites of these pathways hence perturbation of tryptopan metabolism could result in increased tryptophan excretion with decreased xanthurenic acid and TCA cycle intermediates (as observed by NMR).

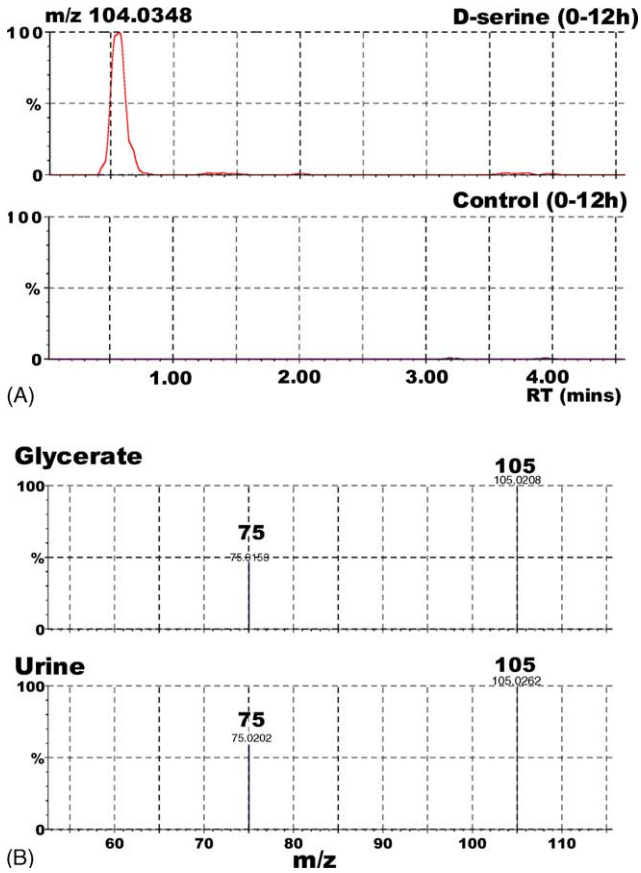

Fig. 7. HPLC–MS analysis; negative ion. (A) Extracted ion chromatogram for *m*/*z* = 104 (proposed to be hydroxypyruate) in control and treated urine samples at 0–12 h post-dosing. (B) MS/MS of *m*/*z* 105 in urine and for glycerate.

Of interest is a recent report that treatment of rats with a PPAR alpha agonist which causes hepatic peroxisome proliferation leading to an increase in excretion of *N*-methyl nicotinamide and *N*-methylpydidone-3-carboxamide, end products of tryptophan metabolism, with a down regulation of ACMSD (Ringeissen et al., 2003) suggesting a relationship between peroxisomal metabolism and tryptophan catabolism. D-Serine is metabolised in peroxisomes in proximal tubule cells, suggesting that some of the changes observed in tryptophan and its metabolites may reflected altered peroxisomal metabolism induced by D-serine. D-Serine is metabolised in the peroxisomes of the proximal tubules therefore it is possible that D-serine perturbs peroxisomal function thus effecting tryptophan metabolism as observed in the liver with compounds that act directly on peroxisomes.

Further to the suggestion that peroxisomal metabolism may be altered in D-serine treated rats is the observation of decreased excretion of sebacic acid and methylsuccinic acid. The importance of peroxisomes in the metabolism of dicarboxylic acids has been demonstrated in patients with Zellweger syndrome as urinary excretion of adipic acid, suberic acid and sebacic acid increases in these patients who have a complete lack of liver peroxisomes (Bjorkhem et al., 1984). Fatty acids constitute a major source of metabolic fuel for energy production in kidney tissue and it has been reported that acute renal injury to the proximal tubule and medullary thick ascending limb leads to structural and functional alterations that result in reduced expression and activity of mitochondrial and peroxisomal fatty acid oxidation (FAO) enzymes (Portilla, 1995). Acetyl carnitine is involved in the

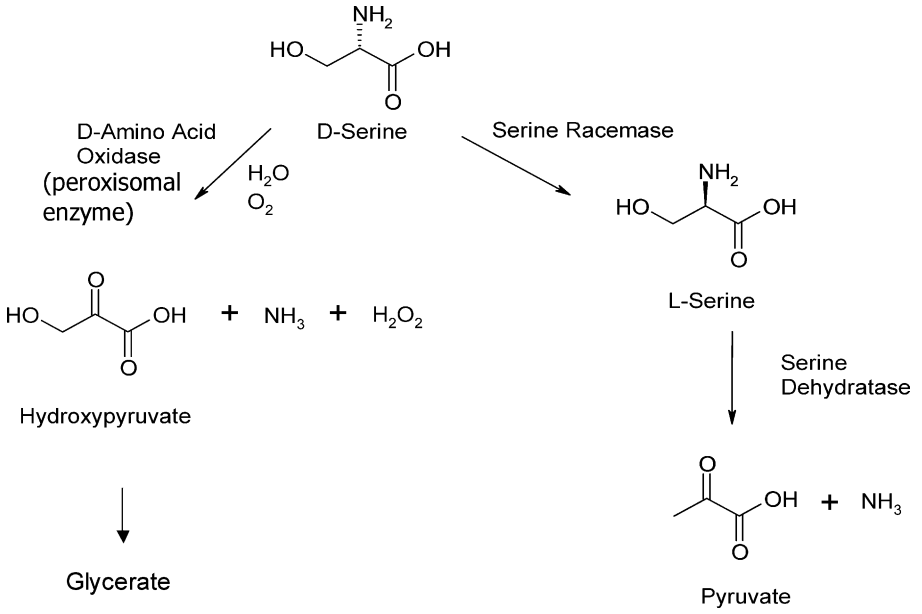

Fig. 8. Metabolism of D-serine.

process of fatty acid oxidation as well thus this may explain the changes observed in this metabolite. The association of dicarboxylic acids with this region of the kidney is further demonstrated by the presence of sodium dependent dicarboxylic transport systems in the proximal tubule (Ullrich et al., 1984).

Serine was observed to increase in the urine at 0–24 h post-dosing, being most prominent at 0–12 h post-dosing. It is not possible to distinguish between D- and L-serine using the analytical techniques used here, however, as serine is not increased during the entire 48 h as for the other amino acids, it is likely that the increased serine is D-serine rather than a general increase in serine due to aminoaciduria. The detection of glycerate and hydroxypyruvate (proposed identity) demonstrate that metabolism via D-amino acid oxidase is occurring in these rats (Fig. 8) as this is the suggested route of metabolism of D-serine. It was not possible to detect these metabolites with NMR and the ability of HPLC–MS-based screening to detect these would be of use in future studies, particularly examining strategies for protection against D-serine-induced renal injury. In addition to the ions that have been identified, other *m*/*z* values were found to be associated with the separation of control and treated data in both positive and negative ion mode, however suitable elemental composition and metabolite identifications were not found. For the characterisation of these ions a more extensive identification strategy needs to be developed, including isolation of the proposed metabolite and analysis using both NMR and MS to provide additional structural information. In addition the construction of databases and further hypothesis driven interrogation of the data may aid to resolve these identities.

In summary, analysis by HPLC–MS has demonstrated that exposure to D-serine results in a marked change in the urinary metabolic profile. Identification of a number of metabolites has provided information concerning the metabolism of D-serine and also proposed perturbations related to peroxisomal function in the kidney.

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