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Interference of hydroxyphenylpyruvic acid, hydroxyphenyllactic acid and tyrosine on routine serum and urine clinical chemistry assays; implications for biochemical monitoring of patients with alkaptonuria treated with nitisinone

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ABSTRACT

Objectives: We have assessed the effect of elevated concentrations of hydroxyphenylpyruvic acid (HPPA), hydroxyphenyllactic acid (HPLA) and tyrosine, on a range of chemistry tests in serum and urine to explore the potential for chemical interference on routine laboratory analyses in patients with alkaptonuria (AKU) treated with nitisinone and similarly implications for patients with hereditary tyrosinemia type 1 (HT-1).

Materials and methods: HPPA, HPLA and tyrosine were added separately to pooled serum from subjects without AKU in a range of assays with Roche Modular chemistries. Effects on urine were assessed by changes in urine strip chemistries after mixing a positive control urine with various amounts of the test compounds and reading on a Siemens urine strip meter.

Results: No significant effect ($p > 0.1$) was observed up to 225 μmol/L of HPPA and HPLA, and up to 5000 μmol/L tyrosine, on any of the serum-based assays including those with peroxidase-coupled reaction systems of enzymatic creatinine, urate, total cholesterol, HDL cholesterol and triglyceride. Both the monohydroxy HPPA, and the dihydroxy homogentisic acid (HGA), at increased urine concentrations typical of nitisinone-treated AKU and non-treated AKU respectively, did however show marked negative interference in strip assays for glucose and leucocytes; i.e. those with peroxide-linked endpoints. The effect of increased HPLA was less marked.

Conclusions: In patients with AKU or on nitisinone treatment and HT-1 patients on nitisinone, urine strip chemistry testing should be used sparingly, if at all, to avoid false negative reporting. It is recommended that urine assays should be organised with a suitable specialist laboratory.

1. Introduction

Alkaptonuria (AKU) is an inborn error of tyrosine metabolism caused by lack of the enzyme homogentisate-1,2-dioxygenase (E.C.1.12.11.5) [1]. The primary metabolic consequence is accumulation of homogentisic acid (2,5-dihydroxyphenylacetic acid; HGA) and other hydroxy phenyl acid intermediates. In AKU, mean(± SD) plasma HGA concentration is 39(± 10) μmol/L compared to < 1 μmol/L in normal plasma [2]; in AKU urine, HGA is at millimolar concentrations (mean 25.2(± 12.5) mmol/L) whereas in normal urine HGA is < 0.25 mmol/L [3,4]. Plasma HGA concentrations may be as high as 50 μmol/L in patients with AKU and at even greater levels following renal failure [2,5,6].

It has been shown that elevated concentrations of HGA have marked negative interference effects on assays involving peroxidase endpoints such as uric acid, glucose, enzymatic creatinine [7–9] and oxalate [10],

Abbreviations: AKU, alkaptonuria; HGA, homogentisic acid; HPPD, 4-hydroxyphenylpyruvic acid dioxygenase; HPPA, hydroxyphenylpyruvic acid; HPLA, hydroxyphenyllactic acid; HT-1, hereditary tyrosinemia type 1

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as well as a positive interference in urine protein assays using reaction with benzemethonium chloride [7]. Other dihydroxy phenol-based reducing agents, such as dopamine and dobutamine, have also been shown to affect hydrogen peroxide-based reactions for serum assays of urate, enzymatic creatinine, total cholesterol and glucose using glucose oxidase [11]. Interference from coloured substances is also possible such as bilirubin (yellow) with the Jaffe creatinine assay (orange) [12]. The use of urine strip testing is well accepted as an indicator of disease, however interferences particularly from reducing substances are well described, with high levels of ascorbic acid (vitamin C) inhibiting reagent strip reactions for glucose, blood, bilirubin, nitrate and leukocyte esterase [13]. The test strip package insert provided by the manufacturer, in this case Siemens Healthcare UK, gives information about a range of potential interfering substances, including ascorbic acid and salicylate; in other words, high concentrations of mono hydroxy phenolic acids.

In AKU, the urine darkens on standing as HGA, a dihydroxy phenolic acid and reducing agent, oxidises to benzoquinone acetic acid (oxidising agent) which subsequently forms a dark melanin-like pigment [1]. This intensely coloured urine makes it difficult to correctly interpret colour reactions on urine strip assays. Coloured urine is a well-described problem for urine strip assays [14,15], and it is therefore recommended that tests on such urines should not be reported using strip technology and to use an alternative method of testing if available.

In AKU, treatment with nitisinone, an inhibitor of the enzyme 4-hydroxyphenylpyruvic acid dioxygenase (HPPD; E.C. 1.13.11.27), produces a marked reduction in plasma and urine concentrations of HGA but a concomitant marked increase (up to 40 mmol/L) in the urinary output of mono-hydroxy phenolic acids hydroxyphenylpyruvic acid (HPPA) and hydroxyphenyllactic acid (HPLA) [3]. Additional factors are increases in tyrosine, tyramine and other hydroxy phenolic compounds such as hydroxyphenylacetic acid, 4-hydroxybenzaldehyde and presence of the drug nitisinone [3,16,17]. The impact of such metabolic derangements after therapeutic intervention, requires further assessment on routine chemical measurements in both serum and urine to test for any positive or negative interference. This is particularly relevant as we previously showed that the negative interference of HGA on the enzymatic creatinine assay can compromise assessment of renal function [7].

Here we present the effects of HPPA, HPLA and tyrosine at concentrations similar to those observed in serum of patients on nitisinone treatment, on a range of serum biochemical assays including enzymatic and Jaffe creatinine assays. A wide range of concentrations was used to include increases associated with impaired renal function and end stage renal failure. The effect on urine test strip analysis was also investigated as such assays are readily available and easy to carry out in a non-hospital environment or the field situation [18]. The concentrations for urine testing were in the millimolar range for HGA, HPPA and HPLA, similar to that observed in nitisinone-treated AKU urine. Other hydroxy acid metabolites, 4-hydroxybenzaldehyde and hydroxyphenylacetic acid, and HGA pigment were also investigated. This study therefore represents new insights into the effects of increased concentrations of tyrosine and related metabolites on both serum and urine testing for patients who are affected by AKU and its treatment with nitisinone.

2. Material and methods

Chemical standards were obtained as the Aristar grade from Sigma Chemical Co (Poole, Dorset, UK). Nitisinone was provided by Swedish Orphan Biovitrum International (Stockholm, Sweden). Solutions were prepared using high grade pure double deionised water (Ultra High Q, Elga Products, High Wycombe, UK). Stock solutions of HPPA (50 mmol/L), HPLA (50 mmol/L), tyrosine (50 mmol/L), tyramine (20 mmol/L), 4-hydroxybenzaldehyde (20 mmol/L) and HGA (100 mmol/L) were prepared freshly before use by dissolving in deionised water. The less soluble compounds tyrosine and HPPA were dissolved in 1 mL 5 N sulphuric acid and 1 mL isopropanol respectively, and then diluted with deionised water to a final volume of 10 mL. HGA-derived pigment solution was prepared from a solution of HGA (10 mmol/L) in deionised water, adjusted to pH 8.0 with 2 M NaOH, by ageing over 3 months at 37 °C.

Blood samples were collected from subjects without AKU and were classified as an improvement study status in accordance with local ethical guidelines as part of routine clinical management request profiles. Blood samples were collected in plain tubes (brown top, Sarstedt Ltd., Leicester, UK) for serum-based analytes. Samples of urine from 3 patients with AKU on nitisinone treatment were collected into acid preservative and stored at −20 °C before defrosting and testing as previously described [5].

Interference effects of HPPA, HPLA and tyrosine on serum biochemistry measurements were investigated by taking aliquots (9 parts) of pooled samples from several non-AKU patients to give varying concentrations of analytes, and spiking them with a freshly prepared solution of the various test compounds (1 part) to give final concentrations of HPPA and HPLA at 0, 75, 150, 225 and 300 μmol/L, and tyrosine at 0, 1250, 2500, 3750 and 5000 μmol/L. The samples were assayed over a range of analyte concentrations as indicated in Table 1: albumin, amylase, ALT, bicarbonate, total Ca, cholesterol, HDL cholesterol, CK, chloride, creatinine enzymatic, creatinine Jaffe, CRP, GGT, iron, potassium, LDH, magnesium, sodium, phosphate, total protein, paracetamol, salicylate, total bilirubin, triglyceride, uric acid and urea. The assays were established routine procedures on a Cobas 8000 modular analyser with C701 modules (Cobas-Roche Diagnostics West Sussex, England) unless otherwise stated. A summary of each method has been previously described [7]. The data were presented as % change in analyte activity at increasing concentrations of the test compounds relative to the control with no test compound added.

Interference effects of HGA, HGA pigment, HPLA, HPPA, hydroxyphenylacetic acid, 4-hydroxybenzaldehyde and tyramine on urine chemistries were studied using changes in the Siemens 8 test strip Multistix system (Siemens Healthcare, Surrey, UK) for leucocytes, nitrite, protein, pH, blood, specific gravity, ketones and glucose. The colour change was read on the CLINITEK Status® + Analyzer (Siemens Healthcare GmbH). The analyzer automatically checks each test strip for humidity exposure, common sample interferences and strip identification for Siemens test strips. The instrument was calibrated with negative and positive controls as provided with the kit procedure insert. Solutions of HGA, HGA pigment, HPPA and HPLA, tyramine and 4-hydroxybenzaldehyde, were mixed 1:1 with the positive urine control separately to give final concentrations for each test compound as indicated in Table 2. The 3 urine samples obtained from patients with AKU were also mixed separately 1:1 with the positive urine control and diluted 1:1 with deionised water prior to analysis (measured HPLA range = 7.5–9.4; HPPA range = 7.8–11.3[individual values provided in Table 2 legend]). The positive control diluted 1:1 with deionised water gave the following readings: glucose = 2500 mg/L, ketones = trace, specific gravity = 1.015, blood = moderate, pH = 7.0, protein = 1000 mg/L, nitrites = positive, leucocytes = small (Fig. 1). The data summarised in the Results are presented in comparison with the positive control.

The chemistry of the assays in which potential interference effects were investigated is described (Siemens Healthcare Information):-

The leucocytes test is based on the principle that esterases found in granulocytic leucocytes catalyse the hydrolysis of a derivatised pyrrole amino acid ester to liberate 3-hydroxy-5-phenyl pyrrole. This pyrrole then reacts with a diazonium salt to produce a violet coloured azole dye.

The nitrite test is based on the Greiss reaction in which the nitrite reacts in acid with an aromatic amine (para-arsanilic acid or sulphanilamide) to form a diazonium salt that in turn reacts with tetrahydrobenzoquinoline to produce a pink azo dye.

The protein test is based on the reaction of amine groups in protein

Table 1
The serum analytes with the range of activity are shown and the range of % change from the control values for each compound tested HPPA, HPLA and tyrosine. The range is from no effect (0) to values (±) change as shown in the examples Figs. 2-4. The intra assay variation is shown for low and high controls as well as the limits of uncertainty for each analyte and is a further confirmatory value to assess if values have changed from the respective mean. Alk, alkaline phosphatase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; HDL, high density lipoprotein; LDH, lactate dehydrogenase.

Serum analyte	Analyte range	Min - max % change from control value			CV % intra assay	Overall expanded (relative) uncertainty of precision, U(P) (95% confidence)
		HPPA	HPLA	Tyrosine		
Sodium	116–129	1.4–1.1	0.0–1.7	0.3–1.0	1.2, 1.3	5.9 at 126.5 mmol/L; 5.4 at 161.5 mmol/L
Potassium	4.2–5.4	1.6–1.8	0.0–2.3	0.8–1.2	1.9, 0.9	11.8 at 2.7 mmol/L; 5.6 at 6.1 mmol/L
Chloride	80–91	0.8–6.2	0.0–1.7	0.0–4.0	1.3, 0.9	8.0 at < 100 mmol/L; 5.2 at ≥ 100 mmol/L
Urea	5.5–28.3	0.9–2.0	0.0–3.3	0.1–4.9	2.8, 2.4	14.3 at 3 mmol/L; 9.5 at 22 mmol/L
Creatinine enzymatic	79–1113	0.8–4.0	0.0–3.8	0.0–0.7	2.1, 1.4	7.9 at 58 μmol/L; 5.2 at 460 μmol/L
Creatinine jaffe	71–1115	0.1–3.7	0.1–3.9	1.6–6.2	< 3.0	
Albumin	31–41	1.9–4.1	0.0–5.4	0.0–1.0	2.0, 1.4	13.4 at < 40 g/L; 9.9 at ≥ 40 g/L
Total Bilirubin	3–173	no effect	no effect	no effect	3.7, 1.6	22.6 at < 11 μmol/L; 4.5 at 110 μmol/L
Protein	54–66	0.0–0.3	0.0–1.6	0.4–1.6	1.6, 1.4	8.4 at 51 g/L; 6.9 at 84 g/L
Magnesium	0.61–0.93	0.5–1.6	0.0–2.0	0.3–1.5	1.9, 1.5	10.2 at 0.60 mmol/L; 7.8 at 1.59 mmol/L
Calcium	1.66–2.22	0.7–1.0	0.1–1.2	0.5–2.2	1.5, 1.2	10.0 at < 2.60 mmol/L; 6.1 at ≥ 2.60 mmol/L
Alk phosphatase	57–155	no effect	0.1–1.5	0.0–3.8	2.6, 1.7	25.1 at 58 U/L; 13.2 at 283 U/L
Bicarbonate	14–20	0.7–3.4	0.0–6.1	no effect	3.7, 2.9	22.4 at < 20 mmol/L; 16.4 at > 20 mmol/L
ALT	6.0–22	no effect	no effect	no effect	3.7, 2.2	10.5 at < 50 U/L; 4.3 at > 50 U/L
GGT	12–127	0.7–0.8	0.1–4.1	0.0–2.0	3.0, 1.9	17.4 at 27 U/L; 12.4 at 146 U/L
Phosphate	0.93–3.03	1.2–5.6	0.0–1.2	0.1–0.7	3.9, 1.7	18.3 at < 1.4 mmol/L; 8.6 at > 1.4 mmol/L
Triglycerides	1.2–23.4	0.4–2.1	0.0–2.0	0.1–1.4	2.1, 1.9	10.1 at 0.89 mmol/L; 8.3 at 2.92 mmol/L
HDL cholesterol	0.16–1.4	0.0–1.7	0.1–3.4	0.0–0.6	3.8, 4.5	8.2 at 0.74 mmol/L; 8.7 at 1.47 mmol/L
Cholesterol	2.9–9.6	0.5–1.4	0.0–1.7	0.0–1.7	1.9, 1.8	9.7 at 2.5 mmol/L; 7.2 at 6.6 mmol/L
Iron	9.5–23.4	0.8–1.4	0.5–1.4	−0.8 - 1.2	1.3, 2.5	7.9 at < 20 μmol/L; 8.9 at > 20 μmol/L
Urate	205–655	0.1–3.8	0.1–3.9	0.2–2.7	1.5, 1.3	5.8 at 130 μmol/L; 5.3 at 454 μmol/L
LDH	280–584	1.3–3.7	0.1–2.2	0.4–2.7	1.4, 1.0	4.5 at 100 U/L; 3.6 at 450 U/L
Paracetamol	< 6–8	no effect	no effect	no effect		
Salicylate	< 3	no effect	no effect	no effect		

with tetrabromophenol blue at pH 3, changing from yellow in the absence to shades of green to blue with increasing presence of protein.

The pH test is based on the double indicator principle, with colours changing from orange through yellow to blue in the range pH 5–8.5.

The blood test is based on the peroxidase like activity of haemoglobin, which catalyses the reaction of hydrogen peroxide with tetramethylbenzidine changing from orange to green and dark blue.

The specific gravity test is based on a pKa change of certain pre-treated polyelectrolytes in relation to ionic concentration, colours range from deep blue green through green and yellow green.

The ketones test is based on the development of a pink or maroon colour by the reaction of acetoacetic acid with nitroprusside. The test does not measure beta-hydroxybutyric acid and it is only weakly sensitive to acetone when glycine is added to the reaction. Compounds that contain sulfhydryl groups, such as mercaptoethane sulphonate sodium (Mesna) and captopril, but also L-DOPA, can give atypical colouring.

Table 2
Summary of test compounds and HGA on the Multistix 8 strip urine testing scheme. The concentrations reported are final after 1:1 dilution with the positive control urine. The positive control urine (diluted 1:1 with double deionised water) is used as a guide to changes in response on the strip chemistry. For the urines from patients with AKU treated with nitisinone, final concentrations (mmol/L) as measured by LCMS [3] of HPPA and HPLA were: 2.2,1.3; 1.8,1.7; and 2.4,2.3; for urines 1,2 and 3 respectively. Glucose reported as 100 mg/dL or 5.6 mmol/L and 250 mg/dL or 13.9 mmol/L total protein as 100 mg/dL or 1 g/L.

	Glucose (mg/dL)	Ketones	SG	Blood	pH	Protein mg/dL	Nitrite	Leucocytes
Positive control urine	250	trace	1.02	mod	7.0	100	+ ve	small
HGA (12.5 mmol/L)	100	trace	1.02	-ve	6.5	100	+ ve	trace
HGA (6.3 mmol/L)	100	trace	1.02	-ve	7.0	100	+ ve	small
HGA (3.15 mmol/L)	100	trace	1.02	trace	7.0	100	+ ve	small
HGA (1.57 mmol/L)	250	trace	1.02	small	7.0	100	+ ve	small
HGA (0.78 mmol/L)	250	trace	1.02	small	7.0	100	+ ve	small
HGA pigment (5 mmol/L)	100	trace	1.01	-ve	6.5	100	+ ve	trace
HGA pigment (2.5 mmol/L)	100	trace	1.02	-ve	7.0	100	+ ve	small
HPPA (25 mmol/L)	100	-ve	1.01	small	6.0	100	+ ve	-ve
HPPA (12.5 mmol/L)	100	trace	1.01	small	6.5	100	+ ve	-ve
HPPA (5 mmol/L)	100	trace	1.01	small	7.0	100	+ ve	trace
HPLA (50 mmol/L)	100	trace	1.02	mod	5.0	100	+ ve	small
HPLA (25 mmol/L)	100	trace	1.02	mod	5.0	100	+ ve	small
HPLA (12.5 mmol/L)	250	trace	1.02	mod	6.0	100	+ ve	small
Hydroxyphenylacetic acid (50 mmol/L)	100	-ve	1.03	mod	5.0	100	+ ve	-ve
4-Hydroxy-benzaldehyde (12.5 mmol/L)	250	-ve	1.02	mod	5.0	100	-ve	-ve
4-Hydroxy-benzaldehyde (6.3 mmol/L)	250	-ve	1.02	mod	5.0	100	-ve	-ve
Tyramine (10 mmol/L)	100	trace	1.01	mod	5.0	100	+ ve	small
Tyramine (2 mmol/L)	100	trace	1.01	mod	6.5	100	+ ve	small
AKU urine 1	100	trace	1.05	- ve	5.5	100	+ ve	Small (− ve)
AKU urine 2	100	trace	1.15	small	6.5	100	+ ve	Small (− ve)
AKU urine 3	100	trace	1.10	- ve	6.0	100	+ ve	Small (− ve)

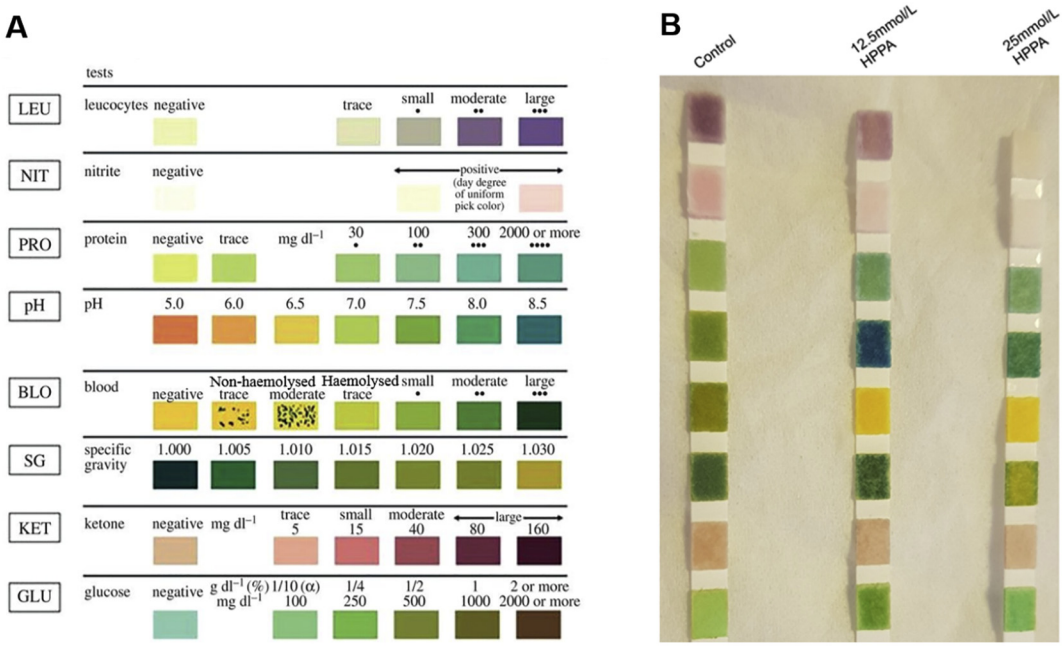

Fig. 1. The 8-multistix Siemens test strip. A: The variation in response for strip chemistry of an 8-multistix Siemens test strip. B: A sample of a test 8-strip after treatment of the positive control with HPPA at 12.5 and 25 mmol/L (final concentration) and shows the marked loss compared with the reference strip of leucocyte from purple to colourless, loss of nitrite, blood from blue to yellow and glucose from green blue to sky blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The reaction detects 0.5 mmol/L acetoacetic acid in urine.

The glucose test is based on a double sequential enzyme reaction utilizing glucose oxidase and peroxidase with a potassium iodide chromogen. The enzyme catalyses the oxidation of glucose by atmospheric oxygen to form D-glucono-δ-lactone and hydrogen peroxide. The peroxide reacts with a chromogen to form a coloured compound from green to brown to indicate glucose concentration [18].

2.1. Statistical analysis

Potential effects of the varying test compound concentrations on the different biochemical assays for serum were assessed using Wilcoxon signed rank tests (paired non-parametric analysis). Assay values obtained at each test compound concentration were compared with values obtained from the controls with no test compound added. The statistical significance P was quoted as > 0.1 and as not significant (NS).

3. Results

Potential effects of HPPA, HPLA and tyrosine were investigated on the various serum assays. No significant effect ($P > 0.1$) or dose relationship was observed up to 225 μmol/L for HPPA on the range of analytes tested including those with peroxidase-coupled reaction systems such as enzymatic creatinine and urate (Fig. 2a,b), as well as total cholesterol, HDL cholesterol and triglyceride (summarised in Table 1). There was no significant effect of HPLA up to 300 μmol/L, the highest concentration tested, on these analytes (Fig. 3a,b). There was also no significant effect ($P > 0.1$) with increasing concentrations of tyrosine up to 5000 μmol/L on any of the analytes (Table 1); the example shown in Fig. 4 is enzymatic creatinine.

The effects of the test compounds on the urine strip assays at each concentration are summarised in Table 2. Solutions of HGA (3.2–12.5 mmol/L), HGA pigment (2.5–5 mmol/L), HPPA (5–25 mmol/L; shown in Fig. 1), HPLA (12.5–25 mmol/L), hydroxyphenylacetic acid (50 mmol/L) and tyramine (2–10 mmol/L) reduced the glucose response from 250 to 100 mg/dL (13.9–5.5 mmol/L). The ketones were generally not affected, except by hydroxyphenylacetic acid (50 mmol/L) and 4-hydroxybenzaldehyde (6.3–12.5 mmol/L) which reduced the response from trace to negative. HPPA (5–25 mmol/L) also reduced the response for the blood and leucocyte assays whereas HPLA had no effect, and 4-hydroxybenzaldehyde (6.3–12.5 mmol/L) reduced the response in nitrite and leucocyte assays. Solutions of HGA and HGA pigment showed a clear dose-response relationship of negative interference on the assays for blood and leucocytes. The urine samples from patients with AKU showed reduction in responses to glucose, blood and leucocytes with a tendency to reduce the ketone response.

4. Discussion

This study has shown that concentrations of the hydroxy acids HPPA and HPLA and the hydroxyphenyl amino acid tyrosine, similar to those found in serum during treatment with nitisinone, have no effect either to inhibit or give a false positive reaction on a wide range of serum-based clinical biochemical assays. The lack of effect by these monohydroxy phenolic acids confirms that they are not as powerful reducing agents as the dihydroxy phenol acid, HGA, previously shown to have marked interference in certain serum-based assays [6]. The reported mechanism for interference by HGA implies effects on the consumption of H_2O_2. The dark pigment produced from HGA may also cause interference as in the urine assay of oxalate with a minor positive effect in the second coupled peroxide measuring enzyme [9]. The mechanism of peroxide scavenging by the phenolic compounds indicates that dihydroxy phenols as well as amino phenols are much more efficient antioxidants than the monohydroxy compounds. Further, the introduction of a methyl group on one of the hydroxyls also reduces the scavenging ability [19,20].

The increased concentration of nitisinone during treatment of AKU patients may be a further source of interference in assays. However, from initial experiments at 0.33 μmol/L, a concentration at the low end of the therapeutic range for AKU treatment, there was no effect on any of the serum-based chemistries. Further work at much higher concentrations similar to therapeutic values of 40 μmol/L in the treatment of hereditary hypertyrosinemia type I is required to confirm lack of effect of this compound [21].

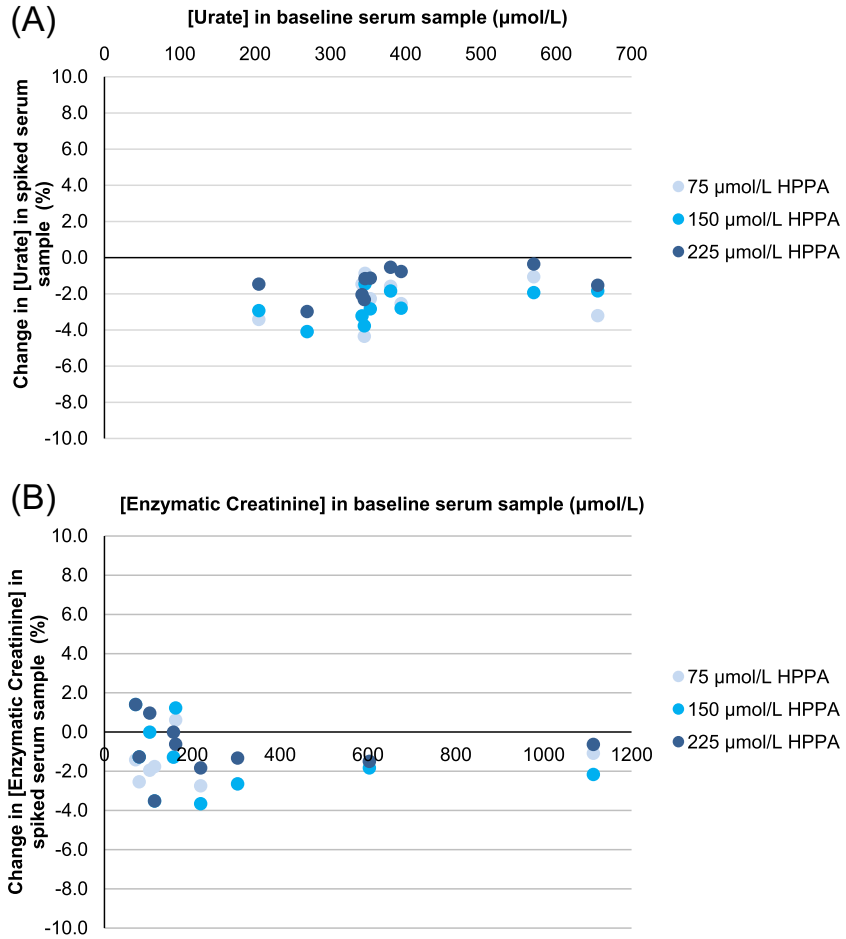

Fig. 2. Representative example of the effect of HPPA on serum-based assays. A: The effect of increasing HPPA on serum urate. B: The effect of increasing HPPA on enzymatic creatinine. The baseline value is the serum value with diluent only and no added test compound. The change in activities was within the error of analysis for these assays.

The high concentrations in urine (> 12.5 mmol/L) particularly of HPPA, but also of HPLA, as found during nitisinone treatment, did show some negative interference on certain urine test strip chemistries. Glucose, blood and leucocyte assays that involve oxidation mechanisms were all partially inhibited in the presence of HPPA. The interference effect is thought sufficient to recommend that urine strip testing should not be carried out on urine from patients treated with nitisinone. Similar precautions must be taken for HT-1 patients treated with nitisinone because of the increased urine output of these acids. Interestingly, loss of the keto group to the hydroxy reduced the effect inferring less effective oxygen scavenging.

The solutions of HGA also showed reduction of the glucose response on the Multistix strip with marked loss of response with whole blood and leucocyte esterase activity at 12.5 mmol/L HGA. Other studies of HGA on glucose, urate and oxalate using oxidase-based procedures, have concluded a direct interference effect via reduction of available H_2O_2 through reaction of HGA with the reagent 4-aminophenazone [7]. Similar in vitro experiments have demonstrated that HGA concentrations > 1 mmol/L inhibit the peroxidase reaction by blocking the colour development with 3-methyl-N-ethyl-N-(beta-hydroxyethyl)aniline and 4-aminoantipyrine. Interference effects of HGA at relatively low concentrations in the assay of 5-hydroxyindoleacetic acid was postulated as a result of oxidation of HGA to the corresponding quinone [22]. In a study on dogs treated with nitisinone, marked increases in urine concentrations of HPPA, HPLA and hydroxyphenylacetic acid were observed [23]. The subsequent testing of the individual acids with urine dipstick strips gave a positive ketone response for HPPA alone, whereas our studies indicated the opposite, with HPPA blocking the urine ketone response and no clear ketone visual colouration when HPPA was tested as a simple aqueous solution. The loss of response to leucocytes and blood in urine may mean a misinterpretation of the presence of a serious renal or bladder infection and reduction in the response to glucose and possibly ketones may imply better metabolic control or even misdiagnose diabetes mellitus so it is important that these urine strip tests are properly managed and interpreted [18]. Hence our concern that in the presence of markedly increased concentrations of these hydroxy organic acids, use of urine strip testing must be carefully carried out if at all.

5. Conclusions

In summary, elevated concentrations of HPPA, HPLA and tyrosine have no significant interference on a wide range of biochemical tests in serum, including those with oxidation/reduction reactions such as urate and creatinine by creatininase. However the markedly increased concentrations in urine of HPPA and HPLA associated with nitisinone treatment and increased urine HGA when not on nitisinone, showed negative interference effects on urine chemistry strip assays, particularly HPPA and HGA on glucose, whole blood and leucocyte esterase activity and will have significant misinterpretation of information if such co-morbidities are present. Based on the data presented, we recommend that for patients with AKU with or without nitisinone treatment and similarly for HT-1 patients treated with nitisinone, strip chemistry testing on urine should not be carried out.

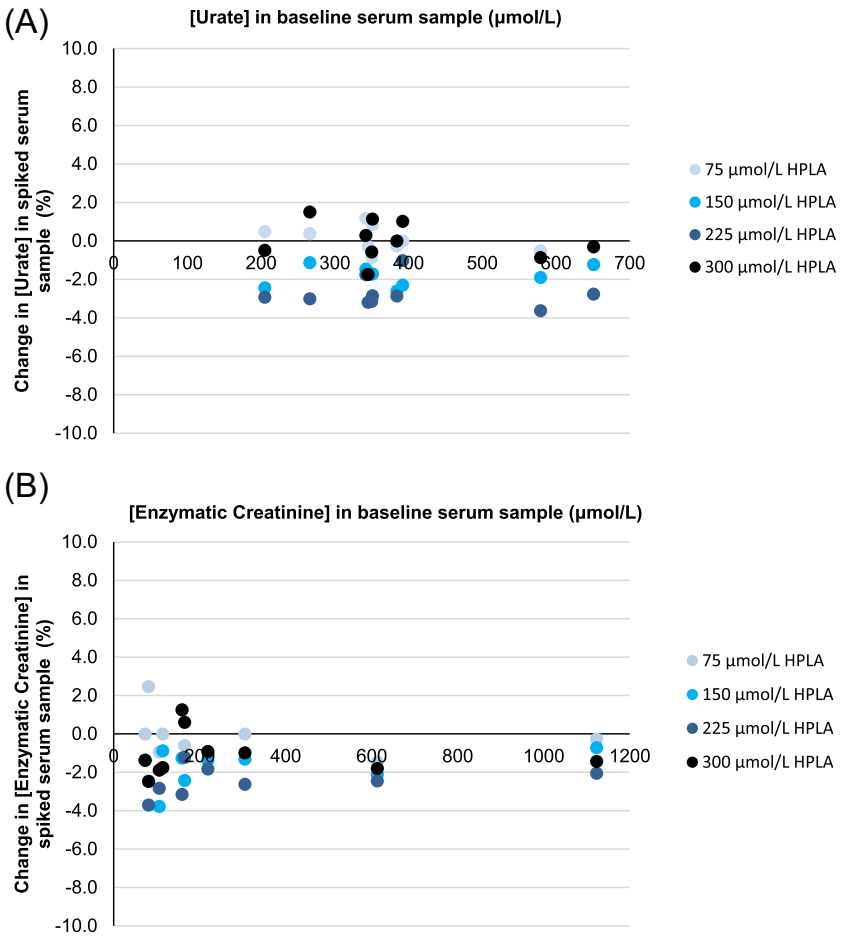

Fig. 3. Representative example of the effect of HPLA on serum-based assays. A: The effect of increasing HPLA on serum urate. B: The effect of increasing HPLA on enzymatic creatinine. The baseline value is the serum value with diluent only and no added test compound. The change in activities was within the error of analysis for these assays.

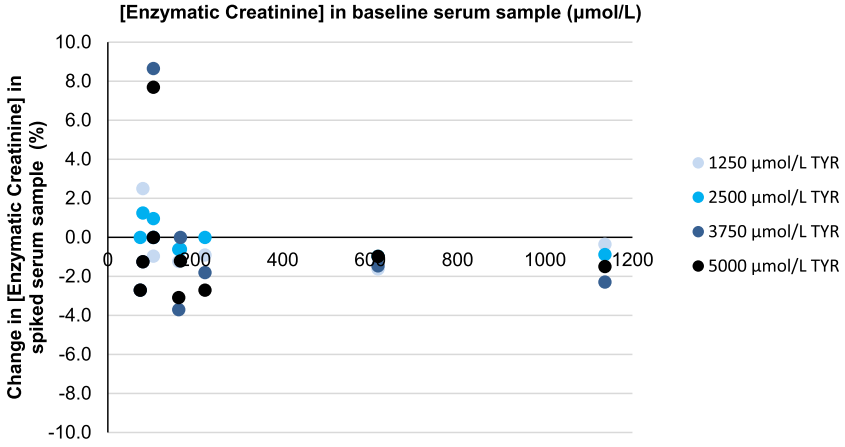

Fig. 4. Representative example of the effect of tyrosine on serum-based assays. The effect of increasing tyrosine on enzymatic creatinine. The baseline value is the serum value with diluent only and no added test compound. The change in activities was within the error of analysis for these assays.

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