AN IMPROVED SCREENING METHOD FOR INHERITED DISORDERS OF PURINE AND
PYRIMIDINE METABOLISM BY HPLC

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INTRODUCTION

HPLC has been used extensively in the identification of genetic disorders of purine and pyrimidine metabolism. Some of the problems which may be encountered in assessing patients, for example on high caffeine intakes, or antibiotics such as septicin, have been reported (1). The spectrum of clinical presentation is broad, ranging from cerebral palsy, immunodeficiency, anaemia, acute renal failure to kidney stones. This means that many patients can be on multiple-drug regimes, which may include purine or pyrimidine analogues, or have recently undergone some clinical manipulation which could severely restrict the chance of making a diagnosis by direct analysis of body fluids. This paper reports an improved method of screening involving better fractionation of the urine and demonstrates how some of the above problems have been overcome.

PATIENTS AND METHODS

Patients, should preferably not be on any form of therapy (or on minimal essential therapy), and kept on a low-purine caffeine-free diet for three days prior to investigation. Therewith a 24 hour urine (toluene preservative) is required with a blood sample taken at the end of the 24 hour period. Data supplied with the sample must include: a) an adequate case history, b) previous and current drug therapy, including blood transfusion if any and date, c) a full family history.

Sample preparation: Venous blood is collected into heparin at room temperature and centrifuged at 1500g and plasma separated immediately. The urine is warmed at 60°C and processed as described below.

Methods: All reference standards (purines, pyrimidines and associated compounds) and other chemicals were of the highest analytical grade available. Solvents were all HPLC grade; water for HPLC was produced by double glass distillation.

Urine fractionation: The amount of urine loaded onto each mini-column depends on the concentration as determined by direct dilution (1/31) with acetate buffer and reverse-phase HPLC (RPLC), and on osmolarity (500 mOsms load 1-2mls urine; 500 mOsms load 3-4mls).

Anion exchange resin (AG 1-X8 100-200 mesh in the acetate form) was added to a depth of 6cm to mini glass columns plugged with cotton wool. Urine (e.g.1ml) (pH adjusted to 10 with 1M NaOH) was loaded to the column and eluted as follows and the corresponding 5ml fractions collected. Fractions:
After the urine, 1ml 0.01M ammonia solution, then distilled water to 5mls (frac 1); then 5ml distilled water (frac 2); then 0.04M HCL (frac 3); then 0.06M HCL (frac 4); then eluent containing 0.02M HCL + 29.2g NaCl/litre (frac 5). Subsequent analysis of the fractions was achieved by RPLC, and conventional chromatography when necessary. Fractions 2-4 may also be freeze-dried then taken up in 100-200ul ammonia solution (fracs 1 and 5 are not suitable due to the high salt content), and these concentrated fractions were then subjected to 2-dimensional electrophoresis and chromatography as described elsewhere.(2)

RPLC: The HPLC used was a dual wave-length (254/280nm) Millipore Waters trimodule system (1) with on-line Diode-array (Waters 990). A 250 x 4.5mm Hypersil 5u ODS-2 column (Hichrom) was used together with the 40mM acetate buffer system and a linear gradient to 20% organic solvent as described previously (1).

UNFRACTIONATED URINE

Fig. 1 shows chromatograms (254nm absorbance) of 5 diluted (unfractionated) urines run on RPLC, illustrating the presence of extra peaks due to paracetamol metabolites, radiocontrast agents used in a renal failure patient, acyclovir, and in a patient with adenine phosphoribosyl transferase (APRT) deficiency.

Abbreviations: Psu pseudouridine; CR creatinine; URA urocanic acid; UA uric acid; HX hypoxanthine; X xanthine; HA hippuric acid; 2PY,4PY pyridone carboxamides; 7MG, 8,7MG 7methyl & 8hydroxy-7methyl guanine; MN methylated nucleosides; AD adenine; PM paracetamol metabolites; RC radiocontrast agent)

RESULTS

Fig. 1 shows chromatograms (254nm absorbance) of 5 diluted (unfractionated) urines run on RPLC, illustrating the presence of extra peaks due to paracetamol metabolites, radiocontrast agents used in a renal failure patient, acyclovir, and in a patient with adenine phosphoribosyl transferase (APRT) deficiency.