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ABSTRACTS

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Reconstruction of Human Hepatocyte Glyoxylate Metabolic Pathways in Chinese Hamster Ovary Cells

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Failure to detoxify the intermediary metabolite glyoxylate in human hepatocytes underlies the metabolic pathology of both PH1 and PH2. The qualitative roles of the enzymes alanine:glyoxylate aminotransferase (AGT, deficient in PH1), glyoxylate reductase (GR, deficient in PH2) and glycolate oxidase (GO) in the metabolism of glyoxylate are well understood, but the relative quantitative importance of how the balance between them is disrupted at the cellular level in PH1 and PH2 is unclear. In order to address this issue, we have generated a series of single, double, and triple Chinese hamster ovary (CHO) cell stable transformants expressing normal and mutant forms of these enzymes in a variety of combinations. Preliminary characterization of these CHO cell lines shows that they express AGT, GR and GO at levels similar to those found in human hepatocytes. In addition, these enzymes appear to be correctly localized (i.e. AGT and GO mainly to peroxisomes, and GR to cytosol and mitochondria). Indirect metabolic analysis, based on the cytotoxicity of glyoxylate, indicates that we have been able to reconstruct the glycolate-to-glyoxylate, glyoxylate-to-glycolate, and glyoxylate-to-glycine metabolic pathways, correctly compartmentalised, in cells that do not normally express them.

A New Method for Treatment of Hyperoxaluria and Kidney Stone Disease: Pyridoxamine Inhibits Kidney Crystal Formation in Hyperoxaluric Rats

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Kidney stone disease affects about 2 to 3% of the general population in the United States with recurrence rates reaching 50 to 70% in 10 years. Since a majority of kidney stones are made of calcium oxalate, the level of urinary oxalate is one of the major risk factors for stone formation. It has been demonstrated that the lowering of urinary oxalate concentration would be a significantly more efficient way to reduce the risk of stone formation than lowering the urinary calcium. Unfortunately, there is no effective pharmacological treatment of stone disease that targets urinary oxalate concentration.

We proposed a new method for treatment of kidney stone disease based on chemical trapping of carbonyl precursors of oxalate biosynthesis by pyridoxamine (PM). We have shown previously that PM treatment significantly reduced urinary oxalate levels in rat model of hyperoxaluria. In the present work, we have demonstrated that PM can inhibit formation of calcium oxalate crystals in kidneys of these hyperoxaluric animals. For induction of hyperoxaluria, rats received ethylene glycol (0.8%) in drinking water. The crystals were analyzed in the fixed kidney tissues using the polarized light microscope. Blind analysis of the slides was conducted independently by two experts. The development of hyperoxaluria in ethylene glycol-treated animals was accompanied by a dramatic increase in kidney crystal formation compared to controls. A degree of crystal formation was different depending on kidney anatomical area ($p=0.009$). The most prominent crystal formation occurred in papilla followed by medulla and cortex. When hyperoxaluric rats were treated with PM, their urinary oxalate excretion decreased by about 50%. The crystal formation in kidneys of these PM-treated animals was significantly reduced ($p=0.03$). These preclinical results show promise for the therapeutic use of PM in kidney stone disease.

Mitochondrial GRHPR

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The gene mutated in patients with primary hyperoxaluria type II (PH2), GRHPR, is a multifunctional oxidoreductase that has glyoxylate reductase (GR) and hydroxypyruvate reductase (HPR) activities. The tissue distribution of specific enzymatic activity for GRHPR was analyzed. Liver was found to be the greatest source of GRHPR, followed by kidney (25% of liver), and then lung, colon, bladder, and lymphocytes, which all had about 10% of the activity of liver. These data demonstrate that the tissue distribution of GRHPR is more widespread than previously thought and raise questions about the role of GRHPR in glyoxylate metabolism in non-hepatic sites. We next investigated GRHPR intracellular localization. GRHPR (enzymatic activity and immunoreactive protein) was located in purified mitochondria from HepG2 cells, frozen liver, and peripheral lymphocytes. Further analysis of the molecular biology of GRHPR demonstrates the presence of a putative mitochondrial localization sequence that is not in the amino terminus of the protein. Transfection of COS 1 cells with GRHPR truncation constructs followed by immunoblot analysis of purified mitochondria demonstrates that this sequence targets the protein correctly. However, only full-length protein possesses enzymatic

activity. We hypothesize that mitochondrial targeted GRHPR is important for detoxification of glyoxylate produced by the mitochondrial hydroxyproline catabolism that occurs in hepatic and renal cells or by other unidentified mechanisms in non-hepatic cells.

Renal Antioxidant Status and Urinary Enzyme Levels in a Rat Model of Hyperoxaluria (HYP) and Chronic Renal Failure (CRF)

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The role of oxidative injury in renal tubular damage was evaluated in chronic hyperoxaluria and CRF induced by hyperoxaluria. Male Sprague-Dawley rats were randomly assigned to one of our treatments as follows: 1) control (CON); 2) 0.75% ethylene glycol for 4 weeks (HYP); 3) unilateral nephrectomy (UNI); and 4) unilateral nephrectomy plus 0.75% ethylene glycol for 4 weeks (CRF). After 4 weeks, 24-h urine samples (U) were collected and kidneys (K) were harvested for enzyme analysis. Results are shown in the table below:

ENZYME	CON	HYP	UNI	CRF	SEM
ALP (U/24 h) U	3.08	3.91*	3.64	4.90*	.22
8-Isoprostane (ng/24 h) U	3.61	3.69	3.03	3.19	.52
GSTμ (μg/g creatinine) U	36.7	52.7	26.9	52.5	7.7
GSTα (μg/g creatinine) U	39.1	56.5	25.1	24.5	5.9
NAG (mIU/24 h) U	375.7	490.0	403.9	482.6	36.9
GPx (μmol/min/ml) K	3.59	2.63*	3.32	1.48*	.25
SOD (U/ml) K	1674	1415	1405	623*	132
GST (nmol/min/ml) K	121.2	115.0	114.5	77.5*	7.2
Catalase (μmol/min/ml) K	9.49	8.70	11.5	3.4	1.2

*Indicates values significantly different (P<.05) from control values.

The data show that the overall antioxidant capacity in HYP rats is comparable to CON rats after 4 weeks of hyperoxaluria. However, this capacity is attenuated in rats with half the renal mass (CRF) although lipid peroxidation, as judged by 8-isoprostane, is still minimal.

The Peroxisomal Import of Human AGT Requires the Presence of Species-Specific Additional Targeting Information

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Although the molecular basis of AGT mitochondrial mistargeting in PH1 is fairly clear, its normal targeting to peroxisomes is far less well understood. Our previous studies have shown that AGT is imported into peroxisomes via the peroxisomal targeting sequences type 1 (PTS1) pathway. However, the C-terminal tripeptide PTS1 of human AGT has a number of very unusual properties that suggest the presence of additional peroxisomal targeting information within the AGT molecule. For example, in this study we found that unlike the PTS1s of rat, cat, guinea pig and rabbit, AGTs, the PTS1 of human AGT is insufficient to direct the peroxisomal import of the reporter protein GFP. In addition, although able to target rat and guinea pig AGTs to peroxisomes, the human PTS1 is unable to do the same for cat or xenopus AGTs. In an attempt to identify the location of this extra targeting information, we have constructed a variety of molecular hybrids between human AGT and GFP, and between human AGT and xenopus AGT. These have been expressed in COS cells and their subcellular distribution determined by (immuno)fluorescence microscopy. The results show that the region from residue 306 to 361 in human AGT is necessary for its correct peroxisomal targeting, in addition to the C-terminal PTS1. The exact role of this region is unclear, but it may stabilise the interaction between AGT and the PTS1 receptor Pex5p, either directly or indirectly, by way of an adaptor molecule.

D-Glycerate Dehydrogenase Activity in Buccal Cells and Blood Mononuclear Cells

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A definitive diagnosis of primary hyperoxaluria type 2 (PH2) is currently made by the analysis of glyoxylate reductase (GR) activity in a liver biopsy. Most cells express GR activity, suggesting that a less invasive procedure utilizing more readily available cells could be used to determine GR deficiency. We have assessed methods for determining GR activity in buccal cells obtained from cheek swabs and in blood mononuclear cells (BMC). Sensitivity and specificity determinants indicated that D-glycerate dehydrogenase activity assayed by an HPLC procedure was the best index of GR activity in these cells. Purified beef heart lactate dehydrogenase was found to have significant GR activity with NADPH as a cofactor, but lacked DGDH activity which utilizes $NADP^+$ as a cofactor. An assay of 10 normal individuals indicated that buccal cells contained a DGDH activity of 0.387 ± 0.319 nmoles/min/mg protein, whereas BMC contained 1.05 ± .69 nmoles/min/mg protein. The higher activity in BMC and their much greater yield indicated that these cells would be preferable for providing an index of GR activity in body tissues. Buccal cells would suffice if a blood sample is not readily available. These results suggest that the assay of DGDH activity in buccal cells or BMC should provide a definitive diagnosis of PH2.

Inhibition of Crystal Adhesion to Renal Epithelial Cells by Urinary Macromolecules is Impaired in Male Stone Formers.

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Adhesion of urinary crystals to renal tubular cells could be a critical event in kidney stone formation. The present study was performed to determine if the urine from stone forming (SF) individuals lacks crystal adhesion inhibitory activity. A first morning whole urine (WU) sample was obtained from 24 SF subjects (17 males and 7 females) and 24 age-, race- and sex-matched controls (C). Urine was centrifuged and an ultrafiltrate (UF) free of macromolecules (MW >10 kDa) and 10X concentrate (U_{conc}) were prepared. Supplementing UF with increasing amounts of U_{conc} (0.25X, 0.5X, or 1X) progressively decreased crystal binding to cells. This effect was blunted only in the male SF group as compared to controls (P<0.10 for WU, P<0.05 for UF plus 0.5X or 0.25X U_{conc}). To identify the responsible macromolecule(s), COM crystals were coated with the U_{conc} and adherent proteins were released and probed by Western blot. COM crystals coated with U_{conc} from controls contained 3.5-fold more Tamm-Horsfall Protein (THP, *P<0.01). Coating of crystals with other proteins in U_{conc} did not consistently differ between SF and C as a group. For all subjects, COM crystal coating by urinary prothrombin fragment 1 (UPTF1) (P<0.05) and crystal adhesion inhibitor (CAI) (P<0.10) correlated with inhibition of crystal binding, whereas coating with osteopontin (OPN) correlated with increased cell binding tendency (P<0.05). In conclusion, urinary macromolecules can coat COM crystals and block their adhesion to renal cells. This inhibitory activity seems to be blunted in male SF individuals. THP, OPN CAI, and UPTF1 may all play a role in renal cell-urinary crystal interactions.

A New Sensitive Spectrophotometric Assay for Plasma Oxalate

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An accurate and precise measurement of plasma oxalate is useful in the diagnosis and management of primary and enteric hyperoxaluria. To achieve sensitivity for detecting normal oxalate concentrations (1-3 nmol/L) an assay using oxalate oxidase immobilized on a coil was developed several years ago but is cumbersome and cannot reliably detect values in the normal range. Thus, a new method for plasma oxalate was pursued. Using a Beckman-Coulter spectrophotometer with enhanced sensitivity (0.0001 OD), a liquid-based assay was developed. This enzymatic assay is based on the reduction of oxalate using oxalate oxidase to release hydrogen peroxide, which reacts with a dye to give a colored end-point. The new method is linear (1-50 µmol/L), with precision within 13% CV at 1 µmol/L and mean recovery of 84%. Acidified unfiltered plasma samples are stable through day 14 when frozen while the frozen filtrates are stable through day 28. A normal value study defined a new reference range as <1.8 µmol/L. Comparison between the coil-based assay with the new assay (1-100 µmol/L) revealed the newer method to have on average 189% higher values than the older method (R=0.9906; y=1.1975x +1.901). This difference is likely due to differences in buffers, which in the older assay resulted in spuriously low values. In summary, a new rapid, reliable assay for plasma oxalate has been designed. The assay is safer, easier to perform and more sensitive with improved discrimination between normal and abnormal results.

Treatment with Potentially Oxalate Degrading Bacteria in Patients with Crohn's Disease: Does it Really Help in Reducing Urinary Oxalate Excretion?

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Introduction: The incidence of urolithiasis/nephrocalcinosis is increased in patients with Crohn's disease. The main culprit is secondary, absorptive hyperoxaluria, but treatment of this condition is difficult. A new treatment option with potentially oxalate degrading bacteria sounded promising, and hence we started treatment with such bacteria. We already showed, that Oxalobacter formigenes can reduce urinary oxalate excretion via intestinal oxalate elimination, now, we were interested to see, whether lactic acid bacteria or Enterococcus faecalis, both reported to be capable of degrading oxalate, would either reduce intestinal oxalate absorption, or intestinally eliminate accumulated oxalate.
Methods: We included 20 patients with M. Crohn, secondary hyperoxaluria and stone disease in our study. So far we included 6 patients (4 male and 2 female) aged 5–62 years. Kidney function was normal in all patients. Before treatment with lactic acid bacteria or Enterococcus faecalis baseline evaluation including 24 h urine analysis and a [$^{13}C_2$]oxalate absorption test were performed. Plasma oxalate levels were obtained and stool specimens were analyzed for the most common intestinal bacterial groups. The patients were taking either 2 x 45 drops of Enterococcus faecalis solution (Symbioflor 1, n=4) or 2 x 1 package lactic acid bacteria powder (SymbioLact comp) preferably with their two main meals for 4 weeks, followed by a short time follow up of 2 weeks. One ml Symbioflor 1 contains cells and autolysate of 1.5 – 4.5x10^7 Colony forming Units (CfUs) Enterococcus faecalis. Each package of SymbioLact comp. contains 5x10^8 CfU of Lactobacillus acidophilus, Bifidofacterium bifidum, Lactobacillus casei and Streptococcus lactis. Stool samples and 24 h urines were weekly collected and analyzed, a second oxalate absorption test and plasma analysis were performed at week 5.
Results: We had previously treated a 47 year old patient with extreme short bowel syndrome and urolithiasis (50-80 CaOx-dihydrate stones per year) with lactic acid bacteria. Urinary oxalate excretion decreased with increasing intestinal colonization and, as a result, no more stone passages were reported. In this study, cell counts of Enterococcus faecalis or lactic acid bacteria, respectively, tended to increase in the particular treatment groups. However, we were so far only able to significantly decrease urinary oxalate excretion in one patient under Enterococcus faecalis treatment. In all other patients, urinary oxalate excretion remained stable, as did the plasma oxalate levels. Intestinal oxalate absorption was elevated in two patients before treatment (13.8 and 25.3 % normal < 10) and in one thereafter (16.6 %).
Conclusion: As previously shown in healthy subjects under a high oxalate diet, other potentially oxalate degrading bacteria than Oxalobacter formigenes seem not to be able to significantly reduce the urinary oxalate excretion. However, as the intestinal oxalate absorption was normal or only slightly elevated, we wondered where the hyperoxaluria came from, with patients being on a normal oxalate diet. There are of course more questions remaining and we hope to get better answers, with more patients included in our study.

Toxicity of Calcium Oxalate Monohydrate in Normal Human Proximal Tubule Cells

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Ethylene glycol intoxication can induce a proximal tubular necrosis through its metabolism to oxalic acid, which precipitates in the presence of calcium as calcium oxalate monohydrate (COM) crystals. Our initial studies using normal human proximal tubule (HPT) cells in culture showed that COM, but not the oxalate ion, produced cytotoxicity in toxicologically relevant concentrations. COM, but not the oxalate ion, also induced hemolysis of rat RBC. Aluminum citrate, at 0.2 mM, completely prevented the COM-induced damage to both the RBC and HPT cells, and was more potent than other citrate salts. Aluminum citrate did not solubilize COM, rather acted through a physicochemical interaction with the surface of the COM crystal. COM altered the thermotropic phase transition of phosphatidylserine (PS)-composed liposomes, without affecting phosphatidylcholine liposomes, suggesting a direct interaction between COM and membrane PS. This interaction was reduced by co-treatment with aluminum citrate. The results indicate that COM is responsible for loss of cellular membrane integrity and that crystal-induced toxic damage to proximal tubule cells could be a key step in the acute renal failure induced by ethylene glycol. Also, the ability of aluminum citrate to ameliorate the toxicity from oxalate should be further studied.

Comparison of the Kinetic Properties of Lactate Dehydrogenase and Glyoxylate Reductase in Glyoxylate Metabolism

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Two enzymes, lactate dehydrogenase (LDH) and glyoxylate reductase (GR) are thought to metabolise glyoxylate. GR catalyses the conversion of glyoxylate to glycolate while LDH is believed to synthesise oxalate.

Detailed kinetic studies using recombinant human LDHA, the isoenzyme predominantly expressed in the liver, and recombinant GR were performed. Kinetic studies were carried out at pH 7.5 for both reduction and oxidation reactions in 100mM potassium phosphate buffer. NADH/NADPH were used as cofactor with glyoxylate and hydroxypyruvate as substrates for the reduction reaction. For oxidation reactions glycolate and glyoxylate were used with NAD as co-factor.

Based on Km and catalytic efficiency values, LDHA was more effective at hydroxypyruvate to L-glycerate conversion than gly-

oxylate to glycolate. GR catalysed the reduction of glyoxylate to glycolate more efficiently than LDHA.

Human LDHA did not utilize glycolate as substrate. LDHA oxidised glyoxylate more efficiently than its reduction. Kim and catalytic efficiency values for glyoxylate oxidation by LDHA were similar to those for GR/glyoxylate reduction. This similarity suggests that, in vivo, glyoxylate oxidation may be dependent upon relative concentrations of NAD and NADPH.

Primary Hyperoxaluria Type 1 in 2 Lebanese Families. Clinical Presentation and Report of a Novel Mutation.

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Primary hyperoxaluria (PH) is an autosomal recessive disorder. Different mutations, in various population, were identified leading to variable degrees of AGT catalytic activity and immunoreactivity. Two unrelated Lebanese families with type 1 PH are reported. In one family, clinical features consisted of an acute complicated renal insufficiency, while in the second, chronic renal insufficiency followed by recurrence of hyperoxaluria on the allograft kidney was noted. Molecular study of the AGXT gene was performed. EDTA blood samples were collected for genetic studies and DNA was extracted from lymphocytes by standard methods. Informed consent was obtained from each family member before blood sampling and DNA analyses.

Exploration of the entire coding sequence of the AGXT gene revealed the presence of a homozygous non-sense mutation the R122X in patients of both families. The substitution of the nucleotide C486→T make the transformation of an arginine to a stop codon. Mutation analysis may be specific to a population and may replace enzymology at the present time, obviate the need of liver biopsy. It would also enable prenatal diagnosis in families at risk and detect asymptomatic individuals. It can be proposed as a screening test when faced with patients with high suspicion of type 1 PH or to their siblings and be considered confirmatory in specific population such as the Lebanese one described.

Calcium Oxalate Saturation in Hemodialysis Patients with and without Primary Hyperoxaluria

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Calcium oxalate supersaturation of the blood is associated with deposition of crystals in the tissues. We measured the serum levels of oxalic acid (Ox), citric acid (Cit), calcium (Ca), and magnesium (Mg) to estimate the saturation in 112 hemodialysis patients without primary hyperoxaluria and 2 boys with primary hyperoxaluria (PH). Serum levels of Ox and Cit were determined by high performance capillary electrophoresis, while Ca and Mg were measured by ICP spectroscopy. The serum levels of Ox, Cit, Ca, and Mg in hemodialysis patients were 44.9±16.5 μmol/l, 138.1±54.9 μmol/l, 2.30±0.28 mmol/l, and 1.07±0.18 mmol/l, respectively, while the levels in patients with PH were 58.6±50.1 μmol/l, 134.8±107.4 μmol/l, 1.99±0.80 mmol/l, and 1.44±0.77 mmol/l, respectively. Serum CaOx saturation (SS) was significantly correlated with serum Ox level. Most patients showed metastable supersaturation, which was associated with a serum Ox level of more than 30 μmol/l. The serum saturation exceeded the formation product in some specimens from PH1 patients. Serum CaOx saturation [SS(CaOx)] showed a significant positive correlation with the levels of [Ox], [Ca], and [Cit]: $[SS(Ox)] = -0.3562 + 34.634 \times [Ox] + 0.394 \times [Ca] - 0.483 \times [Cit] + 0.101 \times [Cit]$, (all mmol/l, $r = 0.9848$, $p<0.01$). In conclusion, the serum Ox level is a good indicator of CaOx saturation and should be monitored accurately to avoid hyperoxalemia.

Polyacrylates Inhibit Calcium Oxalate Crystal Formation In Vitro and In Vivo

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Polyanionic polymers were shown to inhibit calcium oxalate crystal (CaOx) formation in vitro and in vivo (Nakatani et al, 2002; Osswald et al, Urolithiasis 1989). In the search for more potent inhibitors of CaOx crystal formation a series of alkanecarboxylic acids were tested in vitro with the continuous flow crystallizer (CFC) according to Finlayson (1972) and in vivo by assessing renal CaOx deposits in rats fed a vitamin B6-deficient chow plus 1% ethylene glycol in the drinking water for 5 days. Using an energy-dispersive x-ray microanalysis we found no contamination of the calcium crystals with phosphorus in the kidney slices indicating that the particles are composed of CaOx alone.

The most potent compound, Goe 6045, a polyacrylate with a molar weight of 70 kD, inhibited in the CFC CaOx crystal formation with an EC 50 of 10 nM. Crystal growth rate was 0.39 ± –0.02 in controls and 0.15 ± -0.01 micro m/min with Goe 6045, respectively. In rats CaOx crystal deposits in the kidney were reduced by more than 90% following administration of Goe 6045 at a dose of 10 mg/kg/24 hours. The compound was infused i.v. via an osmotic minipump starting at the beginning of the lithogenic diet.

Our data of an inhibitory action of polyacrylates against CaOx crystal formation in vitro and in vivo may provide a basis for a new therapeutic principle in the prevention of kidney damage in primary hyperoxaluria.

Primary Hyperoxaluria Type One – An Unprecedented Presentation at Birth

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Primary hyperoxaluria type 1 is a rare autosomal recessive, peroxisomal disorder. To our knowledge, no published data are available on onset in foetus and neonatal presentation. A term male baby, born by emergency caesarean section to Asian parents with parental consanguinity. Uneventful antenatal history, no risk factors for infection. The baby presented at 5 hours of age with cyanotic episodes, hypotonia, unexplained tachypnea and tachycardia. After excluding other causes, later diagnosed as PH1 based on urinary oxalate, glycolate level, increased oxalate creatinine ratio, renal calcinosis, and echo dense parenchyma on renal ultrasound. The CT scan of brain showed left middle cerebral arterial infarct with unilateral enlargement of ventricle and left porencephalic cyst, inferred as an antenatal event. Baby improved on diuretics, water supplementation, pyridoxine, prophylactic trimethoprim and Albright solution. Presently coping well with follow-up care of community paediatric team. Presentation of PH1 in intrauterine life or at birth is an extremely rare event. Suspicion of possibility could be life saving. Antenatal diagnosis is not easy since blood and amniotic fluid biochemical tests are nonconclusive in foetus and AGT enzyme activity detection needs foetal liver biopsy. Implications – this case raises an issue of "is there any treatment possible for the foetus (intra uterine) if diagnosed early in pregnancy and should the genetic counselling include the possibility of intrauterine damage with associated long term morbidity in its contents along with the possibility of different mutations in the same family".

Insights into Understanding Hyperoxaluria from Studies on Stone-Free Normo-Oxaluric Black South African Subjects

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Although urolithiasis occurs in South Africa's white population to the same extent as in other western societies, it is extremely rare in the black population. Previous studies in our laboratory have shown that the typical diet of this group is markedly hyperoxalurogenic yet their oxaluria levels are within the normal range and are lower than those in whites. This suggests that unique gastrointestinal handling mechanisms for oxalate might operate in the black race group. Ten healthy male subjects from each population group provided fecal samples which were analysed for the colonization and utilization of oxalate degrading bacteria using standard microbiological techniques. Results showed that both colonization and utilization were significantly higher in the black group.

We conclude that investigation of the gastrointestinal factors which protect the stone-free black population of South Africa against hyperoxaluria has the potential to provide insights into understanding this condition in the global community, and how to handle it.

SSCP Analysis of Exons 4 and 7 of the AGXT Gene and Its Value for the Genetic Diagnosis of Primary Hyperoxaluria type 1 (PH1)

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Definitive diagnosis of PH1 requires the analysis of alanine:glyoxylate aminotransferase activity in a liver biopsy sample. Molecular genetic analysis may reduce the number of patients requiring a biopsy. We have previously shown that limited screening of the AGXT gene for the 3 commonest mutations (c.33_34insC, c.508G>A, and c.731T>C) identifies 2 mutations in 34.5% of PH1 patients, avoiding the need for a liver biopsy. The 508A and 731C mutations are detectable by SSCP analysis, which also allows the simultaneous identification of additional mutations. A wider diagnostic strategy encompassing this approach has been evaluated. PCR and SSCP analysis of exons 4 and 7 of the AGXT gene was conducted in 269 biopsy confirmed PH1 patients. Eight additional mutations were identified in exon 4 of which 5 were novel (c.481G>T, c.481G>A, c.473C>T, c.497T>C, and 445-454 del GCTGCTGT) and 6 in exon 7 of which 3 were novel (c.757T>C, c.776+1G>A, and c.725insT). Functional studies of the mis-sense mutations are underway.

Using the combined screening approach, at least 1 mutation was found in 194 (72.1%) patients. A molecular diagnosis (detection of 2 mutations) was possible in 116 patients, giving a clinical sensitivity of 43.1%. In total these mutations account for 55% of mutant alleles in the patient cohort.

Development of a Cell-Based Bioassay to Screen for Drugs that Stabilise Alanine: Glyoxaylate Aminotransferase

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Most missense mutations in alanine:glyoxylate aminotransferase (AGT) in PH1 are predicted to lead to misfolding and decreased stability of the enzyme, leading to its mistargeting, aggregation and/or accelerated degradation. One approach to the rational treatment of PH1 would involve the administration of drugs that counteract the effects of these mutations by stabilizing AGT. In order to search for such drugs, we have designed a novel cell-based system in which U2OS cells are stably transformed with normal GO and mutant AGT. The screen is based on the observation that the cytotoxicity of glyoxylate is much greater than that of its immediate metabolites, such as glycolate, oxalate and glycine. Glycolate is not toxic to untransformed U2OS cells, whereas it is highly toxic (albeit indirectly) in cells transformed with GO, due to its conversion to glyoxylate. Indirect glycolate toxicity is directly proportional to the level of expression of GO. Normal AGT protects the cells from this indirect glycolate toxicity, but mutant AGT (for example that containing the Pro11Leu and Gly170Arg replacements that lead to its peroxisome-to-mitochondrion mistargeting) does not. Preliminary evidence suggests that non-specific chemical chaperones enhance the ability of mutant AGT to protect GO-transformed cells from indirect glycolate toxicity. This finding indicates that this system has potential for screening more specific drugs emanating from our in silico AGT structure-based drug design programme.