A cell culture system for studying kidney stone disease

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Keywords: kidney stone disease, cell culture system, cDNA arrays, gene expression changes

Abstract

Background Adenine phosphoribosyltransferase (APRT) deficiency is associated with 2,8-dihydroxyadenine (DHA) kidney stone disease in humans and in a mouse model for this disease. The renal deposition of DHA is associated with the expression of genes involved in tissue injury. To determine the molecular basis for tissue injury, we investigated gene expression alterations in cultured normal human kidney cortical epithelial (NHK-C) cells exposed to DHA crystals.

Methods First strand cDNAs were synthesized from mRNAs isolated from treated and untreated cells. These cDNAs were hybridized to membrane-bound human cDNA arrays, and the relative changes in gene expression in treated versus untreated cells were quantified.

Results The expression of four genes, α-catenin, integrin α3, and integrin β6, and platelet-derived growth factor B (PDGF-B) was elevated following exposure of NHK-C cells to DHA.

Conclusions These findings suggest that DHA crystals stimulate the expression of specific genes in cultured renal epithelial cells. DHA crystals may affect cell-cell or cell-matrix interactions or alter the cell structure, and these changes may ultimately contribute to renal injury.

Aim

The interaction of nephrotoxic substances with individual renal cells cannot be studied in intact kidneys. We therefore used cultured renal epithelial cells, which constitute the major portion of the renal mass, to study the response of these cells to DHA crystals. The aim of the study was to identify the molecules that are activated at the earliest stages of the interaction between DHA crystals and renal cells.

Introduction

Adenine phosphoribosyltransferase (APRT) deficiency results in the excretion of adenine and its oxidation product, 2,8-dihydroxyadenine (DHA), in the urine. DHA is extremely insoluble and its deposition in the kidney can result in kidney stone disease. However, the mechanisms of crystal-induced renal injury are largely unknown. Cultured renal epithelial cells have been widely used as models to study the response of cells to crystals such as those of calcium oxalate monohydrate (COM), the most common cause of kidney stone disease.

The association of crystals with renal epithelial cells is considered a potential factor in the process of renal stone formation, which may ultimately cause kidney obstruction. The retained crystals may promote stone growth and cause tissue injury. In cell culture models, COM crystals stimulate the expression of specific genes. The pattern of gene expression may provide important information about the pathological role of crystals in kidney stone disease.

We used cultured normal human kidney cortical epithelial (NHK-C) cells as a model to identify changes in gene expression following the exposure of these cells to DHA crystals.

Methods

DHA crystals

DHA crystals were prepared by dissolving DHA powder in 1 M NaOH and then slowly neutralizing the solution with HCl. DHA crystals formed after incubating the solution overnight in the refrigerator. The solution was centrifuged and the crystals washed with water to remove the salt, and then dried in an oven.

Cell culture

NHK-C cells were kept in serum-free medium for 24. Fresh serum-free medium containing DHA crystals was then added. At specific time points after crystal addition, the medium was removed and the cells washed and frozen at -70°C. Cells that had not been exposed to DHA crystals were used as controls.

cDNA synthesis and hybridization

Total RNA was isolated from the treated and the untreated cells, and first strand cDNAs were synthesized. These cDNAs were labeled with radioactivity and then hybridized overnight to membrane-bound human cDNA arrays. The membranes were washed and then exposed to x-ray film. Changes in gene expression were normalized to that of a control gene (glyceraldehyde-3-phosphate dehydrogenase, GAPDH).

Results

In unexposed cells, hybridization signals were detected for 27% of the 588 cDNA elements present on the cDNA arrays, but not for any of the negative controls. Relative to the expression of the control gene (GAPDH), these signals were classified into high, moderate, or low abundance.

In NHK-C cells exposed to DHA crystals, the expression of three adhesion molecules (α-catenin, integrin $\alpha 3$, and integrin $\beta 6$) and platelet-derived growth factor B (PDGF-B) was markedly elevated compared with unexposed cells.

Conclusions

In cultured renal cells, DHA stimulates the expression of specific gene products, including adhesion molecules and PDGF-B. These molecules may interfere with cell-cell or cell-matrix interactions, or alter the cell structure. These alterations may contribute to crystal-induced renal injury.