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CRITICAL ANALYSIS OF GROWTH AND DEVELOPMENT OF CRYSTALS WITH REFERENCE TO MEDICAL APPLICATIONS

Patel Dipeshkumar Babubhai[1] , Dr.Sanjay Rathore [2]

[1]Research Scholar, Dept. of Physics, Sri Satya Sai University of Technology and Medical Sciences, Sehore Bhopal-Indore Road, Madhya Pradesh, India.

[2]Research Guide,Dept. of Physics, Sri Satya Sai University of Technology and Medical Sciences, Sehore Bhopal-Indore Road, Madhya Pradesh, India.

ABSTRACT

Tubular cells and nephron-derived crystal nuclei may play a significant role in determining renal calcification. The most common component of renal stones is calcium oxalate monohydrate, and crystals of COM were quickly bound and absorbed by kidney epithelial cells in monolayer culture (BSC-1 line). It was observed that renal cells and COM crystals interact by the use of scanning and transmission electron microscopes, kinetic investigation of crystals tagged with ['4C]. Electron microscopy revealed that adherent crystals on the apical cell surface serve as aggregation sites. The plasma membrane domains enclosing internalized crystals demonstrated increased binding of exogenous crystals for at least 24 hours after the first cell-crystal interaction. Within lysosomal inclusion bodies, crystals that had been internally ingested seemed to disintegrate during the next five to seven weeks. Many cells with crystals remained in the monolayer at this phase. Adhesion and internalisation have been proven to assist crystals remain in situ, although intracellular breakdown of crystals may represent a hitherto undiscovered protection against kidney calcification.

Keywords: crystals, Calcium oxalate monohydrate, renal calcification, nephron, kidney, tubular cells.

INTRODUCTION

The unsung heroes of modern technology are crystals. It is impossible to have a photonic industry, electronic industry, or fibre optic communications without the use of crystals, which include superconductors, semiconductors, transducers, polarizers,

ultrasonic amplifiers, radiation detectors, and magnetic garnets, ferrites, piezoelectric refractory of various grades, non-linear optics, and crystalline films for microelectronics and the computer industry. [1] A wide range of scientific disciplines are involved in crystal development, including crystallography, physics, and mineralogy. Over the last few decades, crystal growth technologies have become more popular. This is because there has been a lot more demand for materials in technological applications. [2]

One of the oldest ways of crystal formation is the generation of crystals from aqueous solutions. In the manufacture of numerous technologically significant crystals, the crystal formation from low-temperature aqueous solutions approach is particularly popular. Because it works best with materials that are unstable at high temperatures [3] or that undergo phase changes below melting point, it is the most frequently employed method for growing single crystals. Low temperature solution growth takes weeks, months, or even years to complete. Even though crystal growth from solution has been well-developed, it still requires a lot of time, painstaking work, and even a little bit of luck. [4]

COM crystals can't stick to the surface of kidney cells because citrate, glycoproteins, or glycosaminoglycans in the tubular fluid can compete with them. Crystal and renal cell type-specific autocrine factors and proteins, as well as how much potassium there is in the environment, extracellular matrix proteins, are all important for these processes to work. Crystals can stay in cultured renal epithelial cells for weeks before they start to hurt them, but it's not clear if this is the case with intracellular COM crystals and their role in kidney stones. The long-term fate of crystals that had been inside the body and how cells responded to contact with crystals from outside the body were our main goals. [5]

MATERIALS AND METHOD

- **Materials**

Methods were used to create COM crystals from supersaturated liquids. To make radioactive COM crystals, a sodium oxalate solution with a specific activity of 10^5 cpm/ml was treated with oxalic acid and calcium chloride until the solution was

supersaturated. They have a specific activity of between 20 and 60,000 cpm/mg of crystals. Light and scanning electron microscopy were used to evaluate the size and shape of the crystals. Cuboidal to spindle-shaped COM crystals were generally tiny, ranging in diameter from 1 to 2 nm. Heating the crystals to 180°C overnight disinfected them. X-ray crystallography showed that heating had no effect on the crystal structure of COM. Unless otherwise stated, the reagents were acquired from Sigma Chemical Company. The process of crystallisation.

- **Method of crystal growth**

A modified version of an earlier method was employed. A compound microscope was used to examine the growth stages of calcium oxalate monohydrate crystals. At a temperature of 25 °C, crystals formed on a glass slide. In the middle of a glass slide, a drop of gel media at pH 5.02–5.17 was inserted and allowed to turn into gel. About 5 minutes later, the gel had formed. A single drop of magnesium acetate (1:1) solution, 1 M calcium chloride, 1 M oxalic acid and were put onto the gel on the glass slide. A chain of events ensued as a result.

$$CaCl_2 + H_2C_2O_4 \rightarrow CaC_2O_4 + 2HCl$$

After a specific period of time, the growing concentration of HCl at the growth site dissolves the calcium oxalate crystals. However, magnesium acetate solution and $CaCl_2$ prevent this dissolution. The crystals that have grown as a result of this process are clearly visible. Under a microscope, the dried glass slide was examined until it was perfectly clear.

- **Cell-to-crystal adhesion**

Crystal adhesion was assessed by aspirating the BSC-1 cell culture medium and replacing it with 5 ml of PBS heated to 37°C. It was necessary to introduce the [^{14}C] COM crystals into the buffer in order to reach an initial concentration of 200 j.tg/ml (47.2 g/cm^2 of cells) and to keep the solution constantly agitated at 1500 revolutions per minute in order to avoid aggregation of the crystals. It took five seconds of gentle agitation of the culture dishes to evenly disperse the crystals that then sank to the cell monolayer's surface. Three times with PBS were used to wash cells after two minutes

of buffer removal (5 ml). Finally, the radioactivity was detected by scraping cells into a scintillation vial, which was then filled with 6 N HCI and Ecoscint (4.5 and 0.5 ml, respectively).

- **Staining with acid phosphatases**

COM crystals or vehicle alone had been exposed to BSC-1 cells on monolayer cultures for eight days before the cells were rinsed and fixed for 15 minutes at room temperature with freshly prepared 0.037 M sodium phosphate buffer (pH 7.4) containing 0.01 percent sodium periodate and 0.07 percent paraformaldehydes.. Following the manufacturer's directions, naphthol AS-BI phosphate and methylene blue were used to counter stitch the fixed monolayers.

- **Electron microscopy**

In preparation for TEM, cells were rinsed with PBS and then treated for four hours at 4°C with half-strength Karnovsky solution, which included 2.5% glutaraldehyde and 2.5% formaldehyde in 0.2 M cacodylate buffer (pH 7.4). It took an Eppendorf tube one hour to extract the fixed cells from the plate. They were then incubated for an hour in a cacodylate buffer containing 2% Osmium Tetroxide (OTX) to examine the architecture of the cells therein. The extracellular space at the cell's base was examined for the formation of crystals in two different investigations using cells grown on inserts covered with an artificial matrix material. In order to employ TEM, these monolayers did not have to be scraped free of the cells. Using Epon epoxy resin, cells dehydrated in ethanol concentrations ranging from 5% to 35% were placed (absolute). It was done on an ultramicrotome, stained for one hour with uranyl acetate, and then looked at using an electron microscope at 80 kV and a Siemens 101 electron microscope. A light microscope was used to examine it further. Cells on glass coverslips were allowed to air dry for this experiment.

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RESULTS

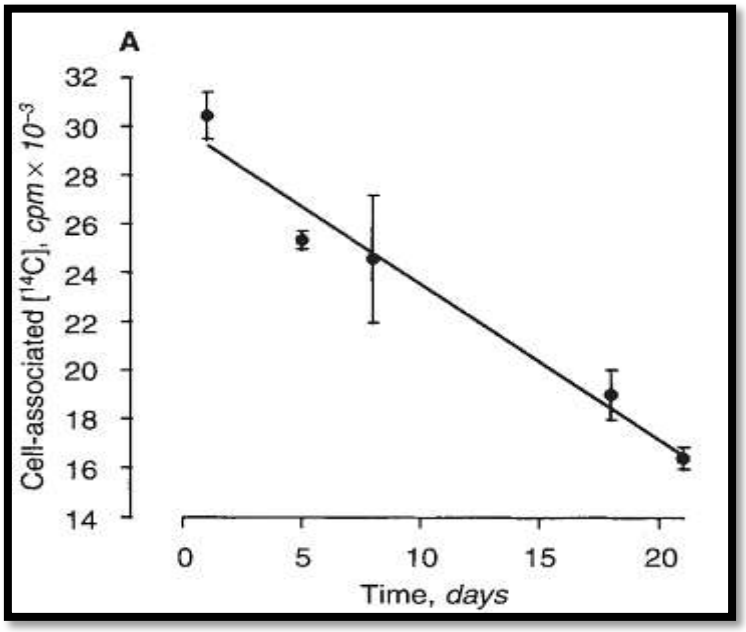

Fig 1: BSC-1 line of monkey kidney epithelial cells and COM crystals

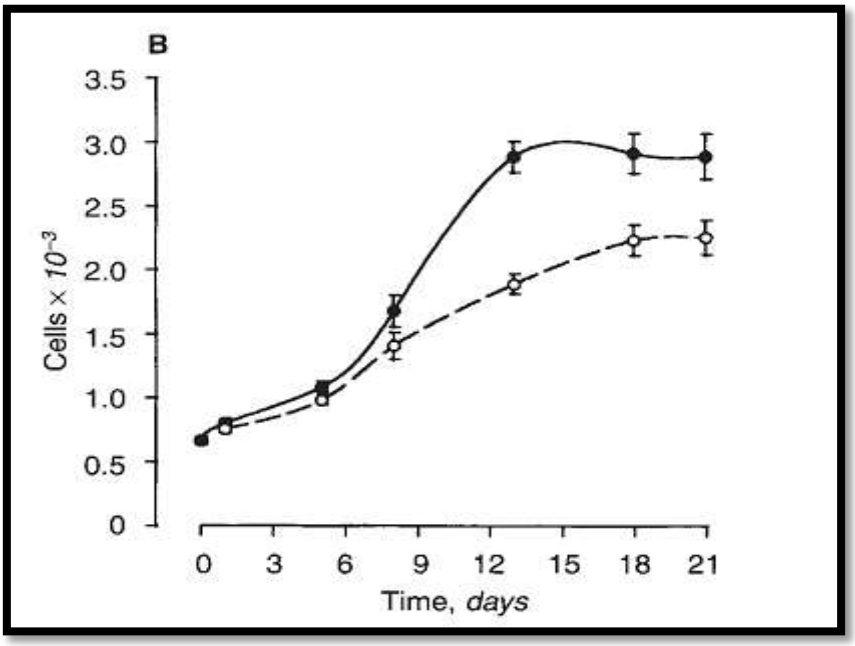

Fig 2. BSC-1 line of monkey kidney epithelial cells and COM crystals

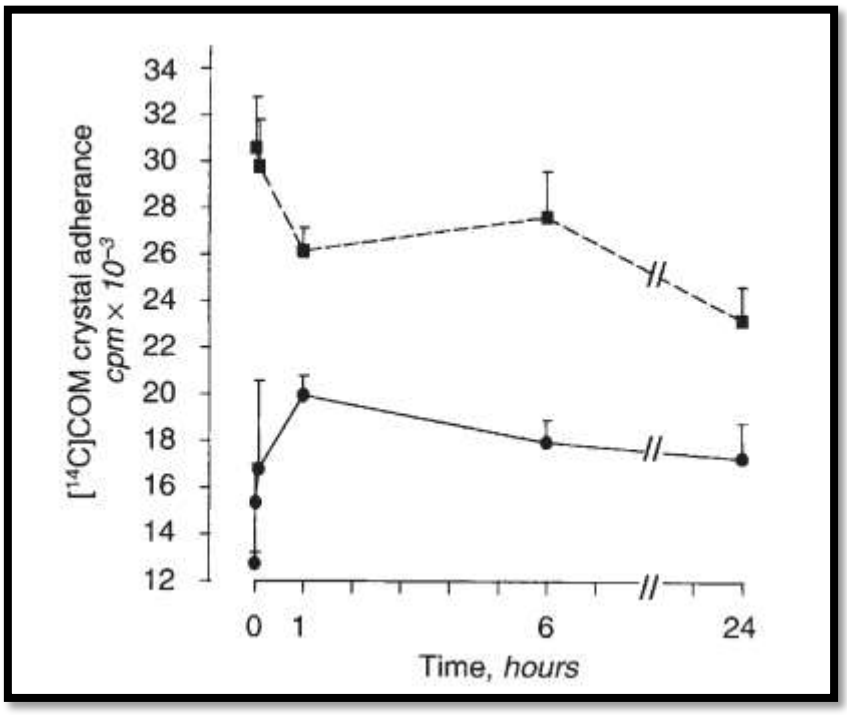

Fig 3. COM crystals adhere to renal epithelial cells

DISCUSSION

According to this study, COM crystals in cultured renal epithelial cells shrink in size. Even though internalised COM crystals seem to disintegrate inside the monolayer, this process may take weeks or months in culture. As such, pathological conditions might limit the renal cells' ability to dissolve COM crystals, causing in their buildup in what is known as the "calcification nidus." Anchored COM crystals may operate as a favored location for the binding of additional crystals as soon as they connect to the apical cell surface and become adherent. Excess oxalate contributes to the production of calcium oxalate monohydrate stones. Oxalates produce oxygen free radicals in renal epithelial cells, resulting in lipid and protein peroxidation, which damages the cell membrane.

CONCLUSIONS

COM crystals are taken in by cultivated renal epithelial cells and then slowly broken down in lysosomal inclusion bodies. Regardless of the rationale, crystal-containing cells have a higher affinity for them and/or have more binding sites for them, making their surfaces suitable locations for the attachment of other crystals. As a consequence, clusters of neighboring crystal-laden cells form in the monolayer with increasing time. If the breakdown of crystals within cells is delayed or poor, it might cause crystals to persist inside cells and create kidney calcification that isn't healthy.

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