Kidney Stones: A Stone That Never Rolls Away

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Kidney stone disease, also called renal calculi, is a common, painful disease which results from the combination of numerous risk factors such as age, gender, lifestyle and family history. The prevalence of kidney stones varies greatly between geographic locations. In India, 12 % of the population are affected with this disease with incidences of patients generally low in the south while high in the northwest regions. The scary thing about kidney stone disease is that even after availing proper treatment, there is 35 – 50 % chance for recurrence of stone. Of the various types of stones, calcium oxalate (CaOx) stones occur in the major population of patients. Other types of stones include uric acid, cystine and struvite stones. Since, CaOx stones account for majority of the population, Prof. G. S. Selvam dedicated his entire research career to find possible therapeutic strategies. Knowing that CaOx stones occur at such high frequencies, one may wonder if he/she may develop a stone sooner or later in life. The major risk factor for development of CaOx stone is the increased levels of urinary oxalate excretion. So when does one excrete more oxalate? The answer is straightforward. One who consumes more amount of oxalate will excrete more oxalate. This leads to another question. Which food commodity exhibits high content of oxalate? Foods such as dark chocolates, beverages, nuts, beetroot and green leafy vegetables contain substantially higher levels of oxalate. Besides consumption of such food group, increased levels of urinary oxalate can result due to genetic factors too. Fortunately, the incidences of genetically influenced kidney

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stone formation are sparse. Hence, I too have started my research career wanting to explore various aspects of food influenced CaOx stone formation.

Although the consumption of oxalate rich food caused a significant increase in urinary oxalate excretion, the underlining factor for CaOx stone formation is uncertain. Hence, to understand the origination and development of the disease, the scientific community requires an experimental model. Animals such as mice, rats, rabbits, swine and monkeys are routinely used to study various diseases. Currently, numerous animal models are available to study CaOx stone disease. Unfortunately, stone development in these prevailing experimental models does not occur as a result of ingestion of natural food commodities. Therefore, studying various aspects of food influenced by CaOx stone formation using existing models may not help us complete the puzzle. In an effort to tackle this simple but important problem, I sampled the level of oxalate in various food commodities. On estimating, I identified that spinach, a green leafy vegetable commonly consumed by all exhibited the highest oxalate content. Therefore, I extracted the oxalate present in this vegetable and fed it to laboratory rats in addition to their regular food. Besides this group of rats, I fed another set of rats with the similar amount of chemically synthesized oxalate (a previously established model for inducing CaOx stones). At the end of the experimental period, the animals were euthanized and kidneys were assessed for stones and damage. Interestingly, both rat groups developed CaOx stones but rats that were fed with the commercially synthesized oxalate exhibited severe kidney damage with high number of stones. The increased number of stones can be attributed to the fact that injured tissue favoured stone formation. On the other hand, the oxalate from the spinach extract invoked mild but significant responses. The abrasive nature of the synthetic oxalate was missing in the spinach extract; suggestive of oxalate from natural sources could induce stones with minimal kidney injury. The findings were published in a peer-reviewed international journal (ToxicolMech Methods. 2018 Mar; 28(3):195-204).

Now that the mode for CaOx stone formation was established, another question came to my mind. What is the mechanism for stone formation? The question is valid since the sequence of events that leads to the formation of kidney stone disease remains unclear. Every scientist had proposed a pathway to illustrate stone formation. However, the one thing that was agreeable among all the research groups was the fact that interaction of renal cells with oxalate ions act as precursor for renal epithelial cell injury, crystallization, crystal retention and development of stone. This unpleasant contact between kidney cells and oxalate ions results in free radical generation. Free radicals are dangerous molecules that inflict injury to various cells and tissues. This aspect was extensively studied for decades. Hence, the biological question raised here was, "Besides free radical generation, are there any other factors responsible for CaOx stone formation".

We have all learnt in school that endoplasmic reticulum (ER) is a cell organelle that plays a significant role in protein synthesis, folding, assembly, transportation and maintaining calcium ion homeostasis. When the cell is under threat due to toxins, or viral and bacterial particle invasion, or even aging this organelle is pushed to work harder. Since the organelle is unable to achieve the target set by the cell, the organelle and the cell harbouring this organelle are 'under stress'. Many a time this stress can cause the cell to die. If the level of stress is below the threshold limit, the ER

invokes an adaptive response and thereby the cell survives. Therefore, I wanted to observe how the ER responds to oxalate toxicity.

Computer stimulations were performed to see if the protein responsible for adaptive response interacted with either oxalate or CaOx crystals. The answer was yes. Oxalate ions and CaOx crystals were strangely attracted to this protein, GRP78. This illicit attraction could spell disaster for the cell to survive. The binding of oxalate ions and CaOx crystals to this protein could hinder or alter its functional ability. Since preliminary results were encouraging, the same was studied using kidney cell lines and rat model. The results obtained implied that oxalate toxicity did incite 'stress' on ER (J PhysiolBiochem. 2017 Nov;73(4):561-573). Although the stress incurred on the ER was significant, the implications of this stress on cell death was minimal. This exercise taught me that in case of oxalate toxicity, free radical generation controlled the life and death of the cell and ER stress played second fiddle. However, the identification of ER stress as a factor in CaOx stone disease has provided the scientific community with a new therapeutic target.

Now that free radical generation was identified as the chief cause for kidney stone disease, I desired to develop a new therapeutic strategy to tackle this common but complex problem. Having recognised that oxalate is the major player in the field of kidney stone disease, degrading this compound can bring about great dividends. The ability to degrade oxalate to less noxious substances could benefit a great number of individuals in the biomedical field. Unfortunately, there are no known naturally occurring oxalate degrading enzymes in humans. Fortunately, human gut harbours a collection of microbes known as intestinal microbiota. These intestinal bacteria convert oxalate into carbon dioxide and formate, the latter being further degraded and excreted in the faeces. These bacteria rely exclusively on oxalate, for energy. Hence in the absence of oxalate, these bacteria perish. Since the existing oxalate degrading bacteria are delicate and lack probiotic efficiency, the manipulation of gut flora with oxalate degrading bacteria may enable degrading oxalate and eventually prevent CaOx stone formation.

The discovery of oxalate degrading gene, oxalate decarboxylase (*oxdC*) from *Bacillus subtilis* (*B. subtilis*) raised a new hope to mitigate the increased urinary oxalate excretion. Since, *B. subtilis* is harmful to human health; the oxalate degrading gene alone was isolated and introduced into a well studied species of lactic acid bacteria, *Lactobacillus plantarum*. The genetically engineered *Lactobacillus plantarum*wascapable of degrading oxalate available in intestine i.e. oxalate that was ingested via food commodities. However, this newly developed genetically modified strain cannot alter/degrade the oxalate synthesized within the human system. Thus, an alternative approach is required to alleviate the oxalate that is produced within the individual. Hence, using a common therapeutic tool, I wanted to target both, the oxalate that is ingested by the individual via food and the oxalate that is produced within. In order to achieve this, I chose kidney as the target site for degradation. Since all the oxalate that has been ingested and produced by the body has to come in contact with the kidney, the organ was the chief site to employ my therapeutic tool.

Therefore, I isolated the bacterial gene *oxdC*and introduced it into a human kidney cell. This newly constructed kidney cell line gainedoxalate degrading efficacy. Since oxalate was degraded, the cell was protected from oxidative damage. The results were published in a peer reviewed

journal called, 'Journal of Enzyme Inhibition and Medicinal Chemistry'. However further studies in animal models are essential to establish the effectiveness of *oxdC* as a potential candidate gene therapy against CaOx stone disease.

This body can be compared to a temple. We have to nurture and care for it. We need to eat healthy, drink adequate quantities of water and squeeze in a few exercises daily to lead a healthy life.