

An Investigation Into The Relationship Between In Vitro Stone Growth Rate, Oxalate And Crystal Size Distribution

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The question we posed at the outset of this project was “**What controls the speed of oxalate stone growth?**”

The crucial word here is “**stone**”. From many years work by those studying kidney stone disease, we have a good understanding of the factors which control the speed of calcium oxalate **crystal** formation, whether this is in simple salt solutions, urine-like solutions or urine itself. There has always been a general assumption that what is true of crystals would also apply to stones – but until recently this could not be tested directly.

Our project aimed to use our stone farm to grow multiple calcium oxalate stones, in the laboratory, under controlled conditions. This allowed us to test the effects of varying the calcium and oxalate concentrations and to measure the response to some known low molecular weight crystallisation inhibitors and the response to high molecular urine factors and some proteins. A specific aim was to examine how the speed of stone growth was related to the size and number of crystals being produced.

1. Our previous studies had shown that citrate, which is a well known inhibitor of calcium oxalate crystallization, also inhibited stone growth – at similar concentrations. By contrast, we have now shown that phytate, another crystallization inhibitor, also inhibits stone growth – but at a much lower concentration than required to inhibit crystal formation [1, 3, 5]. This demonstrates a clear distinction between the processes of crystallization and stone growth and because the stone growth is inhibited while the crystallization is unaffected, we can argue that the effect of low phytate concentrations is mediated through its action/affinity for the stone surface.
 - Stone growth can be inhibited, even when crystal formation is unaffected.
 - Phytate effectively inhibits stone growth at physiologically realistic concentrations.
2. It has long been known that human urine contains a number of high molecular weight components which are powerful inhibitors of calcium oxalate crystallization. We have confirmed that this inhibitory activity extends to inhibition of stone growth [1, 2].
 - Nearly whole human urine and urinary macromolecules almost completely abolished **stone** growth.
 - Even under moderate conditions of hyperoxaluria, urine macromolecules exert a strong inhibition of calcium oxalate stone enlargement.

3. By varying the concentration of calcium and oxalate being fed into the stone farm we were able to modify the stone growth rate in line with the expectation that the supersaturation would provide the overall driving force for enlargement [1, 4, 6].
 - The conventional approach to reducing risk of stone formation, that of reducing excessive calcium and/or oxalate excretion and encouraging a good fluid output, will also help to reduce stone enlargement rates.
4. The surface and characteristics of the developing stones are likely to be critical in determining their growth. We had previously suggested from our preliminary evidence that the growth was directly related to the surface area. In a larger analysis we have confirmed this and shown that once a calcium oxalate layer has been deposited, the underlying composition of the core material has no effect on growth [1].
 - This has obvious implications for stone fragmentation and removal procedures.
5. Animal experiments many years ago suggested that brief peaks of oxalate excretion could trigger stone growth, which would then continue to grow while in a much lower oxalate environment. These experiments have never been confirmed or further expanded upon but they have important implications for individuals at risk of occasional or sporadic periods of hyperoxaluria. We devised a similar experiment with the stone farm and while we were able to trigger stone growth by high oxalate levels, this was not maintained in low oxalate conditions [6]. Although our experiment was in vitro and lacked the biological component, we were better able to control the urine oxalate concentration and reproduce alternate periods of hyperoxaluria and normal oxaluria.
 - Under our conditions, stone growth could be abolished by reducing the oxalate concentration. If this were to apply in vivo, then any periods of reduced oxaluria are likely to be beneficial in terms of reducing overall stone growth rate.
6. There are essentially two mechanisms by which stones can enlarge; through direct crystal growth at the stone surface or by aggregation and incorporation of crystals which developed in suspension. In order to discriminate between these two mechanisms we examined the relationships between stone growth rates, crystal growth rates and crystal nucleation rates. We observed that there was a significant negative correlation between the crystal nucleation rate and crystal growth rate, a significant positive correlation between stone growth rate and crystal nucleation rate and a significant negative correlation between stone growth rate and crystal growth rate [1, 4, 7].
 - This suggests that the primary enlargement mechanism for stones grown in the stone farm is through aggregation rather than direct surface growth.
 - This is the first quantitative evidence to support this proposed mechanism.

In summary, and answering our initial question, we have shown that the important factors controlling calcium oxalate **stone** growth are solution chemistry, suspended crystals, aggregation, surface area and inhibitors present in urine.

Publications resulting from this project.

1. JP Kavanagh, PN Rao (2007) In Pathogenesis of Stone Disease, Proceedings of 1st International Urolithiasis Research Symposium, Edited by J Williams, American Institute of Physics Conference Proceedings, Springer, NY. Lessons from a stone farm. In press.
2. A McSorley, A Jones, AK Webb, PN Rao, JP Kavanagh (2007). In Pathogenesis of Stone Disease, Proceedings of 1st International Urolithiasis Research Symposium, Edited by J Williams, American Institute of Physics Conference Proceedings, Springer, NY. Calcium stone growth in urine from cystic fibrosis patients and healthy controls. In press.
3. NK Saw, K Chow, PN Rao, JP Kavanagh (2007). Effects of inositol hexaphosphate (phytate) on calcium binding, calcium oxalate crystallization and in vitro stone growth. J. Urol. In press
4. JP Kavanagh (2006). Supersaturation and renal precipitation – the key to stone formation? Urol. Res. 34, 81-85.
5. NK Saw, JP Kavanagh, PN Rao (2005). Inhibitory effects of phytic acid on calcium oxalate crystallization and stone formation in vitro. Urol Res 33, 136. (abstract)
6. NK Saw, S Gilpin, J Dixon, JP Kavanagh, PN Rao (2005). The influence of variations in calcium and oxalate concentrations on stone growth in vitro. Urol Res 33, 134. (abstract)
7. NK Saw, S Gilpin, J Dixon, JP Kavanagh, PN Rao. (2005). The relationship between crystals in suspension and stone growth in a stone farm. Urol Res 33, 134. (abstract)

(Three other papers, dealing with the effects of macromolecules, and expanding on the data in abstracts no 6 and 7 are being submitted).

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