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Research Article

FEASIBILITY OF A STRATEGY TO PREVENT GOUTY ARTHRITIS THROUGH LIMITING CRYSTALLIZATION OF MONOSODIUM URATE

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ABSTRACT

Background: Crystallization of monosodium urate (MSU) is the cause of gout as well as is the cause of about 10% of kidney stones. The focus of this paper is on altering the crystallization of MSU, which occurs in the affected joint space. It is generally accepted that the inflammation caused by the MSU crystals leads to the clinical signs of gout: swelling, redness, and pain. Developing treatment and prevention strategies are hampered by not knowing the exact mechanisms. It is known, however, that the inflammatory phase can be controlled by limiting the size and amount of crystals formed. It is also known that only 2 to 36% of hyperuricemic individuals get gout; suggesting that there are chemicals found in the body that can prevent or limit crystallization in hyperuricemic individuals. This study was designed to look at the ability of various chemicals to modify the crystallization of MSU.

Methods and findings: It was found that vitamins (riboflavin, pyridoxine HCL, and β-carotene), some dyes (methylene blue, and fuchsin), calcium, and xanthine were able to limit crystal size by adsorption on the surface of the growing crystal. Both niacin and calcium were able to limit crystal size by altering the solubility of MSU. Lysozyme was able to degrade the crystals to limit their size.

Conclusions: Although these compounds show promise for drug therapy, there are still many steps required to determine if these compounds could be used clinically to significantly reduce the number of MSU crystals larger than 0.5 μm, and do so with minimal side effects. It also would require further tests to determine, if the difference in serum level of these compounds, among hyperuricemic individuals, is responsible for determining the likelihood of getting a gout attack.

Keywords: Gout drug therapy, Monosodium urate crystallization, Size induced inflammation

INTRODUCTION

Crystallization of monosodium urate (MSU) is the cause of gout as well as is the cause of about 10% of kidney stones (Roddy, *et al.*, 2010; Reichard, *et al.*, 2015 and Dumick, *et al.*, 1999). Gout is the most common inflammatory arthritis at about 1% of the population

(Roddy, *et al.,* 2010; Litwin, *et al.,* 2007 and Lawrence, *et al.,* 1998) (5% of arthritis patients) with about 10-14% of the population having kidney stones (Curhan GC, *et al.,* 2007).

In both cases, size and shape of the crystals affects the clinical presentation. For kidney stones this determines residence time in the various parts of the body from the kidney to excretion through the urinary system. For gout there is some belief that the surface chemistry is important (Kam, *et al.,* 1992; Kam, *et al.,* 1994 and Kanevets, *et al.,* 2009), but size (mostly diameter) of particulate or fibers is what leads to macrophage activation and the inflammatory response in other cases (Feldman, *et al.,* 2000; Shanbhag, *et al.,* 1994; Gonzalez, *et al.,* 1996 and Kossovsky, *et al.,* 1983).

The focus of this paper is on altering the crystallization of monosodium urate and therefore will focus more on gout, since kidney stones tend to not be pure MSU (Reichard, *et al.,* 2015 and Dumick, *et al.,* 1999). The results, however, can be applicable to kidney stones but would require further testing.

Again, although the exact mechanism for gout is not known, it requires the formation of MSU crystals in the affected joint space (Roddy, *et al.,* 2010). It is also generally accepted that interaction of MSU crystals with leucocytes leads to inflammation and the production of chemicals that increase the amount of MSU crystals, which serves to continue and amplify the cycle leading to the clinical signs of gout: swelling, redness, and pain (Lawrence, *et al.,* 1998). It is still, however, not agreed upon what causes MSU crystallization as well as how the MSU crystals trigger the inflammatory response.

Prevention strategies can be based on either controlling the crystallization phase or limiting the inflammatory phase; with current drug therapy mostly related to the inflammatory phase (Litwin, *et al.,* 2007 and Wallace, *et al.,* 1972). Developing treatment and prevention strategies are hampered by not knowing the exact mechanisms; also making it difficult to understand why certain strategies work.

All current prevention drugs reduce the uric acid concentration (or increase its solubility) by increasing excretion or reducing production (suppressing purine metabolism [uric acid is the end-product]) (Litwin, *et al.,* 2007; Wallace, *et al.,* 1972 and Howell, *et al.,* 1963). In some cases, however, this can actually trigger an attack (possibly by creating more nucleation sites) (Shoji, *et al.,* 2004). The inflammatory phase, in these cases, is controlled by preventing or limiting the amount of crystals formed (Litwin, *et al.,* 2007; Seegmiller, *et al.,* 1965 and Wilcox, *et al.,* 1975).

Treatment can also attempt to reduce the serum uric acid level as well as be aimed at suppressing the inflammatory response or trying to dissolve the crystals. Again, in some cases reducing the serum uric acid levels can paradoxically actually make an attack worse (Shoji, *et al.,* 2004). The anti- inflammatory drugs tend to target production of inflammatory compounds such as prostaglandins (Litwin, *et al.,* 2007 and Wallace, *et al.,* 1972) or reduce phagocytosis typically by reducing the production of chemotactic factors (Litwin, *et al.,* 2007 and Wallace, *et al.,* 1972). Also uric acid kidney stones have been successfully dissolved, in 70-80% of the cases, by lowering the pH with citrate (Degan, *et al.,* 2007).

A prevention strategy that has not been fully explored is to control the crystallization process to limit the inflammatory process. A potential drug intervention comes from the fact that only 2 to 36 % of hyperuricemic (above saturation levels of uric acid in their serum) patients, with approximately 5–10 years of follow-up, undergo MSU crystal formation (or possible form enough large MSU crystals to cause gout) (Litwin, *et al.,* 2007; Lin, *et al.,* 2000 and Hall, *et al.,* 1967). So there are probably chemicals (as well as environmental factors like pH) found in the body that can prevent or limit crystallization (Lin, *et al.,* 2000).

Instead of reducing uric acid concentration or increasing its solubility, crystallization could be limited by reducing the nucleation rate or affecting growth rate (Figure 1) of the crystals independent of saturation level (Litwin, *et al.,* 2007 and Wilcox, *et al.,* 1975). This strategy has been hampered by agreement about requirements for MSU crystals to elicit a gout attack.

There are a number of researchers who believe the response to MSU crystals is immunological. This comes from the fact that isolated MSU crystals are typically coated with immunoglobulins; with surface concentrations decreasing as inflammation resolves, with concomitant increases in apolipoprotein B surface concentration (Ortiz-Bravo, *et al.,* 1993 and Cherian , *et al.,* 1986). Also the cationic Fab portion of the antibodies bind to urate with the Fc portions pointed away and exposed (Kozin, *et al.,* 1980). The Fc portions then may play a role both in the ability of the crystal to activate complement as well as the ability of Fc-receptor-bearing cells to phagocytose crystals and undergo cell activation (Ortiz-Bravo, *et al.,* 1993 and Terkeltaub, *et al.,* 1983). This would lead to treatment options related to preventing complement activation.

It is, however, also suggested that activate resident tissue macrophages, which secrete inflammatory cytokines including IL-1β (Busso, *et al.,* 2012 and Martinon, *et al.,* 2006), are the starting point leading to complement activation and infiltration of neutrophils, with production of additional pro-inflammatory mediators such as the arachidonic acid products PGE2 and LTB4 (Choi, *et al.,* 2005).

There has been a long-standing debate over the importance of configuration versus chemistry in determining response to foreign materials, in many different clinical situations; besides gout; including asbestosis toxicity, fiber meshes for hernia repair, silicone oil around breast implants, and particulates around joint replacements. It has been suggested that the more stable the surface chemistry of the foreign material, the more important the configuration becomes (Feldman, *et al.,* 2000; Shanbhag, *et al.,* 1994; Sanders, *et al.,* 2000 and Kilpadi, *et al.,* 2000); especially if the substance is phagocytized (Nagse, *et al.,* 1995). It has been shown that if the fibers or particles are stable then both the inflammatory response and angiogenic response are relatively similar independent of material or surface charge (Shanbhag, *et al.,* 1994; Sanders, *et al.,* 2000; Kilpadi, *et al.,* 2000; Nagse, *et al.,* 1995; Black, *et al.,* 1992; Sanders, *et al.,* 2002 and Gonzalez, *et al.,* 1996). The initial protein response may be different, but quickly the surface is coated to elicit a non-specific response (Feldman, *et al.,* 2000; Shanbhag, *et al.,* 1994 and Sanders, *et al.,* 2000). In blood, however, the initial protein adsorption has a great effect on whether the clotting cascade is triggered, since this cascade happens in seconds versus the hours to days for cell activation (Feldman, *et*

al., 2000). Soluble chemicals released from foreign materials by diffusion or degradation, however, can quickly reach cells; with chemistry, in many cases, controlling the response (Feldman, *et al.,* 2000).

It appears that diameter (or the smallest dimension) is the key determination of macrophage activation for fibers and particles that cannot be phagocytized (with below about 50 μm the transition point) (Feldman, *et al.,* 2000; Shanbhag, *et al.,* 1994 and Sanders, *et al.,* 2000) most likely due to the size where macrophages and/or giant cells can surround the material with or without phagocytizing it (Feldman, *et al.,* 2000). Phagocytized particles tend to have another transition below 1 μm, which is probably also related to volume of particulate; with 0.5 μm being the transition point cited for MSU crystals (Feldman, *et al.,* 2000; Shanbhag, *et al.,* 1994; Nagse, *et al.,* 1995; Black, *et al.,* 1992; Sanders, *et al.,* 2002 and Gonzalez, *et al.,* 1996). So a prevention strategy is to limit the amount of MSU crystals larger than 0.5 μm. Since there are probably chemicals (as well as environmental factors like pH) found in the body that can prevent or limit crystallization, this study was designed to look at the ability of various chemicals to limit the crystallization of MSU.

MATERIAL AND METHODS

MSU Preparation

Since MSU is subject to both oxidation and bacterial attack, it was necessary to prepare it just prior to its use. The MSU crystals were made using a modification of Seegmiller's method (Seegmiller, *et al.,* 1965 and Wilcox, *et al.,* 1975). In this technique 1.006 gm of reagent grade uric acid (J.T. Baker Co.) was dissolved in 194 ml of boiling water with 6 ml of 1N NaOH. The pH was adjusted to 7.4 (physiological pH) by adding additional NaOH. The solution was filtered, covered, and allowed to stand at room temperature for about nine hours. The pH of the solution was obtained and the crystals formed were collected by filtration and washed several times with distilled water. The crystals were then dried in an oven at 60°C until a constant weight was obtained (typically about 3 hours).

MSU Identification

A light microscope with polarizing light was used to check the negative birefringence of the crystals and the characteristic needle shape. X-ray diffraction (XRD) was performed using Cr Kα radiation. SEM analysis was done to observe changes in morphology.

Crystallization Studies

The MSU crystals were prepared the same way but a known amount of the chemical being tested was added after the first filtration and before the solution was allowed to stand for nine hours. Any pH change was also noted prior to covering and letting the solution stand. Each

chemical additive was tested at a variety of concentrations up until saturation. Before adding the chemical the filtrate was divided into equal portions to accommodate the number of concentrations to be tested.

Table 1: Chemical additives used to effect MSU crystallization.

Vitamins and Minerals
β-carotene
thiamine (B_1)
L(±) ascorbic acid
riboflavin (B_2)
pyridoxine HCL (B_6)
niacin (B_3)
vitamin pill
calcium

Found in Foods
glucose
caffeine
citric acid
starch
glycine
monosodium glutimate
β lactose
nicotinamide
pyruvic acid

Associated with Gout
$Ca_3(PO_4)_2$
$CaSO_4$
ethyl alcohol
dextran
fuchsin
lysozyme
briallant green
DL lactic acid
xanthine
methylene blue

Also Found in the Body
potassium chloride
adenine
L-leucine
liver residue
pancretin
pepsin
typsin
urea
penicillen

Again, the crystals formed were analyzed using light microscopy, XRD, and SEM. Changes in color were also noted (indicative of adsorption on the crystal surface). The chemical additives used are found in Table 1. The compounds listed are divided into four areas (although some can be in more than one category): vitamins and minerals, found in food, other chemicals found in the body, and chemicals associated with gout.

RESULTS

The results will be described broken into the groups in Table 1. The results of compounds that had an effect on MSU size are found in Tables 2-4 grouped by the categories in Table 1.

Table 2: The effect on MSU crystallization from compounds previously tried.

Methylene blue		Fuchsin		$CaSO_4$		$Ca_3(PO_4)_2$	
gm/L	**size (µm)**	**gm/L**	**size (µm)**	**gm/L**	**size (µm)**	**gm/L**	**size (µm)**
0.001	25 ± 10	High	20 ± 10	1.3	33 ± 5	0.067	30 ± 15
0.0001	70 ± 40			0.33	8 ± 5	0.039	26 ± 5
0.00005	120 ± 70	Medium	30 ± 20	0.31	15 ± 7	0.033	60 ± 20
0.00001	160 ± 60			0.9	26 ± 10	0.011	80 ± 20
control	180 ± 80	Control	80 ± 40	Control	70 ± 30	Control	115 ± 50

Table 3: The effect on MSU crystallization from other compounds associated with gout.

Lysozyme		Xanthine	
gm/L	**size (µm)**	**gm/L**	**size (µm)**
high	25 ± 10	high	7 ± 3
control	200 ± 80	control	150 ± 20

Table 4: The effect on MSU crystallization from vitamins and minerals.

riboflavin		niacin		β-carotene		pyridoxine HCL	
gm/L	**size (µm)**	**gm/L**	**size (µm)**	**gm/L**	**size (µm)**	**gm/L**	**size (µm)**
2.4	25 ± 10	High	25 ± 5	High	15 ± 10	high	10 ± 5
0.8	50 ± 10		13 ± 5				
0.3	80 ± 30		20 ± 10				
0.1	80 ± 30	Low	90 ± 30				
Control	150 ± 70	Control	120 ± 70	Control	80 ± 40	control	90 ± 30

Chemicals Associated with Gout

There were a number of the chemicals (Table 2) that had been previously examined by others (Wilcox, *et al.*, 1975 and Handbook of Chemistry and Physics, 1970-1971) and shown to alter nucleation or growth rate of MSU crystals. Some of these substances, however, are dyes and would probably have limited use clinically.

For methylene blue, as the concentration increased to 0.001 gm/L the crystal size decreased from 180 ± 80 µm to 25 ± 10 µm. Also at concentrations above 0.0001 gm/L the crystals tended to aggregate in a network rather than individual needle crystals. With increasing methylene blue concentration the MSU crystal color changed from white to blue to light purple; with no significant change in crystal structure (based on XRD).

Over the concentration range tested, fuchsin caused a change in size as well as the total amount of crystals formed. As the concentration of fucshin increased, the crystal size decreased from 80 ± 40 µm to 20 ± 10 µm; with no significant change in crystal structure (based on XRD).

The effect of calcium was studied using both $CaSO_4$ and $Ca_3(PO_4)_2$. Both compounds altered the size and amount of MSU crystals formed. Also both seemed to have an intermediate concentration that led to the smallest crystals and least amount of total precipitate (0.33 gm/ml for $CaSO_4$ and 0.039 gm/ml for $Ca_3(PO_4)_2$). The crystals also appeared to be the thinnest and most curved when they were the smallest. The XRD data seemed to indicate a MSU-uric acid crystallization transition above these intermediate concentrations. This corresponded to a change in peak intensities indicating calcium or calcium compound incorporation into the MSU crystals; altering the structure.

Brilliant green, dextran, and ethyl alcohol were also tested in this group and found to have no appreciable effect in this study.

Other Compounds Associated With Gout

Lysozyme (a lysosomal enzyme found in macrophages) was able to decrease the crystal size (from 200 ± 80 μm to 30 ± 20 μm); without significant changes in the crystal structure. The crystals looked more like intertwining strands vs. needle crystals as the concentration increased.

Xanthine, a part of the purine metabolism (the end-product of which is uric acid) was able to decrease the crystal size (from 150 ± 20 μm to 7 ± 3 μm); without significant changes in the crystal structure. The crystals became more irregular and interconnected as the concentration increased.

Vitamins and Minerals

Four of the compounds examined in this group showed an effect on the MSU crystals: vitamin B (2, 3, and 6) and vitamin A (in the form of β-carotene). As the concentration of riboflavin (B2) increased, the crystal size decreased from 150 ± 70 μm to 25 ± 10 μm; with a change in crystal structure (based on a change in relative peak intensities for XRD) as well as a decrease in amount of precipitate. The crystals changed in color from white to dark brown and aggregated in fan-like groups as the concentration increased.

Niacin (B3) also seemed to have an intermediate concentration that led to the smallest crystals (and least precipitate) at around 0.7 gm/L; with size decreasing from 120 ± 70 μm to 13 ± 3 μm. As the crystal size decreased they changed in shape from needles to pollen grains to bacilli. As with calcium, the XRD seemed to indicate a MSU-uric acid crystallization transition above the intermediate concentration; without a change in pH. For β-carotene (vitamin A precursor), an increase in concentration led to a decrease in crystal size (from 80 ± 40 μm to 15 ± 10 μm); without significant changes in the crystal structure. Also for pyridoxine HCL (vitamin B6), an increase in concentration led to a decrease in crystal size (from 90 ± 30 μm to 10 ± 5 μm); with a change in relative peak intensities for XRD.

DISCUSSION

Model Justification

The modified Seegmiller method (Seegmiller, *et al.,* 1965 and Wilcox, *et al.,* 1975) was useful in comparing effects on MSU crystallization at different concentrations for each additive. Using the same filtrate for all the concentrations, for a given additive (including the control), helped assure that the only variable being changed was the additive concentration. Further, changes in pH and MSU concentrations wider than the ranges observed in testing were assessed to assure that these changes had negligible effects on the MSU crystallization.
This method, however, did require alterations from the typical joint environment; allowing only selection of compounds that have the potential to limit the size of MSU crystals in vivo. Further testing would be required to determine the actual effect of these additives at levels feasible in clinical practice. In some cases, this would also be to determine if changes in concentrations among individuals could explain why some individuals with hyperuricemia get gout, while the majority does not.

First using a higher supersaturation level than found in vivo (about 7 versus 2 as a maximum *in vivo*) served to allow crystallization to occur quickly enough that infection or degradation of the uric acid was not an issue. This, however, probably reduced the effectiveness of the additives that adsorbed on the surface of or got incorporated into the growing crystal. Using concentrations up to the saturation level (much higher than feasible *in vivo*) probably helped to assure that compounds that would have an effect *in vivo* would be detectable.

For this model, for the most part, with the possible exception of high calcium concentrations; crystallization appeared to be homogeneous and once started quickly formed crystals that did not change size or shape over time. In vivo, there appears to be at least some heterogeneous crystallization (nucleation that can start on other compounds, tissue, or small MSU crystals). Heterogeneous nucleation is easier than homogeneous and occurs at lower levels of hyperuricemia (Wilcox, *et al.,* 1975). Also years after a gout attack a tophi (a chalky nodule of crystals and cells) can form, which can be a source of heterogeneous nucleation especially if pieces are released. Some believe this release of pieces of the tophi occurs when treatment to reduce uric acid levels are used (increasing the ability of MSU to go into solution) and can trigger a gout attack through heterogeneous nucleation (Shoji, *et al.,* 2004 and Wilcox, *et al.,* 1975). It is therefore unknown how well compounds that can limit crystal size for homogeneous nucleation will effect heterogeneous nucleation.

Not using a buffering solution (although the initial pH was controlled) would have made changes in pH due to the additives easier. Although pH alone did not seem to have a significant effect on MSU crystallization, within the range seen, it helped to determine if changes in the crystal structure seen in XRD were potentially related to a MSU-uric acid crystallization transition.

Cooling to room temperature *vs.* body temperature could have introduced inaccuracy in the model. Some additional experiments, however, were done with compounds that had an effect on MSU crystallization, cooling to 37°; with negligible effects on the results.

Results Interpretation

Despite the limitations of the model, there was a clear difference between the third of the compounds that had an effect vs. those that did not. It appeared that riboflavin, niacin, calcium (as $CaSO_4$ and $Ca_3(PO_4)_2$)., methylene blue, and fuchsin could reduce MSU crystal size in a dose dependent manner. Pyridoxine HCL, β-carotene, lysozyme, and xanthine appeared to reduce crystal size, but only one concentration (plus the control) was used; so a dose dependent response could not be shown. Also all but pyridoxine HCL and β-carotene reduced the amount of precipitate formed.

There seemed to be at least five mechanisms (with some overlap) by which these compounds could limit crystal size: adsorption, syncrystallization (more than one crystal growing together), ion incorporation into the crystal, a modification of the solubility of uric acid, or degradation of the crystal. Adsorption and syncrystallization can be shown by a change in the color of the crystals. Changes in XRD relative peak intensity indicates either syncrystallization or ion incorporation (which can also lead to syncrystallization); with the exact change able to help distinguish between the two. Changes in amount of precipitate and/or changes in pH are indicative of changes in solubility of uric acid. Degradation of the crystal can be seen by changes in shape and smoothness of the crystals. Other have shown the ability of some of these compounds to reduce either growth or nucleation rate (Wilcox, *et al.*, 1975 and Allen, *et al.*, 1965). Although altering the solubility of uric acid would affect nucleation and growth rate, the other four methods of limiting crystal size are not directly tied to nucleation and growth rate; the amount of precipitate, however, is directly related.

Limiting crystal size means stopping growth on each side of the crystal *vs.* just slowing the growth rate. For MSU crystals it is most important to stop growth along the axis of the needle. Coating of the growing crystal with adsorbed compounds or a different crystal (syncrystallization) can alter shape and/or limit size by inhibiting growth on one or more crystal surfaces (Figure 1 shows the effect of having different growth rates on different faces) (Allen, *et al.*, 1965). Either can also result in a color change. There would need to be a minimum size of the second crystal relative to the first to show up in XRD. Incorporation of ions into the growing crystal can change the relative growth rates of crystal surfaces and therefore effects shape and size of the final crystal, potentially through syncrystallization (Allen, *et al.*, 1965). Changing the solubility of uric acid can also alter both the amount of crystals formed and their size.

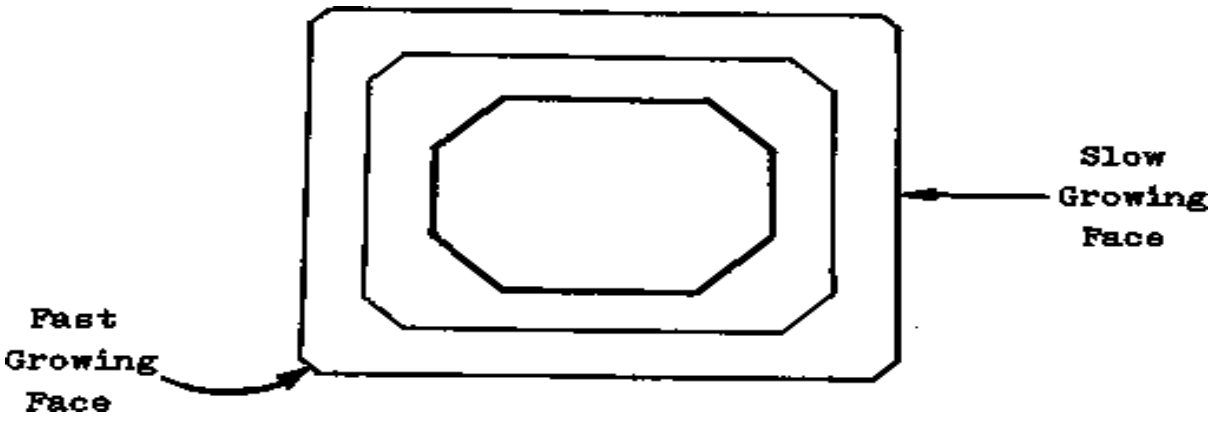
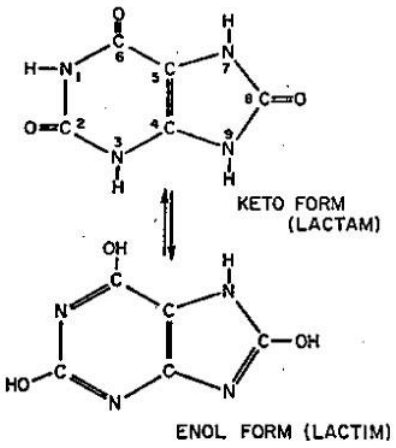


Figure 1: Shape versus face growth rate.

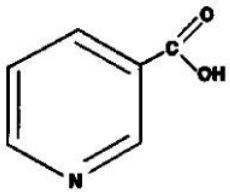
Riboflavin, methylene blue, and fuchsin altered MSU crystals by adsorption on the growing crystal via syncrystallization. Riboflavin was the only one that showed a significant difference in the XRD pattern; which could just be due to the relative size of the riboflavin modified crystals versus the MSU crystals. Allen showed growth inhibition for methylene blue, but not fuchsin. It was suggested that methylene blue competed with sodium or hydrogen urate ions on the crystal surface (Allen, *et al.*, 1965).

Two other vitamins reduced crystal size: pyridoxine HCL and β-carotene; with only pyridoxine HCL significantly altering the crystal structure. Both could be due to adsorption on the surface of the growing crystals with at least pyridoxine HCL due to syncrystallization. Niacin seemed to work by shifting the MSU-uric acid equilibrium altering the solubility of MSU so it crystallized at pH 7.4 vs. normally requiring a pH below 6.5. In this case the pH was not significantly changed due to adding niacin (nicotinic acid). This could be due to the dissociation constant of niacin (1.4 × 10-5) (Handbook of Chemistry and Physics) and/or the incorporation of the excess hydrogen ions into the growing crystal. It is also possible it can cause syncrystallization, since it has a similar structure to MSU (Figure 2).

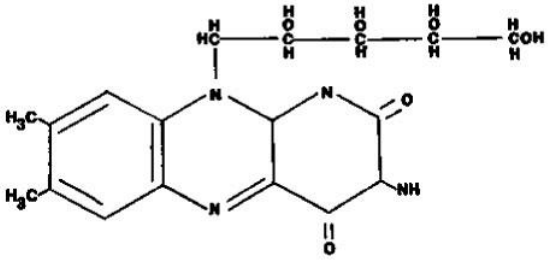


A. Equilibrium forms of uric acid

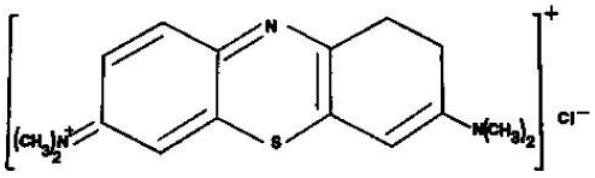
C. Pyridoxine HCL



D. Niacin



B. Riboflavin



E. Methylene Blue

Figure 2: Compounds that reduced crystal size that had a similar structure to uric acid (A). B. riboflavin, C. pyridoxine HCL, D. niacin, and E. methylene blue.

Calcium appeared to also shift the MSU-uric acid equilibrium; possibly by the competitive inhibition of sodium by calcium to uric acid binding sites. The calcium urate crystals also have a different structure than the MSU crystals, which appear to limit the length of the needles. An excess of calcium, however, may serve to promote heterogeneous nucleation (Wilcox, *et al.,* 1975) leading to an increase in crystal size.

Xanthine probably works by syncrystallization or incorporation into the growing crystal due to the similar structure to MSU (Figure 2); however in previous studies (Allen, *et al.,* 1965) it was shown not to alter growth rate. Lysozyme probably works by degrading the crystal, giving the frayed appearance.

Brilliant green and dextran were shown to decrease MSU nucleation rate in previous studies (Allen, *et al.,* 1965), but did not have a significant affect in this study. Also ethyl alcohol was shown to increase nucleation rate (Wilcox, *et al.,* 1975) previously, but did not have a significant affect in this study.

The most effective compounds are ones that get incorporated into the growing crystal via syncrystallization or competing with sodium for changes in the crystal chemistry. It is expected that compounds with a similar pyrimidine structure to MSU, more easily exhibit syncrystallization. This appears to be true for riboflavin, xanthine, pyridoxine HCL, and methylene blue (Figure 2). It also may be the case for niacin. A similar structure alone, however, does not appear to be sufficient due to the many compounds with a similar structure to MSU without any significant change in the MSU crystallization process including: thiamine, nicotinamide, leucine, adenine, and penicillin. It may require a bulky sidegroup like for riboflavin. More sensitive surface analysis techniques or XRD would need to be done to more accurately identify the compounds that exhibit syncrystallization. There are also probably other ions besides calcium that can compete with sodium or other elements to get incorporated into the growing MSU crystals.

Mechanism of Gout

Although not all the steps in the pathogenesis of gout are known, there are some things we do know. First, deposition of monosodium urate (MSU) crystals within joints and connective tissue leads to a localized inflammatory response. A pre-requisite for gout is excessive blood levels of soluble urate, one of the final products of the metabolic breakdown of purine nucleotides (Choi, *et al.,* 2005). Hyperuricemia is typically defined as occurring above the saturation point of MSU, at serum urate levels >6.8 mg/dL (Burns, *et al.,* 2012).

It is also generally accepted that interaction of MSU crystals with leucocytes leads to inflammation and the production of chemicals that increase the amount of MSU crystals, which serves to continue and amplify the cycle leading to the clinical signs of gout: swelling, redness, and pain (Choi, *et al.,* 2005). It is still, however, not agreed upon what causes MSU crystallization as well as how the MSU crystals trigger the inflammatory response.

Treatment of Gout

Again strategies for treatment or prevention of gout either try to lower the uric acid level or decrease the inflammatory response. The goal of this study was to examine the feasibility of a different strategy: reducing the inflammatory phase by limiting the amount and size of the crystals formed. Although it is known that changes in environmental factors such as temperature, pH, trauma, etc. can trigger a gout attack, the fact that these changes are not necessary to trigger gout (Choi, *et al.,* 2005) implies that the chemistry of joint synovial fluid (or surrounding tissue) can alter the probability of a gout attack.

Limiting Crystallization of MSU

It is not known what percent of hyperuricemic individuals who do not have gout still form MSU crystals that do not elicit a gout attack. Also although there is a size transition for eliciting gout it is unknown, if there is an amount of MSU crystals that is also required. It is known that the inflammatory response of macrophages and neutrophils is related to the amount of particle phagocytosis (if the particles are in the appropriate size range) (Nagse, *et al.,* 1995 and Black, *et al.,* 1992). Also the pain level (along with the amount of swelling, redness, and heat produced) is related to the amount of inflammation. Further inflammation can alter the environment (most likely pH) that would increase the MSU crystallization; leading to an autocatalytic ramping up of the response (Choi, *et al.,* 2005). The minimum amount of inflammation, however, to trigger gout symptoms is not known. Interestingly, the heat from inflammation can increase solubility of MSU, which might help to make crystal growth self- limiting (Allen, *et al.,* 1965; Loeb JN, *et al.,* 1972 and Wilcox, *et al.,* 1972). The strategy in this study was to look mostly at compounds that could reduce the size of the crystals as well as look at the ability to reduce amount (volume) of crystals.

Crystal size can be altered by changing the growth rate on one or more faces (Figure 1); particularly along the long axis of a needle crystal. Nucleation rate would just affect the amount of crystals formed. Although nucleation rate and whether it is homogeneous or heterogeneous nucleation are important in triggering gout, the strategy examined here is more related to size of the crystals versus amount (volume) of crystals formed. Some of the impurities did reduce the amount of crystals formed, but the goal (design constraint) of this strategy is not known. This is again tied to not knowing how much inflammation is needed to initiate the response and therefore the amount of crystals needed to lead to a gout attack. For crystal size, however, there is a target value to go below. It is reasonable to expect that the level of inflammation is related to the amount of crystals over the size range and that limiting the volume of crystals might become an additional strategy to use with limiting size, but that was not the focus of this study. Plus using a highly supercooled and highly supersaturated solution is probably not a good way to examine changes in nucleation rate.

Therefore the main mechanism to concentrate on is crystal growth rate. Factors that

affect the rate include: supersaturation, temperature, and mass transfer of the solute to the crystal surface (Handbook of Chemistry and Physics); with the ability of the solute to make it to the surface of the growing crystal the ones that the impurities would affect. The mass transport of solute can be altered by changes in the crystal surface or solution that would alter the diffusion of the solute or the ability of the solute to attach to the surface of the growing crystal. The experimental model used, however, would create an environment where molecular diffusion is rapid enough to not be a controlling factor in growth rate. It also creates an environment that changes in the crystal surface would probably have a reduced effect than *in vivo*.

The fastest growing faces of a crystal will have the smallest area (Figure 1). So the greatest impact on size for MSU crystals is to slow growth on the face at the ends of the growing needle. The shape or habit of growing crystal is related to the structure of the unit cell (triclinic for MSU) but does not necessarily mimic the shape of the unit cell (Seegmiller, *et al.,* 1965; Allen, *et al.,* 1965 and Buckley, *et al.,* 1951). Factors that can change the relative growth rate of the faces and thus the habit include: degree of super saturation, temperature, solvent effects, and impurities (which is the strategy employed here).

There still are many contradictory theories on how impurities can alter growth rate on some or all surfaces of a growing crystal. If the impurity gets incorporated into the growing crystal it can do so by attaching either amorphous or crystalline (syncrystallization) compounds to the surface either in irregular patterns or complete coating of one or more faces. For syncrystallization the new crystal can be similar to MSU with some of the atoms in the crystal lattice being replaced by ions from the impurity or the impurity itself forming a crystal on the surface (Buckley, *et al.,* 1951). The concentration of the impurity as well as how close it is to the MSU crystal (or ions it is replacing) would determine the type of incorporation. E.g. if the ion is close in size (like calcium to sodium) it can alter unit cells; with the number related to the impurity concentration (Allen, *et al.,* 1965 and Buckley, *et al.,* 1951). The interaction between the atoms (like sodium and calcium) as the favorability of incorporating one atom over the other would help determine whether the entire crystal had the new ion incorporated (with the % of new crystal lattices dependent on concentration) or just the surface layer (with the thickness and/or distribution of the new crystal dependent on concentration). Also the impurity can be adsorbed on the surface in an amorphous state; with the thickness and/or distribution of the new compound dependent on concentration. Change in shape and size of the growing crystal can be by having coatings for the entire crystal or having differential coating on different faces of the crystal (Allen, *et al.,* 1965 and Buckley, *et al.,* 1951). The differential coating is believed to be caused by the different force field on each face (in the case of MSU, believed to be caused by the different net ionic charge on each face) (Buckley, *et al.,* 1951). Adsorption of amorphous structures could serve by just blocking sites for crystal growth; with the effect related to the concentration of the impurity (Buckley, *et al.,* 1951).

Ramifications

The study showed the feasibility of specific compounds found in the body to limit the size

of MSU crystals in vivo. This could help explain why only a fraction of hyperuricemic individuals get gout. This could also be used as a prevention medication, if levels are too low in joint synovial fluid. There would need to be many steps before this strategy could be used clinically, but it could not hurt to make sure levels of the B vitamins were at or above RDA requirements in the blood or joint fluid.

These future studies would include testing the ability of these impurities to limit the size (and possibly amount) of MSU crystals in vivo and/or clinically at various dosages with known toxicity levels. It would also be helpful to determine, if there is a difference in the level of these compounds between individuals with hyperuricemia that get gout and those that do not.

Therefore it would have to be determined, if serum levels of these impurities have a significant effect on average MSU crystal size, in hyperuricemic individuals. Further, if the range of serum levels found clinically can alter the average crystal size both above and below the critical size (0.5 μm), in hyperuricemic individuals. Next it should be checked that indeed keeping the size of the crystals below the critical size (0.5 μm) prevent a gout attack.

These studies would also help determine which impurities (or combinations) are the most likely to be used as a treatment. It also may require a treatment tailored to the individuals serum levels of these impurities. Then studies would have to be done to determine the treatment regimens for a given serum level of the selected impurities.

CONCLUSIONS

It appears that the key factor in producing the clinical manifestations in a gout attack is inflammation caused by the MSU crystals. Inflammation not only leads directly to the biggest clinical issue: pain, but also the swelling, redness, and heat associated with a gout attack in joints such as a toe. The inflammation can initially create an auto-catalytic effect to ramp up the response (by decreasing MSU solubility), but also can eventually slow the response by increasing MSU solubility. Treatment to increase MSU solubility or breakdown crystals frequently leads to a ramping up of the gout attack, particularly if there have been previous attacks; most likely due to breaking up an existing tophi that would increase the heterogeneous nucleation rate. So the best current treatment strategies are probably to reduce the inflammatory reaction, but can be coupled with efforts to reduce uric acid levels or increase MSU solubility.

Current prevention is predominantly aimed at reducing serum uric acid levels. This has not always been effective, since hyperuricemia is not sufficient or always necessary to cause a gout attack. Part of our inability to prevent gout is an agreement on the mechanism that triggers the inflammation that leads to a gout attack. The debate centers on the relative importance of chemistry versus size. As for other pathologies caused by small particles or fibers, chemistry should only be important if the material is breaking down or releasing substances. It has also been shown that injecting crystals below a certain size (0.5 μm) does not elicit a gout attack.

The goal of this study was to examine the ability of various compounds (either found in the body or have previously been shown to affect MSU crystal nucleation or growth rate) to limit the size of MSU crystals as well as determine the likely mechanism. Although some compounds were found, there are still many steps required to determine if these compounds could be used clinically to significantly reduce the number of MSU crystals larger than 0.5 μm, and do so at a level that had minimal side effects. It also would require further tests to determine, if the difference in serum level of these compounds among individuals is responsible for determining if a hyperuricemic individual gets a gout attack.

Specifically it was found that vitamins (riboflavin, pyridoxine HCL, and β-carotene), some dyes (methylene blue, and fuchsin), and xanthine were able to limit crystal size by adsorption on the surface of the growing crystal most likely leading to syncrystallization. Calcium also seemed to exhibit syncrystallization with a calcium-urate vs. MSU. Both niacin and calcium were able to limit crystal size by changing the MSU-uric acid equilibrium altering the solubility of MSU. Lysozyme was able to limit crystal size by degradation of the crystals.

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