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Topical Application of 2% Lithium Carbonate Reduces Pain, Swelling and the Chemokine KC-GRO (Murine IL-8) in a MSU-induced Model of Gout in Rat Ankle

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Abstract:

Background: The level of Mono Sodium Urate (MSU) in the human should be ideally kept below 6.o mg/dl. Above 6.8-7.2 mg/dl, crystallization may occur in tissues; resulting in the inflammatory response understood as acute gout attack. Alfred Baring Garrod and Alexander Ure felt that these concretions in the synovial tissues and kidneys of chronic gout could be dissolved. Garrod's 1859 text "The Nature and Treatment of Gout and Rheumatic Gout" should be considered one of the most complete texts discussing the topic from antiquity, through the mid-19th century. The application of lithium carbonate directly to gouty tophi was one treatment Garrod advocated. He felt that the MSU concretion could be dissolved if the more-soluble lithium urate moiety was formed. Garrod demonstrated the solvent effects of lithium by dissolving the gouty tophus in a metacarpal bone dropped into solution. Thomas Edison in 1890 presented "An Account of Some Experiments of Electrical Endosmose to the Treatment of Gouty Concretion". It became evident the dilute solutions of the lithium salts are not solvents for uric acid or urates and results such as Garrod's metacarpal experiment worked only when in concentrated solution. Edison's own lab showed that there were better solvents for urates; and by 1893 lost interest in the use of lithium for dissolution of gouty concretions. The use of Lithium for the treatment of gout persists today only as a historical reference; due in part to the often-cited Abramowitsch text "Treatment by Ion Transfer (Iontophoresis)". This study was undertaken in an attempt to validate the beneficial effects of topical lithium on gout; as seen by the author in his anecdotal off-label use of 2% lithium carbonate in 25 patients.

Methods: A proof-of-concept study was personally-commissioned by the author. Topical application of a 2% lithium carbonate solution was tested in an MSU Acute Gout Model induced via injection into the ankles of rats. Clinical observation of swelling and pain index was determined. Cytokines and chemokines were measured in the synovial fluid. Histopathology was performed after necropsy on the banked ankles.

Results: The observed pain index and swelling due to MSU-induced gout attack was slightly decreased by the topical application of 2% lithium carbonate/10% DMSO solution to male Sprague Dawley rat ankles. Cytokines IL-1b, IL-6, and TNF-a were increased. Unexpected reduction in the chemokine KC-GRO (murine IL-8) was seen. The histopathology showed anticipated findings in the MSU control; edema, synovial hyperplasia, and large rafts of inflammatory cells/WBCs within the synovium of the rat ankle. DMSO vehicle alone reduced appearance of inflammation. The addition of 2% Lithium Carbonate produced a more significant reduction in the thickening of the synoviocytes and the appearance of inflammatory cells within the synovial fluid was also more markedly reduced by the addition of Lithium.

Conclusion: Topical application of 2% lithium carbonate compounded with 10% DMSO reduces the pain, swelling and inflammation of a MSU-induced gout attack in the ankle of male Sprague Dawley rats. The reduction of the chemokine KC-GRO suggests that analogous reduction of IL-8 in the human may explain the beneficial effects seen in the author's anecdotal success using topical 2% lithium carbonate in 25 patients.

Keywords: Chemokines; Synovial hyperplasia; Inflammation

Introduction

The micropathophysiology of the gouty attack in the human joint is well-known; and the specifics of this cascade of events are very well documented [1-9]. It is assumed that the reader is well-versed in the basics; and the author refers the reader to those excellent references. Certainly, as one studies both the historical research of gouty inflammatory arthropathy, and is involved in the treatment of patients.The role of colchicine becomes clear. One of the best articles, in my opinion was written by Dalbeth, et al. [9]. In this excellent review, "Mechanism of Action of Colchicine in the Treatment of Gout" the three pathways associated with the initiation and amplification of the acute MSU-induced gout attack are described in great detail (Figure 1).

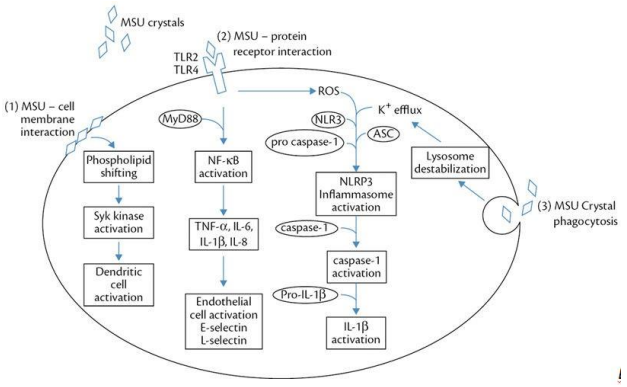


Figure 1: Mechanism of Action of Colchicine in the Treatment of Gout.

This important review can be cross-referenced and data-mined for review of many analogous actions of Lithium. This was the starting point of my search for the potential mechanisms and actions of colchicine that may be analogous to those of Lithium. Of course, we know that colchicine disrupts the microtubules of the pseudopodia that are critical for the migration of the WBCs and macrophages from the endothelium into the synovium [9]. In my early research into Lithium's actions: I came across an article describing its ability to affect the motility of sperm flagellae [10]. Understanding that this locomotion was also directed by microtubular activity; I began to look closer at the similarities and differences between colchicine and lithium. The main action of colchicine is to bind tubulin, and prevent further assembly and subsequent locomotion [11]. Lithium affects locomotion differently, and prevents assembly of microtubules by inhibiting GSK-3b. Many of Lithium's actions are due to its ability to replace magnesium in the enzyme pocket of glycogen synthetase kinase 3-b [12]. As a very reactive alkali metal, Li+ ion readily substitutes for the slightly larger Mg++ ion. Because of its ability to block GSK-3b and other kinases, Lithium affects many pathways [13]. Lithium is known to be neuroprotective and to increase survival of neural and glial cells [14-17]. The action and effects of Lithium far exceed those of colchicine. My observations are tabulated (Table 1).

	Colchicine	**Lithium**
Microtubules	Prevents assembly Binds to tubulin	Li+ reversibly Inhibits microtubules Lithium Blocks GSK-3ß
Endothelial E-Selectin	Decreases adhesiveness	Inhibits
Neutrophil -Selectin	Impairs Adhesion to Vessel	Inhibits
Leukotriene B4	Impairs Diapedesis	Inhibits
NLRP3 Inflammasome	Suppresses Activation	Inhibits
Chemotaxis	Inhibits	Inhibits
Cytokines	Inhibits	+/-
Phagocytosis	Inhibits	Inhibits
TNF-alpha	Diminished endothelium Diminished macrophages	Increased TNFa Expression can cause Granulocytosis
COX-2 and PGE2		Attenuates in some tissues
Mast Cell Degranulation	Inhibits Microtubules	Inhibits
Sensory Nociceptors	May Attenuate Hyperalgesia via Microtubular Inhibition	Blocks Substance P at NK1 Receptor Attenuates GABA Receptor Activates MOR
Nerve Conduction	No Effect	Li+ acts on Voltage Gated Ion Channels blocks efflux from Axon @ K+ Channel Affects Action of Local Anesthetic I Effective Dose ↓ Duration ofBlock↑
Dorsal Root Ganglion	No Effect	↓ NMDA ↓ NK1 ↑ GABA
NeuroProtection	No	current ongoing research in many diseases Significant NeuroProtection
Apoptosis	Induces Cell Death	Reduces Neural & Glial Cell Death Increases Cell Survival
Allodynia	No Effect	Reverses Allodynia via MOR
Fibromyalgia	No Effect	Li+ may be helpful in Tx of Fibromyalgia
Diabetic Neuropathy	No Effect	Li+ 1 Neuro-regeneration & Remyeliation can prevent both motor & sensory components of paclitaxel neuropathy in rats

Table 1: Observations comparing the actions of colchicine and lithium.

The table shown above begins with the relative similarities between colchicine and lithium; and one clearly sees the numerous effects of lithium within the CNS and spine.

Methods

The myriad actions of lithium within the CNS may have implications for pain management in the future; and may be also have both scientific and clinical application to the neural cells and pain pathways within peripheral nervous system. It stands to reason, that topical application of lithium may produce a local response; with only a fraction of the applied dose reaching the serum. In 3 patients tested who were applying 2% Lithium Carbonate in witch hazel to the foot; no detectable concentration was seen in the 8-1.2m Eq/L lab range expected to be therapeutic. Taken together, I feel that the reduction of nerve pain and gouty inflammation seen in patients treated off-label; and reduction in pain, swelling, and KC-GRO (IL-8) seen in the MSU rat model, may be attributed to the 2% Lithium Carbonate applied topically and delivered to the desired target tissues as Li+ ion after passage through the epidermis, without any measurable uptake in serum. The study of Lithium Carbonate topical Pharmaco Kinetics (PK) was beyond the designated scope of this proof-of-concept study (Figures 2-7).

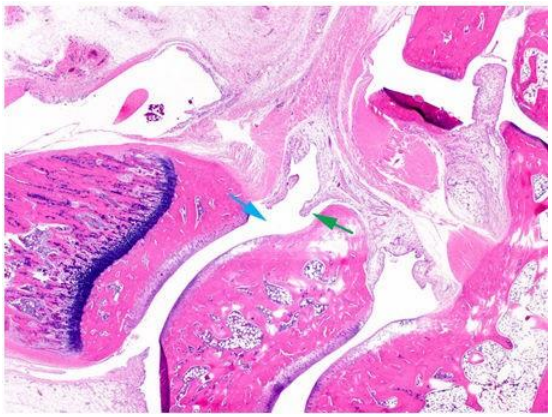


Figure 2: (Normal Joint) there are no inflammatory cells in joint (blue arrow) Normal synovial lining (green arrow).

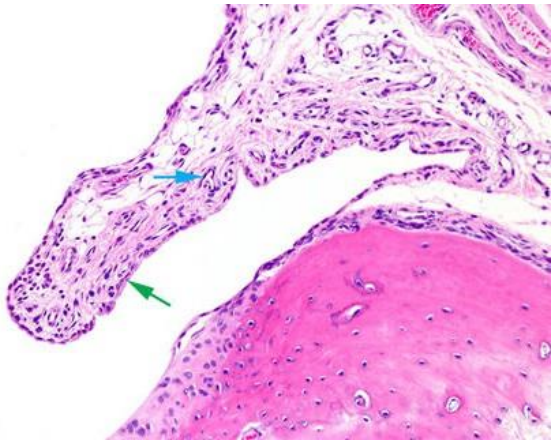


Figure 3: @20x note a single layer of synovial fibroblasts (green arrow) and collapsed vessel (blue arrow) which indicates lack of inflammation in the normal physiologic state of the Sprague Dawley Rat.

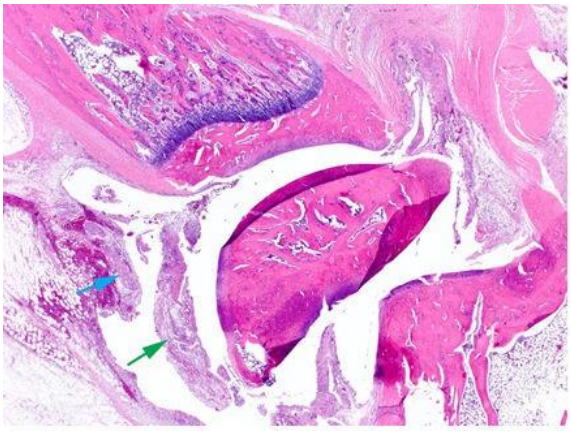


Figure 4: (MSU Gout Attack) Notice the edema within the joint; and take note of the raft of inflammatory cells that have infiltrated and occupy the joint (green arrow) and hyperplasia of the synovium (blue arrow).

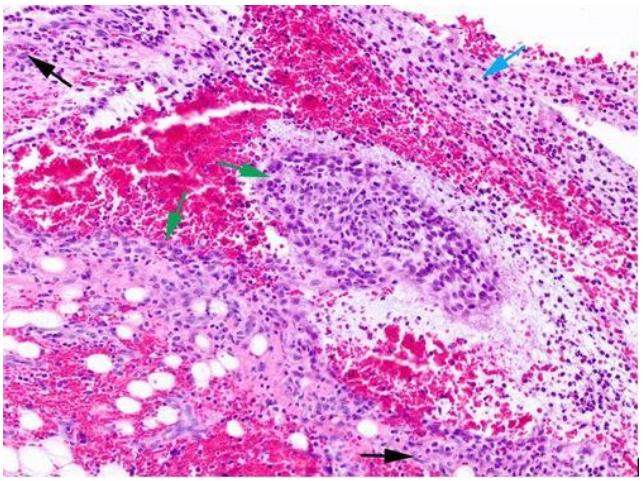


Figure 5: @20X Neutrophils and fibrin are free within the joint cavity (blue arrow). The synoviocytes are proliferative (green arrows) and the capillary endothelium is hypertrophied (black arrows).

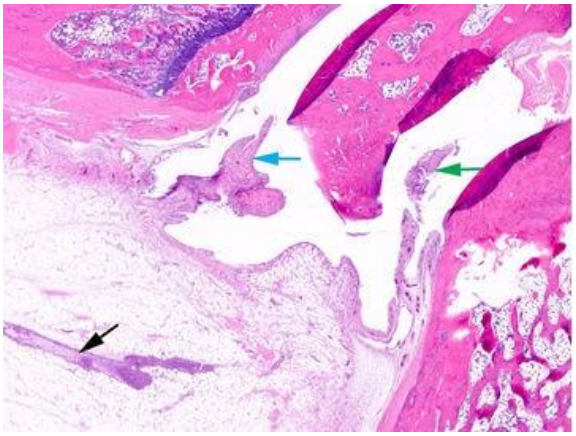
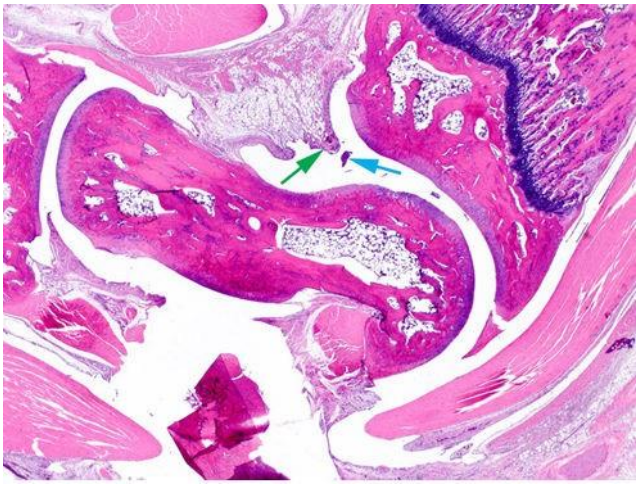


Figure 6: (DMSO Treated) the synovium is focally thickened (blue arrow), but there is less acute inflammation within the joint cavity (green arrow). Presumptive Mono Sodium Urate (MSU) (black arrow) is present.



Figue 7: (Lithium Formulation-Treated) The synovium is less thickened (green arrow) when compared to the Acute MSU attack, and

also is less thickened than the DMSO treated ankle. There is also less acute inflammation and WBCs within the left ankle joint cavity (blue arrow) when compared to DMSO treatment alone.

Results and Discussion

The IL-8 seen in the human is not expressed in the murine model. The murine homologue is called KC-GRO [18-20]. The cytokine CXCL1 that binds the human IL-8, and its receptor CXCL-2 are essential for the development of the acute gouty neutrophilic response to urate crystals in the murine gout subcutaneous air pouch model. In a resting state, neutrophils are rare in synovial fluid. The CXC chemokine IL-8 in one study accounted for more than 90% of the neutrophil chemotactic activity seen [21]. IL-8 is considered an important marker for gout. The IL-8 acts on CXCL1/CXCL2 to recruit neutrophils out of the endothelium [22]. This excellent article by Girbl, et al. illustrates the effects of chemokine signaling on neutrophil recruitment from the endothelium. The 3-d illustrations and animated real-time imaging is remarkable (Figure 8).

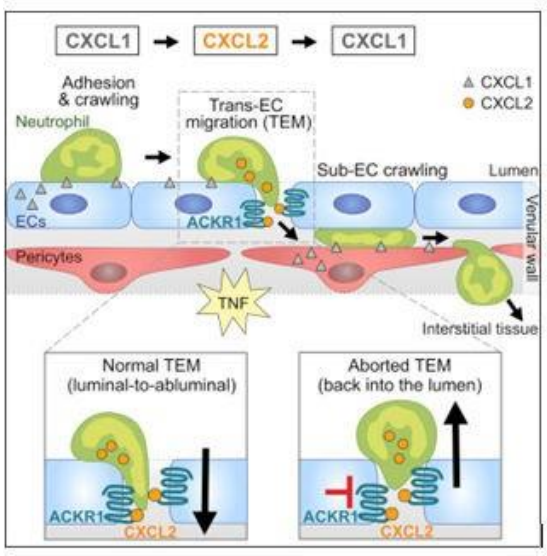


Figure 8: 3-d illustrations and animated real-time imaging is remarkable.

In the author's current study of MSU-induced gout, several cytokines IL-1b, IL-6, KC/GRO, and TNF-a were seen in synovial fluid analysis. The elevation of these cytokines would be expected as a normal baseline inflammatory response to MSU injected into the rat ankle. However, the application of topical lithium carbonate caused an increase in the cytokines IL-1b, IL-6, and TNF-a in synovial fluid. The increase in these cytokines, although somewhat unexpected; may be seen as an indication that Lithium Ions actually affected the joint. Lithium is known to have both anti-inflammatory and neuroprotective effects [14-17, 23]. Lithium acts to modulate TNF and IL-1 induction early in the signaling pathway [24]. The inhibition of GSK3b by LiCl increases the TNF-a protein synthesis by greater than a 3-fold margin in neutrophils [25]. Lithium led to a consistent increase of IL-1b, IL-6 and TNF-a in the serum of 30 subjects tested [26]. There is a large body of data which indicates that under certain experimental conditions lithium also exhibits pro-inflammatory properties e.g., induction of IL-4, IL-6 and other pro-inflammatory cytokines' synthesis [27]. It may be reasonable to attribute the "priming" of the inflammatory response, and induction of cytokines IL-1b, IL-6, and TNF-a in the BRT model to the topical Lithium Carbonate applied.

Attenuation of cytokine KC/GRO was seen in synovial fluid with application of a topical Lithium Carbonate as the test ingredient in the BRT rat MSU model. This was a surprising and also an unexpected finding in light of the increase in the other cytokines that were tested. An NIH-funded study in 1994 established the fact that KC is the murine homologue of human GRO-a; and the KC receptor is also an IL-8 receptor homologue capable of binding both KC and the macrophage inflammatory protein-2 with high affinity [18]. The interaction of KC/GRO (as it binds the IL-8 receptor) triggers neutrophil activity. IL-8 is abundant in the synovial fluid in both acute gout and pseudogout [28,29]. Rapid release of IL-8 and binding to CXCL2 stimulate the adhesion and diapedesis of neutrophils out of the endothelium into the synovium. The CXCL1/CXCL2 interaction is also known to regulate and activate the NLRP3 inflammasome in macrophages [30]. The neuronal inflammatory cytokines CXCL1/ CXCL2 are regulated by GSK3 signaling [31] (Table-2). Lithium is well-known to block GSK3; and its effect on synovial tissues by reducing KC/GRO may be used to treat and prevent gout/pseudogout via IL-8 (Figure 9).

Animal	Ankle Joint	Group	Treatment	IL-1ß (pg/mL)	IL-6 (pg/mL)	KC/GRO (pg/mL)	TNF-a (pg/mL)
6	Right Ankle	1	Untreated	51	48.5	10.9	4.55
7	Right Ankle	1	Untreated	51	48.5	32.4	4.55
8	Right Ankle	1	Untreated	51	48.5	10.9	4.55
9	Right Ankle	1	Untreated	51	48.5	10.9	4.55
10	Right Ankle	1	Untreated	51	48.5	10.9	4.55
11	Right Ankle	2	Untreated	527	644	266	25.8
12	Right Ankle	2	Untreated	838	2690	443	29.7
13	Right Ankle	2	Untreated	466	674	101	9.61
14	Right Ankle	2	Untreated	893	5242	128	19.2
15	Right Ankle	2	Untreated	739	1502	72.8	23.3
16	Right Ankle	3	Untreated	223	3370	61.1	4.22
17	Right Ankle	3	Untreated	744	1330	111	9.99
18	Right Ankle	3	Untreated	265	314	36.6	4.22
19	Right Ankle	3	Untreated	431	2296	54.9	4.22

20	Right Ankle	3	Untreated	51	676	27.9	4.22
6	Left Ankle	1	Untreated	594	7800	816	31.1
7	Left Ankle	1	Untreated	1178	8537	3280	38.4
8	Left Ankle	1	Untreated	364	2631	458	14.7
9	Left Ankle	1	Untreated	51	518	152	4.22
10	Left Ankle	1	Untreated	706	2884	1066	32.2
11	Left Ankle	2	Vehicle	431	505	291	30.1
12	Left Ankle	2	Vehicle	466	1840	287	18.1
13	Left Ankle	2	Vehicle	1563	2729	377	40.6
14	Left Ankle	2	Vehicle	699	14759	170	12.5
15	Left Ankle	2	Vehicle	493	1643	120	29.9
16	Left Ankle	3	Test Article	800	5890	117	14.3
17	Left Ankle	3	Test Article	3586	17496	716	45.2
18	Left Ankle	3	Test Article	1501	2610	166	44
19	Left Ankle	3	Test Article	4691	11099	306	54.7
20	Left Ankle	3	Test Article	567	18013	92.7	10
Assay		LLOQ		102	96.9	21.7	9.1
		ULOQ		8100	8550	728	793
BLQ		<		102	96.9	21.7	9.1
1/2 BLQ		(pg/mL)		51	48.5	10.9	4.55
				IL-1ß (pg/mL)	IL-6 (pg/mL)	KC/GRO (pg/mL)	TNF-a (pg/mL)

Table 2: High Levels of IL-6, TNF-a, and IL-1b seen in MSU gout model amplified by topical lithium carbonate.

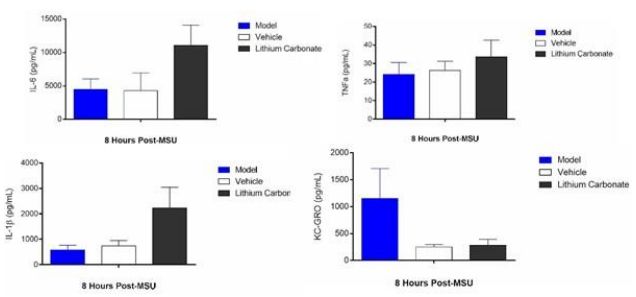


Figure 9: KC-GRO (murine IL-8) seen in MSU Gout Model IL-8 in Synovium is unexpectedly reduced by topical lithium carbonate.

Conclusion

This study demonstrates for the first time, the ability of topically-applied 2% Lithium Carbonate to decrease pain, swelling and inflammation of MSU-induced gout in the ankles of male Sprague Dawley rats.

This study also measured for the first time, the reduction of KC-GRO (murine IL-8) in the synovial fluid of gouty ankles of male Sprague Dawley rats induced by the topical application of 2% Lithium Carbonate.

The histopathology of this study validates the ability of topical 2% Lithium Carbonate to favorably affect the MSU-induced gouty ankles of male Sprague Dawley rats. The topical application of 2% Lithium Carbonate reduced KC/GRO (IL-8) in the rat synovial fluid; but the histopathology confirmed that topically-applied Lithium reduced inflammation, synovial hyperplasia and cellular infiltration of WBC's into the joint . This has implications far beyond gout, and also pseudogout in my opinion. It is my opinion, that our study validates the ability of topical Lithium Carbonate to reduce the amount of KC-GRO in the synovium; and may prove effective as blocking the critical amplification of the CXCL1/CXCL2 cascade seen in the crystalline inflammatory arthropathies of gout and pseudogout. The recent literature and our research, have demonstrated the importance of KC-GRO and CXCL1/CXCL2 in gout, pseudogout, OA, and RA. The indications for the use of Topical Lithium Carbonate might be extended to the inclusion of other forms of chronic neuroinflammatory pain, and the chronic pain associated with degenerative arthritis. This attenuation of inflammation may be achievable by blocking a complex cascade of events in the tissues around/within the synovium.

Disclaimer

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