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Observational study of Calcium Folate

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Abstract

Calcium Folate is the calcium salt of the vitamin folic acid which is involved in the metabolism of proteins and DNA. Folic acid is sensitive to acidic-oxidative conditions and, in the presence of Ca^{+2} as well, is metabolically transformed into pterins and calcium pterins. This pilot observational study describes the use of CaFolate-derived CaPterins in the treatment of certain immune-related ailments. Thirty-one patients with various immune-associated disorders were directed to take *ad libitum* 25-76 mg of CaFolate (300-900 µg folic acid equivalent) orally per day, and to collect personal and medical assessments. The users reported no flu/colds (13/13 reporting none during the cold and flu season), greater energy/stamina (8/8 reporting increased), less arthritis pain (10/11 reporting improvement), and diminished mouth sores due to chemotherapy (2/2 reporting significant clearance). These observational findings identify certain clinical endpoints for future matched group, randomized, double-blind, placebo-controlled clinical trials.

Keywords

Calcium Folate, Calcium Pterin, Folic Acid-Based Immunotherapeutics, Immune-Related Disorders, Pterins

1. Introduction

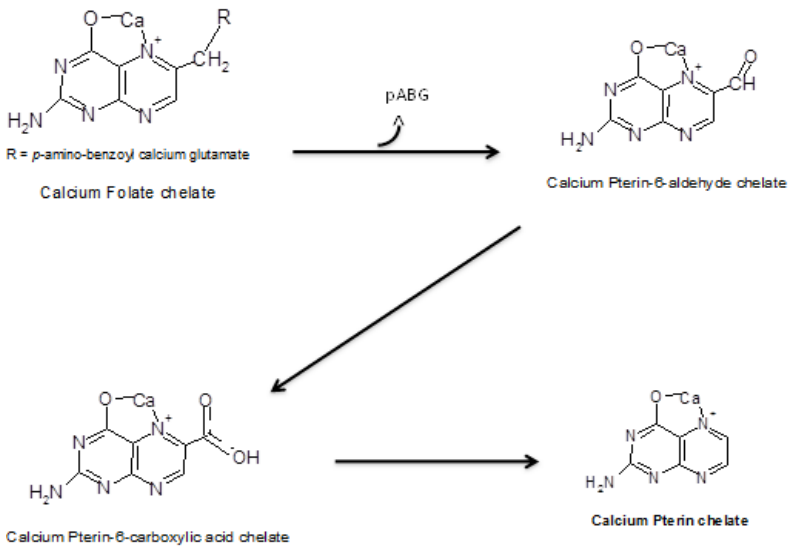

Figure 1. *Acid-oxidation of folic acid yields a series of pterins [12], shown here as Ca^{+2} chelates. Calcium folate in water yields calcium pterin-6-carboxylate, as determined by mass spectrometry [1].*

The structure of Calcium Folate has the same hetero-aromatic pterin ring as its three pterin and calcium pterin metabolic products formed in water (Figure 1) [1]. It has been reported that various pterins can act as immuno-modulators and that they are present in the blood and tissues of mammals [2, 3]. In particular, the compound pterin is excreted into the urine of cancer patients in elevated amounts relative to normal persons [4]. When combined with calcium, oral pterin demonstrates in mouse models anti-tumorgenic [5-7], anti-viral (hepatitis B) [8], and anti-diabetic [9] efficacy, and anti-tuberculosis mycobacterial activity in an *in vitro* model [10]. These studies also included the testing of dipterinyl calcium pentahydrate (DCP), a dimer of pterin linked together with calcium which breaks down to CaPterin in a pH < 4 environment (stomach). Therefore, DCP exhibits similar immuno-modulatory and anti-tumor activities as the monomeric CaPterin. DCP and CaPterin have both been shown to inhibit indoleamine 2,3-dioxygenase (IDO) with *in vitro* PBMCs (peripheral blood mononuclear cells) [5, 11]. IDO inhibition and other cytokine modulations are the likely mechanisms of action of the CaPterins *in vivo*.

1.1. Anti-Tumor Responses with Calcium Pterins [5, 6]

CaPterin has been shown to generate a dose-response relationship reaching a tumor growth inhibition of 63% at 21 mg/(kg day) with MDA-MB-231 breast tumor xenograph growth in nude mice after 43 days of oral administration. Pterin alone at 21 mg/(kg day) was found to have no antitumor activity.

DCP (calcium pterin dimer) at 23 and 69 mg/(kg d) also strongly inhibited MDA-MB-231 breast tumor xenograph growth in nude mice, with 75% and 50% tumor growth inhibition, respectively. Interestingly, calcium chloride dihydrate showed a significant 75% inhibition attributable to the high dietary folic acid intake of the mice (Human Equivalent Dose = 11 mg/day). No toxicity was observed in these studies.

1.2. Hepatitis B Virus (HBV) Mouse Model Study of the Calcium Pterin Dimer, DCP [8]

DCP dosing was found to effect a significant dose-response reduction of liver HBV DNA as measured by Polymerase Chain Reaction (PCR) in female HBV mice in the 2.3 to 23 mg/(kg d) range. 23 mg/(kg d)) DCP showed an 83% inhibition, comparable to adefovir dipivoxil (ADV) at 10 mg/(kg day), currently used for the treatment of Hepatitis B.

DCP-driven decreases in HBV DNA (PCR), coincided with immunologically-related decreases in indoleamine 2,3-dioxygenase (IDO) activity (measured by serum kynurenine to tryptophan ratio), and the chemokine MCP-l; and increases in serum GM-CSF.

1.3. The Calcium Pterin Dimer, DCP, Inhibits Intracellular M*ycobacterium Tuberculosis* Growth in Human Monocytes [10]

DCP significantly induced Mycobacterium tuberculosis killing by enhancing the antimycobacterial activity of human monocytes. In this in vitro system, DCP appears to induce mycobacterial killing via MIP-1β and nitric oxide-dependent effects. Hence, DCP acts as an immunoregulatory compound enhancing the antimycobacterial activity of human monocytes.

1.4. Orally-Administered DCP (Calcium Pterin Dimer), Improves Oral Glucose Tolerance in Diet-Induced Obese (DIO) Mice [9]

Dipterinyl calcium pentahydrate (DCP) was tested as a novel therapeutic for Type 2 diabetes. Female DIO C57B/6J mice, fed a high-fat diet, were administered DCP in 0.4% carboxymethylcellulose for 21 days. Blood glucose was followed during the dosing period, and an oral glucose tolerance test (OGTT) was carried out on day 21, along with measurements of plasma indoleamine 2,3-dioxygenase (IDO) metabolites (tryptophan and kynurenine), and certain cytokines and chemokines. 7 mg/(kg d) DCP reduced OGTT/AUC (area under OGTT curve) by 50% ($p < .05$).

2. Material and Methods

The investigators received medical and personal reports from the study participants as to their reactions to CaFolate regarding the various ailments each subject was following. Case details and other personal information were not collected, all identities were coded, and informed consents obtained.

Based upon NIH recommended doses for folic acid [13], 31 non-randomized subjects, aged 42-84, in varying degrees of health were directed to take, *ad libitum*, one to three capsules of CaFolate daily. Each capsule contained 300 μg folic acid + 75 mg $CaCl_2 \cdot 2H_2O$, and the excipients tricalcium phosphate, microcrystalline cellulose, gelatin, silicon dioxide, and magnesium stearate.

3. Results and Discussion

Subjects aged 42-84, in varying degrees of health, were directed to take one to three capsules of calcium folate daily

Table 1. *CaFolate study*

Subject ID	Indication	Reported Effect of Calcium Folate	Comments
1	Lupus erythematosus	Improvement	Corroborated by MD
1	Chemo Mouth Sores (MTX)	Improvement	Corroborated by MD
1	Arthritis	Less Pain / improvement	
1	Flu/colds	None since taking CF from 9/2012 to 4/2013	
2	Hepatitis C, undergoing anti-viral therapy	Possible Improvement	100% viral clearance after 2 weeks of CF (correlation)
2	Chemo Mouth Sores (from anti-viral therapy)	Improvement	
2	Flu/colds	None since taking CF from 2/2012 to 4/2013	
3	Colds	CF stopped cold	
4	Flu/colds	None since taking CF	
4	Mononucleosis/Lyme Disease flare-up	No apparent efficacy	
5	Energy/stamina/mental clarity	Improvement	
5	Flu/colds	None since taking CF from 12/2012 to 6/2013	
6	Flu/colds	None since taking CF from 1/2013 to 4/2013	
7	Flu/colds	None since taking CF from 9/2012 to 6/2013	
7	Energy	Improvement	
7	Arthritis	Less Pain / improvement	
8	Energy	Improvement	
9	Energy	Improvement	
9	Flu/colds	None while on CF	
10	Energy	Improvement	
11	Energy/calm	Improvement	
11	Fatigue	Improvement	
11	Arthritis	Less Pain / improvement	
11	Flu/colds	None since taking CF from 9/2012 to 6/2013	
12	Insomnia	Worse	Exacerbated by magnesium taurate
12	Leg pain	Improvement	
13	Type 2 diabetes	Possible Improvement	
14	Arthritis	Less Pain / improvement	
15	Arthritis	Less Pain / improvement	
16	Flu/colds	None while on CF	
17	Flu/colds	None while on CF	
18	Flu/colds	None while on CF	
19	Arthritis	Less Pain / improvement	
20	Type 2 diabetes	Stabilized	
21	Energy	Improvement	
22	Arthritis	Less Pain / improvement	
23	Arthritis	Less Pain / improvement	
24	Arthritis	Less Pain / improvement	
25	Advanced Stage IV Brain CA; patient near expiration	Increased apatite corroborated by attending MD	Expiration after several weeks [a]
26	Kidney CA	Remission	Tumor resected; benign
27	Bladder CA; patient deemed terminal	Remission	Alive after > 1 year
28	Flu/Cold	Improvement	
29	Lyme Disease; arthritic complications	Improved joint flexibility	3 capsules of CF in one dose exacerbated inflammatory flare-up [a]
30	Gastroenteritis	Mitigation	User on 2 capsules CF/day for 2 weeks mitigated gastroenteritis relative to family member not on CF
31	Arthritic hands	Inconclusive	User reported mild level 2 headaches after 1 week of CF; possibly unrelated to CF [a]; CF discontinued

Footnote: [a] Confounding from other supplements and medicines taken simultaneously.

The data from the observational study participants yielded the following results from those taking CaFolate (Tables 1 and 2):

- 13/13 subjects reporting on colds or flu had none during the cold and flu season.
- 10/11 subjects reporting on arthritis pain found improvement.
- 8/8 subjects reporting on overall energy and stamina found these factors were increased.
- 2/2 subjects with chemotherapy-induced mouth sores reported significant improvement.

Reported side effects by the cohort of 31 subjects include:

- 1 report of insomnia.
- 2 reports of inflammatory responses in two individuals with chronic inflammatory states due to Lyme Disease.
- 1 report of a mild (level 2) headache, possibly due to other confounding factors.

Table 2. *Summary of CaFolate observational data*

Number of CaFolate Users Reporting (N)	Condition	Percent Reporting Positive Response with CaFolate
13	Flu/colds (during cold/flu season)	100%
8	Energy/Stamina	100%
11	Arthritis	91%
2	Chemo Mouth Sores	100%
1	Lupus erythematosus	100%
1	Leg Pain	100%
1	Hepatitis C	Inconclusive
1	Mononucleosis/Lyme Disease	Inconclusive
2	Type 2 diabetes	Stabilized
1	Brain CA	Increased apatite
1	Kidney CA	Remission
1	Bladder CA	Remission
2	Lyme Disease	Possible induced inflammatory response; anti-arthritic
1	Gastroenteritis	Mitigated

Every person entering the study, and all pertinent reports, are tabulated in Table 1. Flu/colds (N=13) and arthritis (N=11) dominate the positive findings (100% and 91% reported positive effects, respectively). The increased energy and stamina reports (N=8; 100%) are encouraging as well. The magnitudes of these percentages are sufficiently compelling within the objectives of this observational study that elaborate statistics are not needed. The small number of reports for the other indications summarized in Table 2 renders these data unrobust.

The major robust findings presented herein are based upon all the response data from every one of the 31 subjects entering the observational study. The strengths of these reported findings serve to overcome many of the confounding issues (differing treatments, lifestyles, etc.) present in an observational study such as this. However, the findings do support moving ahead with the testing of calcium folate.

3.1. Abbreviations

CaFolate: calcium folate; CaPterin: calcium pterin; DCP: dipterinyl calcium pentahydrate; PCR: polymerase chain reaction; DIO: diet-induced obesity; OGTT: oral glucose tolerance test; IDO: indoleamine 2,3-dioxygenase; AUC: area under curve.

4. Conclusions

Based upon these pilot observational study results, CaFolate is an excellent candidate for matched group, randomized, double-blind, placebo-controlled clinical trials. The most promising immunotherapeutic indications for these clinical trials would be 1) colds/flu, 2) arthritis, and/or 3) chemotherapeutically-induced mouth sores. Successful results from these trials can lead to rapid clinical application and expanded utilization with patients.

Potential conflict of interest:

The author holds stock and stock options in SanRx Pharmaceuticals, Inc., which has patent pending claims for calcium folate.

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