Microcurrent Experimental Results.

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Hypothesis 1. FSM alters LOX induced inflammation using an accepted model of inflammation?

Test hypothesis using an ear inflammation model – Arachadonic acid induced swelling.
Male SKh-1 albino hairless mice aged 6 weeks. Ears were measured with a micrometer, 5µl of 25mg/ml of Arachadonic acid in ethanol, was painted on both sides of the ear and both ears were treated. FSM was applied immediately after it had dried and measurements were taken at 1 hour post application. The mean ear swelling for the change in thickness was determined and presented in a table.

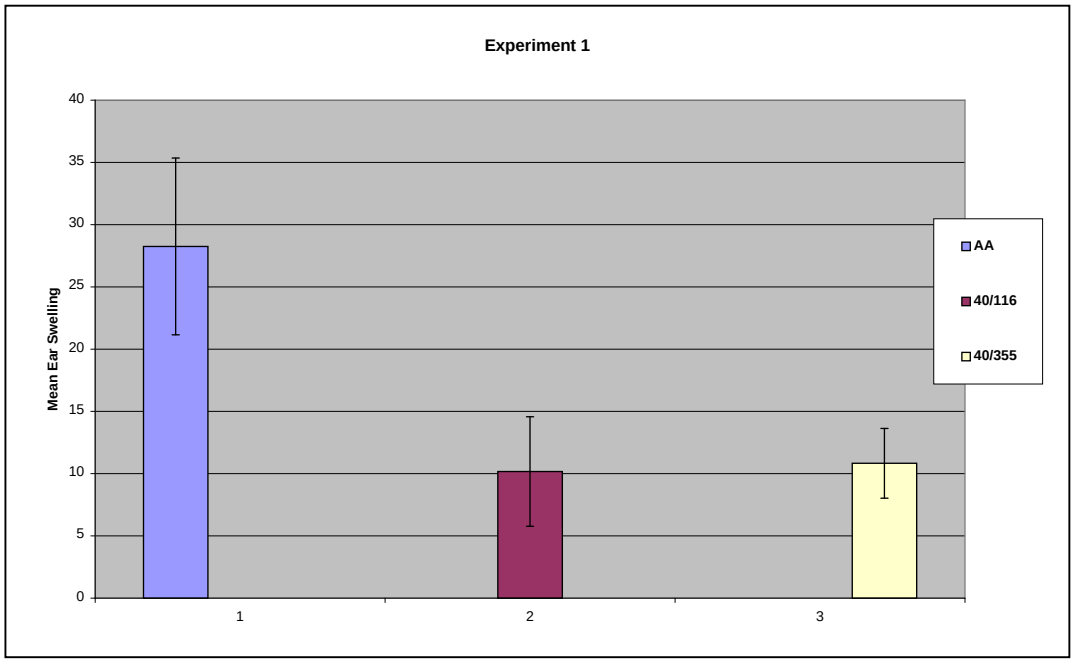
4 minutes of frequency 40/116 at (200microamps) immediately after painting of AA gives a 70% reduction in ear swelling.

4 minutes of frequency 40/355 at (200microamps) immediately after painting AA gives a 70% reduction in ear swelling

The response is time dependent as 1min gives no protection, 2 mins gives a 50% protection and 4 mins gives 100% protection. Data Appendix 1.

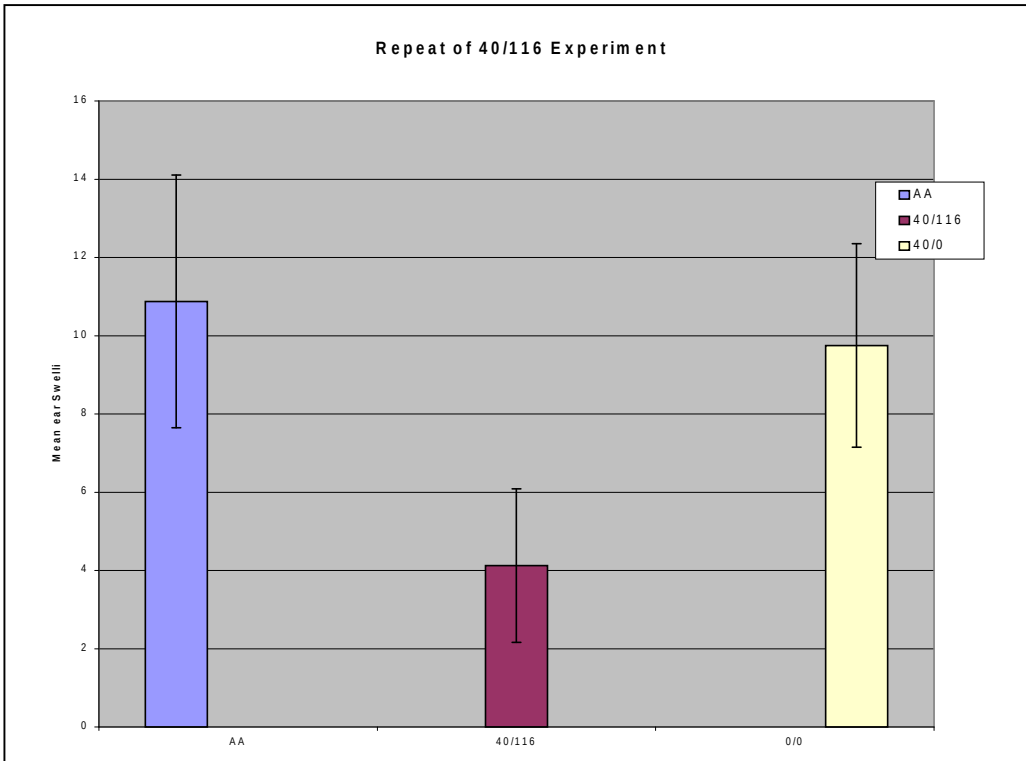
Conclusion.
Frequency Specific Microcurrent can effectively reduce AA induced ear swelling demonstrating its utility in applications for the treatment of acute inflammation, (Table 1.)

Table 1.



Repeat of 1st experimental results using 40/116 (Inflammation in the immune system)

Test hypothesis using a ear inflammation model – Arachadonic acid induced swelling.
Male SKh-1 albino hairless mice aged 8 weeks. Ears were measured with a micrometer, 5µl of 25mg/ml of Arachadonic acid in ethanol, was painted on both sides of the ear and both ears were treated. FSM was applied immediately after it had dried and measurements were taken at 1 hour post application. The mean ear swelling for the change in thickness was determined and presented in a table.



Conclusion.
Results for this experiment were measured blinded to Zero (0) time measurements and treatment group. In this experiment a control with no frequency set on the A/B channels was included. These animals also acted as a control for the FSM treatment method. The 40/116 group again showed a 62% reduction in ear swelling compared to both the positive and negative control. Again all animals responded to treatment. This experiment conclusively demonstrates the efficacy of the 40/116 frequency in relation to its anti-inflammatory property. As the collection of data in this experiment was blinded and hence without bias it further supports the utility of FSM.

Hypothesis 2. **a.** Frequency is an absolute requirement for the observed anti-inflammatory response., **b.** the frequency is specific for target and tissue.

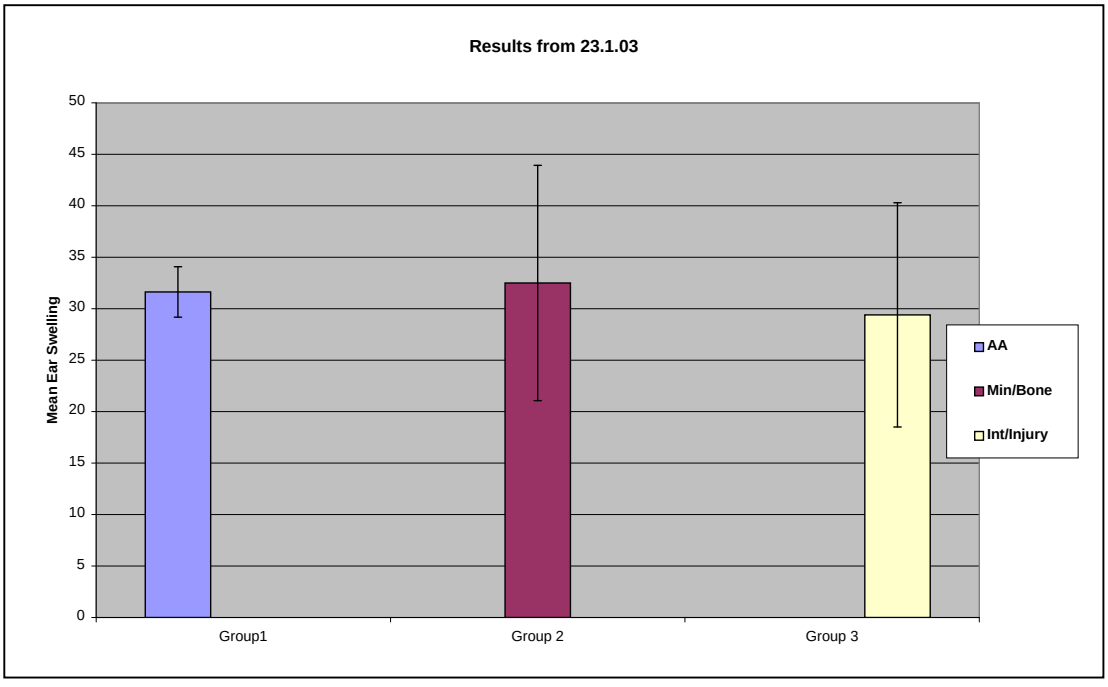
4 minutes of frequency 0.1/0.1 at (200microamps) immediately after painting of AA gives **no reduction** in ear swelling

4 minutes of frequency Mineral/Bone at (200microamps) immediately after painting of AA gives no **reduction** in ear swelling

4 minutes of partial Intermediate protocol frequency at (200microamps) immediately after painting of AA gives **no reduction** in ear swelling

Conclusion.
Current alone was found to have no effect on inflammation, the mean ear swelling was the same as for AA. Frequency was found to have specificities in both A/B channels and as seen in Table 1, ie particular frequencies are required for the reduction of inflammation in the model. (see Table 2).

Table 2.

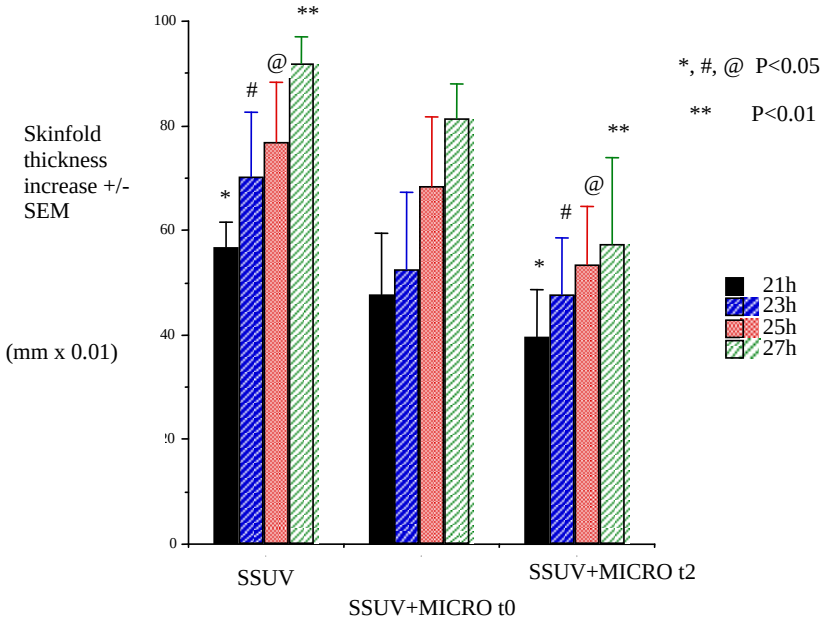


Hypothesis 3. **FSM can alter the UV induced skin fold oedema associated with sunburn.**

Female albino SKh-1 mice were irradiated for 28.95 minutes under a solar simulated light source. This was equivalent to two MED's of UV (This is enough UV to produce a sunburn response as measured by increased skin fold thickness (skin oedema) which reaches a maximum at 24 hours after UV exposure. Groups of 4 mice were exposed to UV and treated with FSM immediately after and 2 hours after UV exposure. Mice were treated simultaneously using a box modified for FSM treatment. The frequencies used were 40/116 for 4 minutes at 200µamp; 40/355 for 2 minutes at 200µamp and 40/103 for 2 minutes at 200µamp. The results are presented in Table 3.

Table 3.

Development of Erythema/Oedema following 2MED of SSUV, with microcurrent immediately after (to) or at 2h

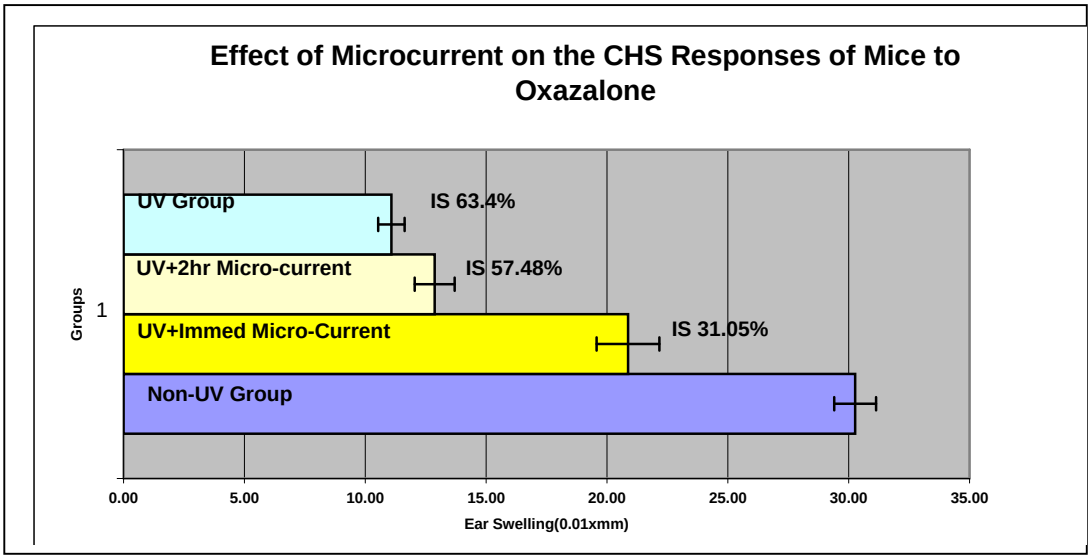


Conclusion.
FSM at the frequencies tested was shown to have a partial effect on Skin Fold Thickness indicating some protection against the UV induced sunburn response. The result at 2 hours post UV was statistically significant to P=0.01. It is highly probable that other specific frequencies could have even greater effects on this model of inflammation.

Hypothesis 4.
FSM can alter UV induced systemic immunosuppression. Demonstrating the immunomodulatory effect of FSM on Th1 immunity.

The mice that had been used in the sunburn experiment, Hypothesis 3, were then sensitised with Oxazalone a well known chemical sensitiser. This method measures the ability of an animal to produce an immune response to the contact sensitiser (CHS response) and is a measure of systemic immunity as challenge with Oxazalone, at a site away from the sensitisation site, will induce an inflammatory response. UV is a known immune suppressor of this ability to mount an immune response on subsequent challenge to a chemical sensitiser.
If FSM is able to overcome UV induced immune suppression it is proof of its activity on the systemic immune system. It also suggests that a Th1 response has been induced and that a systemic memory to the Oxazalone has developed. The results are in Table 4.

Table 4.



Conclusion.
FSM when given immediately after UV was shown to have a protective effect on UV induced immune suppression. This clearly demonstrates systemic immune modulation.

Experimental Conclusion.

These experiments clearly demonstrate the efficacy of FSM in these well characterised models of inflammation and immunity. The key findings are as follows.

1. Four (4) mins of 40/116 or 40/355 will produce a greater than 60% reduction in ear swelling in a recognised model of inflammation.
2. The effect is repeatable with 40/116 and the result was determined blinded demonstrating efficacy.
3. Frequency was shown to be required to obtain a result and frequencies appear to be specific.
4. A statistical significant reduction in inflammation was also observed in a second inflammation model.
5. A systemic immunomodulatory effect was demonstrated in relation to FSM's ability to reverse immunosuppression to UV as measured by CHS responses to oxazalone.

References to validate models.

1. Kim SY, Son KH, Chang HW, Kang SS, Kim HP . Inhibition of mouse ear edema by steroidal and triterpenoid saponins. Arch Pharm Res 1999 Jun;22(3):313-6.

Abstract
Certain steroids and triterpenoids isolated from diverse plant families were known to possess anti-inflammatory activity. In the course of finding new anti-inflammatory natural products, some steroidal and triterpenoid saponins were isolated and evaluated for their anti-inflammatory activity using in vivo mouse ear edema test. At the oral dose of 100 mg/kg, several steroidal saponins and triterpenoid saponins such as hederagenin glycosides showed significant inhibition of ear edema (20-37% inhibition), though less potent than indomethacin and hydrocortisone.

2. Qian C, Hwang SB, Libertine-Garahan L, Eckman JB, Cai X, Scannell RT, Yeh CG . Anti-inflammatory activities of LDP-392, a dual PAF receptor antagonist and 5-lipoxygenase inhibitor. Pharmacol Res 2001 Sep;44(3):213-20

Abstract
Leukotrienes (LTs) and platelet-activating factor (PAF) are important mediators of inflammation and allergy. LDP-392, a novel dual PAF receptor antagonist and 5-lipoxygenase (5-LO) inhibitor, has been identified. LDP-392 is 17.9-fold more potent than zileuton (5-LO inhibitor) in the RBL cytosolic 5-LO assay, and equally potent as MK 287 (PAF receptor antagonist) in the human platelet PAF receptor binding assay. The in vivo dual activities of LDP-392 were confirmed by measuring the inhibition of ex vivo LTB(4)production in rats and PAF-induced hemoconcentration in mice. Intravenous administration of LDP-392 demonstrated greater inhibition than zileuton, BN 50739 or MK 287 on arachidonic acid-induced ear edema and protected mice from LPS-induced lethality. Topical administration of LDP-392, in a dose-dependent manner, inhibited TPA-induced ear edema in mice and UVB-induced erythema in guinea-pigs. These data suggest that LDP-392, as a dual PAF receptor antagonist and 5-LO inhibitor, may be of greater clinical effectiveness. Copyright 2001 Academic Press.

3. Ueda H, Yamazaki M Anti-inflammatory and anti-allergic actions by oral administration of a perilla leaf extract in mice. Biosci Biotechnol Biochem 2001 Jul;65(7):1673-5

Abstract
The anti-inflammatory and anti-allergic activity of perilla leaf extract was investigated. The oral administration of perilla leaf extract to mice inhibited two types of acute inflammatory models, arachidonic acid-induced ear edema and 12-o-tetradecanoylphorbol-13-acetate-induced ear edema. Oral administration of perilla leaf extract also inhibited the contact dermatitis model, oxazolone-induced ear edema, by affecting sensitization.

4. Danno K, Ikai K, Imamura S.Anti-inflammatory effects of eicosapentaenoic acid on experimental skin inflammation models. Arch Dermatol Res 1993;285(7):432-5

Abstract
Anti-inflammatory effects of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) were examined on three models of skin inflammation induced in mice by topical application of an arachidonic acid (AA) solution, ultraviolet-B (UVB) irradiation, and contact sensitization with dinitrofluorobenzene. Ear oedema reactions induced by AA and UVB irradiation were significantly suppressed in mice fed a daily dose of 300 mg/kg EPA for 2 weeks. The contact hypersensitivity reaction was not impaired by EPA. None of the skin reactions was significantly

inhibited in mice fed DHA or safflower oil. The results suggest that EPA, but not DHA, has anti-inflammatory effects on AA- and UVB-induced acute inflammation reactions.

5. Kotyuk B, Raychaudhuri A, DiPasquale G.
Effect of anti-inflammatory compounds on edema formation and myeloperoxidase activity in the arachidonic acid-induced ear model in the mouse. Agents Actions 1993;39 Spec No:C46-8

Abstract
The arachidonic acid (AA)-induced ear edema model in the mouse has been demonstrated as an effective in vivo experimental tool to screen compounds showing anti-inflammatory activity. Since neutrophil influx is a component of the inflammatory reaction, we have modified this assay by quantitating myeloperoxidase (MPO) levels which reflect neutrophil accumulation in the edematous biopsies of the mouse ear. Our work has shown that orally administered 5-lipoxygenase inhibitors, dual inhibitors (CO/LO), and steroids dose-dependently inhibit both edema formation and MPO activity, whereas oral activity is not seen with NSAID's. There is a good correlation between the inhibition of edema formation and of MPO activity by these compounds. Thus, measurement of MPO, in addition to the AA-induced edema in the mouse ear, can provide another parameter to profile potential anti-inflammatory compounds.

Appendix 1.