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Combination anti-inflammatory therapy: synergism in rats of NSAIDs/corticosteroids with some herbal/animal products

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Abstract—A useful function of any complementary medicine is to supplement some of the benefits from other treatment modalities. In rats, extracts from Indian celery seed and the NZ green-lipped mussel are powerful nutraceuticals that (i) amplify the potency of salicylates and prednisone for treating pre-established chronic inflammation (arthritis, fibrosis) and (ii) reduce the steroid's gastrotoxic and lymphopenic side effects. Such combinations might also be useful for treating inflammatory components of (a) osteoarthritis caused by microcrystalline hydroxyapatite (BCP) and (b) pseudo-gout, associated with calcium pyrophosphate crystals; that are usually refractory to monotherapy.

Key words: Celery (seed) extracts; collagen-induced arthritis; (N.Z.) mussel extracts; prednisone; BCP/zymosan-induced fibrosis; steroid-sparing activity.

1. INTRODUCTION

Complementary/alternative medicines (CAM) are now the subject of considerable re-appraisal (Mills and Bone, 2000; Ernst, 2001; Rotblatt and Ziment, 2002; Bratman and Girman, 2003). However, evaluations of non-prescription therapies often do little justice to some of their truly complementary, i.e., supportive characteristics, such as covering an insufficiency or failing of another therapy. This may be difficult to assess and then accept, especially in the current regulatory climate which generally opposes the licensing of drug combinations.

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Table 1.
Overview of three nutraceutical medicines, as studied in rats

Tradition	Source AI agents	AI*	'Steroid-sparing'	Analgesic	Gastroprotectant
Ayurvedic (India)	Celery seed *Apium graveolens*	+	+	+	+
Maori (New Zealand)	Green-lipped (GL) mussel *Perna canaliculus*	+	+	−	+/−**
Aboriginal (Australia)	Fat from emu *Dromais novaehollandiae*	+	+	+/−**	+/−**

* Anti-inflammatory activity, preventing arthritis development.
** Dependent on degree of fractionation.

As this report shows, it is feasible to explore the potential of drug–CAM combinations for treating inflammatory disorders using stringent pharmaco-assays in laboratory rats.

There are few modern drugs for inflammatory disease so polyvalent as the classic synthetic drugs: aspirin (used for over a century) or prednisone (nearly a half-century old). These are still widely consumed or prescribed when not much else seems efficacious. It is worth looking at ways to live with the side-effects of these two classes of drugs. Each of these classic drugs has spawned an extended family of pharmaceutical second- or third-generation synthetic agents designed to be either more potent for local delivery (supersteroids for asthma or dermatitis) or less toxic for systemic use (COX-2 inhibitors). These are expensive, still need regulation and are unlikely to be available to the 80% of the global population living in the Third World.

This study examines some natural products derived from three systems of traditional medicine (TM) practised in non-Western cultures, that are as yet relatively neglected (Table 1). The intrinsic anti-inflammatory activity of the animal products (from a shellfish and a bird, the emu) has been discussed elsewhere (Whitehouse *et al.*, 1997, 1998).

Here we outline some of their potential to augment both aspirin-like drugs and corticosteroids — effectively reducing both dosage and side effects of these classical anti-inflammatory drugs.

For evaluating arthritis remedies, the choice of animal model(s) may be critical for generating apposite data concerning the real power of new monotherapies (e.g., biologicals) or novel drug combinations, that might realistically fulfill expectations in a subsequent clinical trial. It is not too difficult to identify anti-inflammatory agents that suppress development of an experimental arthritis in rodents. However, to curtail the arthritis, once fully expressed, is quite another achievement.

Two models of chronic inflammation in rats were used to verify the value of combinations of steroid with steroid-supportive nutraceuticals; namely (1) the

collagen-induced polyarthritis in rats, relevant to rheumatoid arthritis (Trentham, 1982) and (2) the persistent inflammation engendered with insoluble calcium salts (Denko and Whitehouse, 1976) of relevance to osteoarthritis (and perhaps pseudogout).

2. MATERIALS AND METHODS

Studies in rats were carried out according to protocols approved and supervised by the University of Queensland (UQ) Animal Ethics Committee Number 5. Outbred Wistar and inbred Dark Agouti rats were obtained from the UQ Central Animal Breeding Facility, Pinjarra Hills.

2.1. Collagen-induced arthritis (CIA)

Collagen type-II from bovine trachea was dissolved in 10 mM acetic acid (2 mg/ml) and emulsified with an equal volume of Freund's incomplete adjuvant, FIA. On day 0, 250 μl of this emulsion was injected ($= 5 \times 50$ μl inoculations) intradermally into the tailbase of Wistar rats, using a 30 g needle. No booster injection was needed. CIA was assessed by paw swelling measured on day 15 and at earlier times as appropriate. An arthritis score (scale 0–4+) was also compiled for each animal, based partly on signs of inflammation in all four paws and along the tail but also including assessment of other clinical signs of general well-being or otherwise (mobility, piloerection, low-grade fever, grooming, etc.). Prophylactic treatment was administered either (i) continually from day 0 for 15 days or (ii) at late stages of development, from day 9–14 inclusive. Therapeutic treatment was given, after the disease was optimally established, from days 15–18.

2.2. Adjuvant-induced arthritis

Female Wistar rats (160–180 g) were injected subcutaneously with 0.7 mg heat-killed *M. tuberculosis* (human) dispersed in 0.1 ml squalane. Treatment was initiated on day 15 for three days.

2.3. Chronic response to persistent local irritants

The rear paws of rats were injected with irritants dispersed in isotonic saline ($\leqslant$0.2 ml): zymosan-A, 0.5 mg; hydroxyapatite, 5 mg; calcium pyrophosphate, 10 mg. Paw swelling was measured after 3 h, 24 h and daily thereafter. Test treatments were usually administered orally after three h, when the initial oedemic response had peaked and the more chronic responses leading to fibrosis had begun.

2.4. Assessment of some side effects

Animals were fasted overnight after final dose of treatment and challenged next day with 50 mg/kg oral ibuprofen (given as the OTC formulation, Nurofen®) to probe

the resistance of the gastric mucosa to an NSAID. Animals were killed after 2.5 h, their stomachs rinsed and the number of mucosal haemorrhagic lesions enumerated. Spleens and thymi were removed and weighed to assess lymphoid involution as another marker of steroid intoxication.

2.5. Therapeutic agents (sources)

Aspirin (Monsanto), prednisone, dexamethasone, diflunisal, naproxen (Sigma, St. Louis, MO, USA); meloxicam (Boehringer-Ingelheim); celery seed extracts = alcoholic (Celtech® Beagle, Nerang, Qld, Australia), and supercritical fluid extract therefrom (Pharm-Med, Chapel Hill, Qld., Australia); extracts of green-lipped (GL) mussel = stabilised powder (Biomex®, MacLab, Nelson, New Zealand) and a lipid extract prepared therefrom (Lyprinol®, Blackmores, Sydney, NSW, Australia). Therapeutic grade refined emu oil (Dromaiol®) was supplied by Turner Research Labs (Narellen Vale, NSW, Australia).

2.6. Other reagents

FIA, hydroxyapatite (grade I), zymosan-A and some batches of collagen type-II (Sigma); another sample of collagen type-II was a gift from Dr. G. Slim (Industrial Research, Wellington, New Zealand). Calcium pyrophosphate dihydrate (CPPD) crystals were prepared from sodium pyrophosphate and calcium chloride (Denko and Whitehouse, 1976), selected for optimal irritancy and minimal endotoxin contamination.

3. RESULTS

3.1. Prophylactic treatment of collagen-induced arthritis (CIA)

Table 2 indicates the intrinsic anti-inflammatory activity in suppressing CIA development of two celery seed extracts: alcoholic (A-CS) and a further concentrate prepared from A-CS by supercritical fluid extraction with liquified carbon dioxide = S-CS (Butters *et al.*, 2002). The similar efficacy of GL mussel extract has been described previously (Whitehouse *et al.*, 1997).

This is in marked contrast to dosing rats with some triglyceride oils (olive, fish), used as neutraceuticals and frequently claimed to be of benefit in arthritis, which actually hastened arthritis development (by day 12) and consistently amplified its magnitude as measured on day 15.

3.2. Therapeutic treatment of established CIA

Table 3 shows that combinations of low-dose prednisone with celery or GL mussel extract, which were each not effective used singly, rapidly engendered significant arthritis-remitting activity. Since the average paw swelling of the arthritic animals

Table 2.
Prophylactic treatment of collagen-induced arthritis in rats

Treatment	g/kg	Mean signs of arthritis (day 14)		
		Increase in rear paw thickness (mm)	Arthritis score	Rats/group
None	–	0.58 (0.17)	2.0+	6
A-CS[a]	0.3	0.21 (0.12)	0.7+	4
S-CS[a]	0.05	0.08 (0.07)	0.8+	4
Fish oil/N[b]	1.8	1.12 (0.18)	2.8+	3
Fish oil/US[c]	1.8	1.08 (0.22)	3+	3
Cod liver oil	1.8	1.67 (0.17)	3.7+	3
Olive oil[d]	1.8	1.23 (0.26)	3+	5

Oils given orally for 15 daily doses; celery seed extracts for 6 daily doses only commencing on day 8.

[a] Alcoholic (A) and supercritical (S) fluid extracts from Indian celery seed.

[b] Pikasol, Norway.

[c] MaxEPA, USA.

[d] Light cooking oil, Spain.

Table 3.
Therapeutic treatment of pre-established collagen-induced arthritis in rats

Treatment	Dose (mg/kg)	Reduction in rear paw thickness (mm)	Reduction in arthritis score	Gastric lesions[a]
None	–	0.23	(−0.2+)	38
Prednisone (Pr) alone	2.5	0.18 (0.07)	0.2+	26
Pr + A-CS	2.5/300	0.82 (0.09)	0.9+	11
Pr + S-CS	2.5/50	0.93 (0.13)	1.3+	15
Pr + Lyprinol[b]	2.5/20	0.99 (0.12)	1.5+	30
Pr + BioMex[c]	2.5/300	1.05 (0.29)	1.7+	3
Meloxicam (Mx) alone	5	(−0.02) (0.07)	0.2+	23
Mx + Lyprinol	5/20	0.44 (0.21)	0.3+	16
Mx + BioMex	5/300	0.59 (0.13)	0.9+	19
A-CS alone	300	(−0.25)	(−0.3+)	10
S-CS alone	50	0.10	(−0.3+)	0
Lyprinol alone	20	(−0.08)	(−0.3+)	27
BioMex alone	300	(−0.03)	(−0.3+)	17

Treatment given for 3 days only. Animals challenged on day 4 with ibuprofen (50 mg/kg) to probe gastric resistance. Measure change rear paw thickness and arthritis score after 3 daily doses. Initial paw swelling was 1.1–1.3 mm; arthritis score = 2.7–3.2+. Data are means (SEM) from groups of 4 or 6 rats. Numbers in parentheses represent increase over the no-treatment controls ($n = 4$ rats/group).

[a] Mean number haemorrhagic lesions with ibuprofen, 50 mg/kg after fasting overnight (on the third day).

[b] Lyprinol from the GL mussel = lipid extract.

[c] Stabilised whole mussel powder.

Table 4.
3-Day combination therapy for rats with pre-established adjuvant-induced arthritis

Dose (mg/kg)	"Allopathic" treatment	With	Reduction in			Mean number of gastric lesions[a]	Weight change (g)
			Rear paw (mm)	Front paw inflammation	Arthritis score		
10	Hi Prednisone	—	0.07 (0.05)	0	0	44	+1
2.5	Lo Prednisone	—	0.06 (0.11)	(−0.7+)	(−0.3+)	32	2
		Ly	0.76 (0.13)	1.1+	1.3+	13	+8
		A-CS	0.60 (0.13)	0.9+	0.8+	13	+7
		S-CS	0.45 (0.19)	0.8+	0.8+	11	+3
		Dromaiol	0.59 (0.17)	0.8+	0.9+	21	+3
200	Aspirin	—	(−0.16) (0.07)	(−0.6+)	0.2+	20	+2
		Ly	0.40 (0.22)	0.6+	0.3+	10	−2
80	Diflunisal	—	(−0.11) (0.07)	0.3+	0.1+	29	+5
		Ly	0.48 (0.14)	1.4+	1.0+	0	+3
		A-CS	0.65 (0.11)	1.3+	0.8+	5	+10
		S-CS	0.53 (0.15)	1.0+	1.0+	8	+9
150	Mefenamic acid	—	(−0.09) (0.15)	0.3+	0.4+	13	+6
		Ly	0.61 (0.17)	0.3+	0.7+	0	+9
		BM	0.56 (0.21)	1.2+	0.9+	2	+12
20	Lyprinol alone		0.15 (0.11)	0.6+	−0.1+	17	+4
300	BioMex alone		(−0.07) (0.21)	0.4+	0	0	+3

Dose for 3 days only, fast overnight and probe for gastric resistance with ibuprofen (see Table 3). Data are means (SEM) from groups of 4 or 6 rats. A-CS = alcoholic celery seed extract (300 mg/kg); S-CS = supercritical fluid extract from celery (50 mg/kg); Dromaiol (emu) = 900 mg/kg; Ly = Lyprinol (20 mg/kg); BM = BioMex (300 mg/kg) from GL mussel.

[a] Mean number haemorrhagic lesions with ibuprofen, 50 mg/kg after fasting overnight (on the third day).

before treatment was 1.2 ± 0.09 mm ($n = 60$ rats), whilst the normal paw thickness was 7.3 mm, some of these 'remissions' with reductions in paw thickness $\geqslant$0.9 mm were quite dramatic in appearance. Reductions in paw volume would be even more dramatic; see Appendix A.

This response was all the more significant because (i) several NSAIDs were ineffective; (ii) much higher doses of prednisone ($\geqslant$10 mg/kg) were required, if used alone, to have any impact on paw size but also inducing unacceptable side effects (weight loss, virtual extirpation of thymus and heightened susceptibility to NSAID-gastropathy); and (iii) the synergist alone, like the NSAIDs alone, were ineffective here — in contrast to their ability to suppress arthritis development when used at an earlier time, e.g., celery extracts (Table 1) and the mussel extracts (Whitehouse *et al.*, 1997).

3.3. Therapeutic treatment of established adjuvant-induced arthritis

Tables 4 and 5 show that this synergy was reproducible in another model of experimental polyarthritis, induced with a mycobacterial adjuvant. Here it was

Table 5.
"Souping up" salicylate for treating established adjuvant-induced arthritis in rats

Dose of additive (per kg)	Treatment p.o.	Reduction in			Mean number of gastric lesions[a]	Weight change (g)
		Rear paw (mm)	Front paw inflammation	Arthritis score		
2 ml	Olive oil (OO) alone	0.10	0	0.2+	31	+4
200 mg	Na salicylate and OO	(−0.07)	(−0.5+)	(−0.9+)	33	+5
20 mg	– and Lyprinol[b]	0.48 (0.12)	0.7+	0.5+	0	+8
1 ml	– and Dromaiol[b]	0.67 (0.12)	1.6+	0.7+	16	+10
100 mg	– and S-CSE	0.45 (0.17)	0.5+	0.5+	2	+3
20 mg	Salicyl alcohol and Lyprinol	0.05	(−0.2+)	(−0.1+)	ND	+6

Dose for 3 days only, fast overnight and probe for gastric resistance (see Tables 3 and 4).

Dose of sodium salicylate or salicyl alcohol = 200 mg/kg per day. OO = olive oil carrier for active lipids.

[a] Mean number haemorrhagic lesions with ibuprofen, 50 mg/kg after fasting overnight (on the third day).

[b] Inhibition of leukotriene synthesis by human PMN's.

possible to replace low-dose prednisone with (i) some relatively weak but non-gastropathic NSAIDs, e.g., diflunisal, mefenamic acid and sodium salicylate, better known as analgesics rather than anti-arthritic agents, or (ii) other NSAIDs with low gastrotoxicity, e.g., meloxicam. Also, the Lyprinol could be replaced with whole stable GL mussel powder or celery seed extracts or an active emu oil (given orally).

The combination of salicyl alcohol (saligenin) with Lyprinol did not remit arthritis, showing that Lyprinol needed to given with a proven NSAID to successfully treat established arthritis.

3.4. Experimental fibrotic inflammation induced by zymosan

The yeast cell wall protein-carbohydrate complex, zymosan, induces a biphasic response when injected into a rat's paw, i.e., an initial rapidly developing oedema (partly reduced by cyproheptadine) which subsides after 2–3 h and is succeeded by a slow developing but persistent local fibrosis. To distinguish anti-fibrotic activities from acute anti-inflammatory effects, all treatments were given after 3 h.

Table 6 again shows that combinations of celery seed or GL mussel extracts together with low dose prednisone or dexamethasone were able to reduce both the delayed fibrotic response to zymosan and the incipient toxicity of these steroids (thymus involution, predisposition to NSAID gastropathy).

3.5. Persistent inflammation with calcium salts found in human joints

The major mineral component of bone, the basic calcium phosphate (BCP) = hydroxyapatite, induces chronic irritation when injected into an air pouch in rats (Dieppe *et al.*, 1976) or into rats' rear paws (Denko and Whitehouse, 1976); the

Table 6.
Persisting rear paw oedema after injecting zymosan or crystalline calcium salts

Treatment	mg/kg	Zymosan[a]		with BCP[b]		CaPP[c]	
		24 h	48 h	24 h	48 h	24 h	48 h
High Prednisone	10			19		20	
Low Prednisone	2.5	0		0		6	
L-Pr. and A-CS	300	58	44	46	41	67	69
L-Pr. and S-CS	50	34	27	57	72	48	75
L-Pr. and BioMex	300	76		66		64	60
L-Pr. and Lyprinol	50	52	39	66	46	8	20
L-Pr. and emu oil	900	63	46	40	48	22	59
Naproxen	50	−32	−11	−9	−7	0	9
N. and A-CS				82	63	0	23
N. and S-CS						0	28
N. and BioMex				59	46	9	17
N. and Lyprinol		0	0	73	70	0	19
N. and emu oil				71	84		

Data are percentage inhibition ($n = 4$/group) measured after 24 and 48 h, treatment given at 3 and 24 h after injecting irritants.

Dose of irritant adjusted to give increase in paw thickness = 1.2 ± 0.11 mm in untreated controls.

[a] Zymosan (yeast cell wall) a trigger for fibrotic inflammation.

[b] Hydroxyapatite as found in OA (not RA) synovial fluids.

[c] Calcium pyrophosphate as found in pseudo-gout.

Table 7.
Divergent anti-inflammatory responses within the one animal

Rat	Treatment[a]	Mean paw swelling in mm (SEM) with			
		BCP after		CPP after	
		24 h	48 h	24 h	48 h
DA	None	1.60 (0.07)	1.50	0.85 (0.05)	1.05
	Naproxen alone	1.46 (0.02)	1.43	0.95 (0.13)	0.85
	Naproxen and BM	0.50 (0.15)	0.70	1.04 (0.06)	0.99
Wistar	None	1.38 (0.13)	1.38	1.22 (0.19)	1.27
	Naproxen alone	1.38 (0.07)	1.45	1.20 (0.13)	1.03
	Naproxen and BM	0.80 (0.17)	0.70	1.22 (0.08)	1.08

Groups of 4 DA or Wistar rats were injected in the left paw with BCP (5 mg) and the right paw with CPP (10 mg).

[a] Naproxen = 50 mg/kg; BioMex (BM) = 300 mg/kg, dosed orally 3 and 24 h after inoculating rear paws with BCP/CPP.

latter response being expressed as a slow-developing but persistent paw oedema. Table 6 shows that this too was reduced by treatment with the same synergistic TM-combinations with low dose prednisone that held down the fibrotic response to zymosan. Rather surprisingly high-dose naproxen plus TM combinations also suppressed this BCP-induced inflammation.

Table 6 also indicates that the inflammatory response to CPPD, the likely cause of pseudo-gout, might also respond to the same steroid-plus synergist combination. However, this CPPD-induced inflammation was refractory to high dose naproxen alone or combinations of naproxen with the same synergists that effectively suppressed BCP inflammation when combined with prednisone. This was dramatically confirmed when hydroxyapatite was injected into one rear paw and CPPD into the other and the animals then dosed with a test combination (Table 7).

3.6. Lymphoid involution

Animals with the zymosan-induced paw oedema suffered modest parallel reduction in thymus and spleen mass after three days, compared to non-inflamed rats. Treatment with prednisone (2.5 mg/kg) reduced thymus/body weight ratio by 30%: this was restored to normal by co-dosing with 13 mg/kg Lyprinol. The equivalent anti-inflammatory dose of prednisone alone (10 mg/kg), i.e., without Lyprinol, reduced the thymus/body weight ratio by over 50%.

4. DISCUSSION

These experimental studies have asked the question: will combination therapies safely and effectively out-perform monotherapy in controlling severe, disabling, inflammation? Few anti-arthritic drugs induce/accelerate remission of established polyarthritis in rats at sub-toxic doses. Not too many treatments are truly effective for preventing the cartilage destruction in rheumatoid arthritis caused by ingrowth of pannus (from hyperplastic synovium).

Endogenous cortisol provides *inter alia* a first line of defence from the ravages of chronic inflammation. When other palliative therapy fails, it is still common practice to introduce a synthetic corticosteroid (CS), e.g., prednisone for systemic use or yet more potent CS for local use (lung, skin). These allopathic steroids are multi-potent and suppress chronic inflammation by several mechanisms, functioning simultaneously or sequentially, which other therapies may only control singly. Thus, the leukotriene-regulant function of corticosteroids, effected by controlling phospholipase-A_2, is largely reproduced by inhibitors of 5-lipoxygenase (5-LOX) supplemented by leukotriene-receptor antagonists. Other valuable properties of corticosteroids, such as inhibiting nuclear factor κB and other promoters of pro-inflammatory cytokines, have not as yet been fully reproduced by currently available drugs in the clinic. Fortunately this latter function seems attainable with low-dose steroids. Some of the effective natural products, e.g., Lyprinol and the more potent emu oils contain 5-, 12- and 15-LOX-inhibitors (W. H. Betts, personal communication).

So we can now begin to write new pharmaco-efficacy relationships along the lines:

(1) Low-dose steroid plus LOX inhibitor (may be) = high-dose steroid alone.

Paradoxically, we can also write another equation:

(2) Low-dose steroid plus steroid 'antagonist' = high-dose steroid for efficacy, while minimising the steroid toxicity. This is achieved in a two-fold manner: direct reduction of the steroid dose needed and supplemented by any properties of the synergist acting as an 'antagonist' to counteract a steroid side effect, e.g., celery extract protecting the stomach (Whitehouse *et al.*, 2001) or Lyprinol protecting the thymus.

In other clinical contexts, the concept of 'steroid-sparing' agent (SSA) is used to describe supplementary prescription therapy for patients, usually asthmatics, who are steroid dependent. Some of these SSA, e.g., methotrexate, auranofin, cyclosporin (Harbison and Whitehouse, 2000), are notoriously toxic and can only be used clinically under strict supervision. However, as shown here, there are natural products/nutraceuticals which might also be considered steroid-sparing, either as (i) amplifiers of potency (i.e., diminishing steroid dosage required) or (ii) protectants from some of the notorious side effects of steroids, e.g., GI ulcers, lymphoid involution, and perhaps osteoporosis. The SSA identified here can probably/generally be regarded as safe (GRAS), being derived from (a) long traditional usage in non-Western systems of medicine and (b) regular foodstuffs in the same and other cultures.

Osteoarthritis is notoriously difficult to treat beyond palliative therapy with analgesics (Hochberg, 2002). The presence of hydroxyapatite crystals embedded in the synovium or phagocytosed by synovial fluid leukocytes may trigger the release of pro-inflammatory/algesic factors (Dieppe *et al.*, 1976; Gibilisco *et al.*, 1985). Corticosteroids are sometimes used intra-articularly (Buchanan and Kean, 2002) when prostaglandin-suppressant drugs prove inadequate. However, both oral and intra-articular steroids may impair what little cartilage regeneration might be occurring (Lash and Whitehouse, 1961).

The proliferation of fibroblast-like synoviocytes, probably responsible for pannus formation and destruction of joint cartilage in rheumatoid arthritis, is driven by macrophage inhibitory factor (MIF). MIF may also activate the expression of phospholipase-A_2 and cycloxygenase by rheumatoid synovial fibroblasts (Lacey *et al.*, 2003). A key factor regulating MIF is its natural antagonism by endogenous/supplemented corticosteroids (Morand *et al.*, 2003).

This realisation again raises the question of how to get the best out of the steroids we know — optimising their therapeutic potential — while successfully controlling their side effects. One answer may be to harness the power of readily available synergistic CAMs with the requisite qualities: reproducible and reliable, adequate preservation, proven efficacy/safety and affordable cost (Butters and Whitehouse, 2003).

Finally, these studies also showed the beneficial effects of some TM-products for reducing, even sometimes abolishing, NSAID-gastropathy in chronic inflammation (see Tables 3–5); demonstrated previously in more acute models of inflammation (Rainsford and Whitehouse, 1980; Whitehouse *et al.*, 2001).

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APPENDIX A

Relationship between paw thickness and paw volume, as indicators of oedemic/fibrotic inflammation (Y. Anissimov, Therapeutics Research Unit, UQ):

- Assuming a cylindrical geometry, the volume of a rat's rear paw = length (l) × area of cross section ($\pi D^2/4$).

  With $V = a \cdot D^2$, where D = diameter, $a = l\pi/4$
  then ΔV approximates to $2\,aD \cdot \Delta D$.

- So the relative change in volume

$$\frac{\Delta V}{V} = \frac{2aD \cdot \Delta D}{aD^2} = \frac{2\Delta D}{D},$$

  i.e., twice the relative change in diameter.

- Restating this, the volume of oedema reduction is double the reduction of paw thickness/diameter.

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