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ANTI-INFLAMMATORY ACTIVITY OF A HOLOTHURIAN (SEA CUCUMBER) FOOD SUPPLEMENT IN RATS

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ABSTRACT

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A human food supplement (SeaCare^{R}) composed of dried extracts from specific varieties of holothurians (sea cucumbers) and a sea plant has been found to have anti-inflammatory activity in both sexes of two strains of rats. It is slightly less active than aspirin (w/w) against the acute carrageenan-induced paw inflammation, but without the gastrotoxicity of aspirin. It is also active against adjuvant-induced polyarthritis in rats on a daily dose schedule.

Keywords: anti-inflammatory, holothurian, sea cucumber, arthritis

INTRODUCTION

The marine animal, Bêche-de-mer, also known as trepang, sea cucumber or sea slug, belongs to the phylum Echinodermata, class Holothurioidea [1,2] of which some 900 species have been identified. They have been used for centuries in Chinese traditional cooking and are considered in some cultures to be aphrodisiacs and important dietary supplements for treating a range of ailments, including high blood pressure. The diversity of species and processing methods has led to products with widely differing properties being sold to specialized markets throughout south-east Asia. The particular product that we examined was prepared from species of holothurians collected in waters off northern Australia [1], is available in Australia and is a registered therapeutic good (SeaCare^{R}). Hitherto, no scientific reports have described any pharmacological properties of this Australian product but some holothurians are known to be toxic to fish [3,4]. The increasing emphasis in the drug industry on safety is leading to renewed interest in screening of natural products, including those used nutritionally in large populations for many years.

In this preliminary study, the food supplement SeaCare and its crude constituent species were examined for anti-inflammatory activity in two rat models of inflammation that have been commonly used for the detection and development of clinically effective anti-inflammatory drugs [5–7]. Indications in this report are that the supplement contains an ingredient that has anti-inflammatory activity.

MATERIALS AND METHODS

The food supplement, SeaCare, manufactured under controlled conditions to reproducible specifications by Pacific Grant Corporation, Townsville, North Queensland, Australia, was prepared from specially selected and processed varieties of Bêche-de-Mer (95% w/w *H. (Microthele) nobilis*, *H. (Microthele) axiologa* and *Stichopus variegatus*) and sea plant (5% w/w *Sargassum pallidum*). SeaCare was packaged in 500-mg capsules each containing protein (421 mg), fat (10 mg), calories (2.0) and <2% rda (US) of the following nutrients: vitamins (A and C), riboflavin, niacin, minerals (Ca, Mg, Zn, Cu, Fe, Na), thiamine. Amino acids present were (in mg per 421 mg protein): Ala 27.5; Val 19.5; Gly 22; Ile 24; Leu 32; Pro 20; Thr 23.5; Ser 21.5; Met 13; Cys 8; Phe 15.5; Asp 44.5; Glu 68.5; Tyr 16.5; Lys 29; His 9; Arg 20; Trp 7. Groups of ten male and female Wistar rats have tolerated single doses of 5 g/kg po body weight without adverse effects during the following 14 days [8].

For administration by intraperitoneal injection (ip), the material was ground in a mortar with pestle, extracted into 0.08 mol/L sterile saline and filtered through filter paper (Whatman No. 1). The transparent filtrate was given as a single dose, without evident local irritation at the site of injection. Oral doses were prepared as a ground slurry in 0.04% Tween-20 (detergent) in water then either briefly centrifuged, or allowed to settle for one hour before decantation, to remove coarse material. These aqueous extracts were administered to male and female dark agouti and hooded rats either in three daily doses at 300 mg/kg, the last dose being administered 45 min before the carrageenan (1 mg in 0.1 ml saline) was injected to initiate the paw oedema [6].

Polyarthritis was initiated in male Dark Agouti or hooded Wistar rats on 'Day 0' by injecting 250 μg heat-killed *Mycobacterium tuberculosis* in 50 μl squalane (a Freund's adjuvant) into the tail base [5,7,9]. Test compounds were given before Day 7 to halt the disease (antiarthritic) or after Day 10 to treat the established arthritis (anti-inflammatory). The polyarthritis is usually manifest from Day 12 as local inflammation and ulceration in the tail, inflammation in all paws and inflammatory lesions on forepaws and ears. Inflammation was evaluated using a micrometer to measure changes in rear paws and tail thickness, and by visual estimates of severity of front paw swelling and inflammation. Animals were sacrificed by cervical dislocation on or before Day 18 and checked for gastric lesions and other non-articular pathological changes.

RESULTS

Table 1 summarizes experimental data from rats in which the carrageenan paw oedema had been established. Doses refer to the amounts of solids used to prepare aqueous extracts.

When the SeaCare formulation was given at 50 mg/kg by ip injection 20 minutes prior to administration of the carrageenan, there was substantial inhibition of the paw inflammation. This effect was equivalent to a larger dose (150 mg/kg) of aspirin (Table 1). This apparent anti-inflammatory activity might have been due to the toxic effects of an irritant in the formulation. At higher ip doses of SeaCare (150 mg/kg), rats also went limp after 2 h, a sign that the material might be hypotensive. Some

irritants are known to show anti-inflammatory properties [10], particularly when given ip. Therefore, we administered the product orally for all further studies, in which this relaxant effect was not seen, even with relatively high doses (1 g/kg po).

Single oral doses of the holothurian supplement (SeaCare) at 300 mg/kg showed some anti-inflammatory activity in the carrageenan paw oedema assay in both sexes (Table 1), with about the same potency as 150 mg/kg oral aspirin. By contrast, many of the non-steroidal anti-inflammatory drugs currently in the market place are $\geqslant 25$ times more potent than aspirin in this assay [11,12].

TABLE 1
Acute anti-inflammatory activity of a holothurian-based food supplement in Dark Agouti rats*

Sex	Dosage (mg/kg)	Route	% Inhibition of paw swelling after 1 h	2 h	3h
Male	1 × 50	ip	53	56	22
	1 × 300	po	31	13	22
	+Mpl**		48	44	31
	3 × 300	po	47	37	30
	Control		0	0	0
Female	1 × 300	po	31	31	08
	+Mpl**		41	37	36
	3 × 300	po	56	51	43
	Control		0	0	0
	Aspirin 100	po	13	09	05
	+Mpl**	po	41	40	23
	150	po	43	30	35

*Groups of 4
**0.5 mg/kg misoprostol

The anti-inflammatory activity of SeaCare was enhanced 1–2 fold, either by giving three oral doses (300 mg/kg) at 48 h, 24 h and 45 min prior to the carrageenan, or alternatively by coadministering po a single dose of misoprostol (Mpl, 500 μg/kg), a prostaglandin-E_1 analogue. These enhancements were seen in both sexes and similar data (not shown) was also obtained using a second strain of rats (hooded Wistar).

For reference, aspirin also showed little activity in hooded rats at 100 mg/kg whereas it was active when given in combination with Mpl [13]. Mpl alone was inactive in this assay at the dose used.

Table 2 shows the anti-inflammatory effect of the supplement given prophylactically to male hooded rats developing adjuvant-induced arthritis. The material was given at 300 mg/kg daily for 16 days, beginning one day before initiating the arthritis and ceasing dosing 14 days after initiation. The crude data represent a modest inhibition of rear paw swelling (37%) and forepaw inflammation (45%) by Day 15. The lesser weight decrease in the treated animals (versus controls) indicates a significant reduction in the cachexia normally associated with injecting adjuvants into susceptible rat strains.

TABLE 2
Inhibition of adjuvant-induced polyarthritis in male hooded Wistar rats by a holothurian supplement given prophylactically

		Signs of arthritis (day 15)			
			Increased thickness (±SEM)		
Treatment*	Dose (mg/kg)	ΔWt (g)	Tail (mm)	Rear paws** (mm)	Forepaw inflammation†
Control (water)	–	−18	3.28 (0.31)	1.39 (0.27)	4+
Supplement	300	−11	3.52 (0.17)	0.88 (0.21)	2.2+
Ibuprofen	80	−06	2.31 (0.61)	0.47 (0.31)	1.2+

*Given orally for 16 days (days −1 to +14) to rats (4 per group)
**Average thickness in uninflamed rats = 6.8 mm
†Scored on scale 0–4+ for increasing severity

TABLE 3
Inhibition of acute inflammation in rats by individual components of the holothurian supplement

Treatment*	Carrageenan oedema* % inhibition after		
	1 h	2 h	3 h
Microthele axiologa	−01	−11	−21
Stichopus variegatus	21	04	−01
Microthele nobilis	31	07	−09
Sargassum pallidum	37	36	21

*Male Wistar hooded rats (3 per group) treated orally with 3 × 300 mg/kg (48 h, 24 h and 45 min prior to carrageenan). Negative values indicate increased swelling relative to untreated controls

TABLE 4
Comparison of anti-inflammatory activity of the whole holothurian supplement versus its individual components in arthritic male Dark Agouti rats

Treatment[a]	Mean increase in arthritis signs over:								
	Days 10–14[b]					Days 14–17[c]			
		Swelling			Inhibition rear paws (%)		Swelling		
	ΔWt (g)	Rear paws (mm)	Tail (mm)	Front paws		ΔWt (g)	Rear paws (mm)	Tail (mm)	Front paws
A.									
Control (none)	−18	1.58	0.18	4.2+	0	−17	0.18	−0.32	0
Whole supplement	−15	0.91	−0.03	0.7+	42	−18	0.68	0.53	2.3+
plus Mpl[d]	−19	0.22	−0.32	+	86	−6	1.15	0.03	1.5+
Aspirin (150 mg/kg)	−02	0.63	0.45	0.7+	60	−9	0.65	0.05	1.5+
B.									
Control (Tween 20 only)[e]	−19	1.98	0.20	2.3+	0	−16	0.15	−0.10	1.5+
Microthele axiologa	−27	2.02	0.28	1.8+	−2	−15	0.26	−0.23	1.5+
Stichopus variegatus	−17	1.06	0.25	1.0+	46	−17	1.09	0.07	1.2+
Microthele nobilis	−2	0.29	−0.04	0.9+	84	−1	1.76	−0.11	1.0+
	(−3	0.79	−0.02	1.4+	60)[f]	(−9	1.16	0.39	1.3+)[f]
Sargassum pallidum	−12	0.04	0.18	0.6+	98	−1	0.54	0.10	2.3+
	(−24	1.74	0.11	2.5+	12)[f]	(−4	0.34	−0.19	1.4+)[f]

[a]Orally 300 mg/kg/day on days 10–13; 4 rats/group
[b]Measured changes between day 10 and 14 in weight (ΔWt), rear paw and tail thickness (mm), front paw lesions. Inhibition of rear paw inflammation is relative to control (0%)
[c]Rebound in signs of inflammation measured over days 14–17, i.e. after ceasing treatment
[d]Mpl = Misoprostol applied transcutaneously (50 μg/kg) in ethanol/propan-1,2-diol (2:1 v/v, 2.5 ml/kg)
[e]No. rats = 8
[f]Processed in the same way as SeaCare mixture

Table 3 presents the effect of individual components of the SeaCare product on the carrageenan paw oedema. Administered orally in three doses at 300 mg/kg at 48 h, 24 h and 45 min prior to carrageenan, three of the components were anti-inflammatory. Their relative activity (w/w) decreased in the order: *Sargassum pallidum* ~ *Microthele nobilis* > *Stichopus variegata*; with *Microthele axiologa* showing no discernible activity.

Table 4 summarizes experiments in which the whole supplement (Table 4A) and its individual components (Table 4B) given orally (300 mg solids/kg each day for 4 days) were evaluated for anti-inflammatory activity against established adjuvant-induced polyarthritis in rats. On this early therapeutic dose schedule (Days 10–13), *Microthele axiologa* again showed no anti-inflammatory action, consistent with results in Table 3. The other three components were all anti-inflammatory, the order of activity decreasing as *Sargassum pallidum* > *Microthele nobilis* ≫ *Stichopus variegata*. However, when these fractions were subjected to the same processing conditions that are used industrially to prepare SeaCare, only the *Microthele nobilis* component retained activity in this assay (Table 4). The anti-inflammatory effect is temporary; a 'rebound' in arthritis signs being evident between Days 14–17 (Table 4) after cessation of the treatment.

DISCUSSION

The holothurian formulation, SeaCare, has weak anti-inflammatory activity in rats when given orally. Even greater activity was observed when the formulation was given ip, but then at least one noxious (hypotensive) side-effect was also detected.

Although the anti-inflammatory activity was somewhat less than that of aspirin, the activity could be increased either by repeated dosing or by coadministration with a synergizing agent. The synergistic activity with the simple prostaglandin-E_1 analogue, misoprostol, in both these acute and chronic models of inflammation suggests that prostaglandins released in the inflammatory milieu might potentiate the activity of holothurian-derived anti-inflammatory agents. This hypothesis needs to be further tested.

The anti-inflammatory data in Tables 3 and 4 show that the commercial food supplement contains only one particularly active holothurian species, *Microthele nobilis*, which constitutes some 50% of the SeaCare product. These findings suggest that a comprehensive effort, to extract and identify the specific compound(s) responsible for the temporary anti-inflammatory effect of the SeaCare formulation, may yield a potential drug candidate of marine origin that is probably present in only trace/small quantities in the aqueous extracts evaluated here.

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