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The Effect of Two Extracts from *Stichopus badionotus* Selenka upon Induced Pleurisy in Rat

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Abstract: The pleurisy model in rats was used to study the pharmacology of extracts from the sea-cucumber *Stichopus badionotus* Selenka. Ten control animals were injected intrapleurally with 0.1ml carageenan (1%) and their pleural cavities were examined. Three groups (six animals per group) were treated with concentrations of 5, 10 and 20 mg/ml of the ethanol extract of sea-cucumber given i.p. and three other groups were treated with similar concentrations of a phosphate-buffer extract also given i.p. A half-hour later the animals were administered carageenan (i.p.). These animals were killed four-hours after the administration of carageenan and their pleural cavities were examined.
The results showed that the extracts were able to reduce pleural effusion, neutrophil counts, and also adenosine deaminase activity significantly and in a dose-dependent manner. The effects were more pronounced with ethanol extract compared to the phosphate-buffer extract.

Key words: Pleural effusion, carageenan , pleurisy, sea-cucumber

Introduction

In Malaysian waters some 25 species of sea-cucumber have been identified. Some species of the animal have been traditionally taken as food and some others are reported to be used for medicinal purposes. Traditionally, sea-cucumber extracts have been reported to be efficacious for wound-healing, such as in gastric ulceration, or when applied externally to skin lacerations. There are also reports of the use of extracts for treating asthmatics, worm infestation, back ache, high blood pressure, and for heightening the libido (Ridzwan *et al.*, 1990; Kaswandi *et al.*, 1993; Ridzwan *et al.*, 1995; Hassan *et al.*, 1996). Reports by Shimada (1969), Anisimov *et al.* (1980) and Kuznetsova *et al.* (1982) found that holothurin, a glycoside extracted from the sea-cucumber showed anti-fungal and anti-tumour activities while Zainuddin *et al.* (1986) reported that the sea-cucumber extract did not have phosphodiesterase inhibiting activity but have the ability to cause de-fusion of tetanic contraction when tested in the cat soleus muscle. In this preliminary study of the crude extract from one particular species, the pharmacological effects of the extract upon induced pleurisy will be studied. This is because its effectiveness in wound healing may involve an anti-inflammatory action. The animal model used is that of pleurisy in rats and several indicators of inflammation or cell damage such as the volume of pleural exudate, the presence of neutrophils and the activity of adenosine deaminase will be looked at.

Materials and Methods

Animals: Forty-six adult male Sprague-Dawley rats weighing between 200 and 300 gm were used.
Sea-cucumber of the species *Stichopus badionotus* Selenka were obtained from the waters of the coast of Kapas Island, in Trengganu state, Malaysia.

Preparation of extracts: The phosphate buffer extract was prepared according to the method of Yasumoto *et al.* (1967). After being sliced open and its internal organs removed, the body wall of the sea-cucumber was blended in phosphate buffer (in a volume = 4 x tissue weight) and extracted at room-temperature (27°C) with pH 7.2 for 5 hours. The filtrate was dried in freeze-drier and the resultant powdered extract was kept at −20 °C until used.
The ethanol extract was prepared according to the work of Shimada *et al.* (1969). The body wall of the sea-cucumber was blended with 95% ethanol (x 5 w/v) and extracted at 50°C for 5 hours. After drying the filtrate, 100ml of benzene was added and the whole was centrifuged at 2500 x g for 20 minutes. The benzene was afterwards removed and the residue was dried. The resultant dry ethanol-extract was taken and kept at −20 °C until used.

Administration of extracts: The phosphate-buffer or ethanol extracts were redissolved in normal saline concentrations of 5, 10 and 20 mg/ml. The extracts were administered by intraperitoneal injections in a volume of 2.0 ml/kg body weight.

Induction of pleurisy: Pleurisy was induced according to the method of Bullivant & Otterness (1988) as modified by Dr. Jalaluddin from the Anatomy Dept., UKM.
Carageenan was prepared as a 1% solution in sterile saline. All animals were injected with this solution intrapleurally half an hour after administration of the extract. Control animals were only injected with carageenan.

Differential count and leukocyte identification: The determination of leukocytes and the differential counts were carried out with the Coulter counter Cell-Dyn 3200SC.

Protein determination: Protein concentrations were determined using the method of Bradford (1976) at a wave-length of 595nm using the Shimadzu UV-160A spectrophotometer.

Determination of adenosine deaminase activity: Adenosine deaminase activity was determined according to the method of Giusti (1974).

Statistical methods: The results were analyzed using ANOVA and Student's t-test, where appropriate for differences between means of the untreated pleurisy-induced group and the groups treated with three different doses of the extracts. The level of significance for the t-test was $p < 0.05$.

Results and discussion

Table 1 shows that the three concentrations of ethanol extract were able to reduce the pleural exudate significantly ($p < 0.05$) and this happened in a dose-dependent manner. The phosphate-buffer extract at 5 and 20mg/ml was also effective significantly ($p < 0.05$). This anti-exudative effect may possibly be due to the effect of saponins known to occur in sea-cucumber extracts. The presence of glycosides (holothurin) has also been reported by Shimada (1969); Anisimov *et al.* (1980) and also Kuznetsova *et al.* (1982) and it is possible that the glycoside holothurin in the ethanol extract is showing an anti-exudative effect since according to Vinegar *et al.* (1982) the early phase of pleural exudate formation is able to be blocked by steroidal anti-inflammatory drugs. According to Van Der Velden (1998), glucocorticoids has been shown to inhibit many inflammation-associated molecules such as cytokines, chemokines, arachidonic acid metabolites and adhesion molecules. Dexamethasone has been shown by Saleh *et al.* (1999) to be able to inhibit the cell migration and exudate formation during acute inflammatory phase of carageenan-induced inflammation.

Table 2 shows that the activity of adenosine deaminase was reduced significantly ($p < 0.05$), after treatment with each concentration of ethanol extract. As for the phosphate-buffer extract, only the concentration of 5mg/ml showed significant effect ($p < 0.05$) but the concentrations of 10 and 20mg/ml were less consistent in effect and just did not reach significance. Adenosine deaminase is a useful marker for cell damage. In many cases of inflammatory response of tissues, the activity of adenosine deaminase (ADA) will increase. This is due to the infiltration of lymphocytes into the inflamed area. Lymphocytes exhibit high ADA activity because part of their function would be to restore the damaged tissue or prevent further damage after an injury. Injury results in the release of adenosine from damaged cells which will cause an influx of calcium into the cell and can lead to cell toxicity (Thomas and Reed, 1989). There have been identified four adenosine receptors to date, namely A1, A2, A2b, and A3 (Olah and Stiles, 1995) and many physiological functions involving adenosine (Kanagawa *et al.*, 1997). Receptor A2 is believed to be involved in anti-inflammatory activity of adenosine (Cronstein *et al.*, 1993). The enzyme ADA will metabolize adenosine to inosine, hypoxanthine and other less harmful metabolites. In many diseases the activity of this enzyme would be high in the affected tissues ; for example in tuberculosis of the lungs, and in many cases of cancer (Van der Weyden and Kelly, 1976; Galanti *et al.*, 1981). The reduction of ADA activity seen in this study is a good indicator of a possible cytoprotective effect of the extract since by reducing the concentration of adenosine, this should lead to a reduction in calcium influx and less cell damage. There is a possibility that a cytoprotective agent exists in the extract of sea-cucumber.

Table 3 shows that the neutrophil count was also reduced by ethanol extract in a concentration dependent manner and was significant ($p < 0.05$) at 20mg/ml. Thus it seems that again this gives an indication of the existence in the ethanol extract of an anti-inflammatory substance because an early indicator of inflammation is the rise in neutrophil count (Davies, 1997). There was no observable effect with the phosphate-buffer

Table 1: The effect of the Ethanol and phosphate buffer extracts of *Stichopus badionotus* Selenka on pleural exudate (ml) of rats induced with pleurisy by carageenan given intrapleural.

Volume of pleural exudate in ml (mean ± s.e.m.)						
Carageenan-treated (controls)	Ethanol extract (mg/ml)			Phosphate-buffer extract (mg/ml)		
	5	10	20	5	10	20
	*	*	*	*		*
0.67 ± 0.11	0.29 ± 0.06	0.21 ± 0.04	0.0	0.30 ± 0.09	0.590.12	0.09 ± 0.03
(10)	(6)	(6)	(6)	(6)	(6)	(6)

* = significant at $p < 0.05$ when compared to control (carageenan-treated) (n) = number of animals in treatment group

Table 2: The effect of the Ethanol and phosphate buffer extracts of *Stichopus badionotus* Selenka on adenosine deaminase activity of pleural exudate of rats induced with pleurisy by carageenan given intrapleural.

Adenosine deaminase activity of pleural exudate in μmol/min/mg protein (mean ± s.e.m.)						
Carageenan-treated (controls)	Ethanol extract (mg/ml)			Phosphate-buffer extract (mg/ml)		
	5	10	20	5	10	20
	*	*	*	*		
271.18 ± 16.58	162.93 ± 14.13	96.78 ± 10.59	76.62 ± 13.72	133.24 ± 38.75	164.91 ± 42.46	145.70 ± 39.27
(10)	(6)	(6)	(6)	(6)	(6)	(6)

* = significant at $p < 0.05$ when compared to control (carageenan-treated) (n) = number of animals in treatment group

Table 3: The effect of the Ethanol and phosphate buffer extracts of *Stichopus badionotus* Selenka on neutrophil count $\times 10^6$ /ml of pleural exudate of rats induced with pleurisy by carageenan given intrapleural.

Neutrophil count $\times 10^6$ /ml in pleural exudate (mean ± s.e.m.)						
Carageenan-treated (controls)	Ethanol extract (mg/ml)			Phosphate-buffer extract (mg/ml)		
	5	10	20	5	10	20
			*			
4.41 ± 1.60	3.99 ± 1.66	1.75 ± 0.46	0.31 ± 0.08	1.99 ± 0.57	5.84 ± 1.06	2.52 ± 0.30
(10)	(6)	(6)	(6)	(6)	(6)	(6)

* = significant at $p < 0.05$ when compared to control (carageenan treated)
(n) = number of animals in treatment group

extract.

The study succeeded in showing the anti-exudative, anti-inflammatory, cytoprotective and anti neutrophil-migration effect in the ethanol extract of the sea-cucumber from the species *Stichopus badionotus* Selenka. It is very likely to be a moderately lipid soluble substance since similar effects were observed also with the phosphate buffer extract but in a less prominent manner.

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References

Anisimov, M. M., N.G. Prokofieva, L.Y. Korotkikh, I.I. Kapustina and V.A. Stonik, 1980. Comparative study of cytotoxic activity of triterpene glycosides from marine organisms. Toxicon., 18: 221-223.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding: Protein assay by dye-binding. Analy. Biochem., 72: 248-254.

Bullivant, M. L. and I.G. Otterness, 1988. Carageenan pleurisy. Methods in Enzymol., 62: 334-338.

Cronstein, B.N., D. Naime and E. Ostad, 1993. The anti inflammatory mechanism of methotrexate: increased adenosine release at inflamed sites diminishes leucocyte accumulation in an *In vivo* model of inflammation. J. Clin. Invest., 92: 2675 – 2682.

Davies, H., 1997. Introductory Immunology. London: Chapman & Hall.

Galanti, B., S. Nordiello, M. Russo and F. Florentino, 1981. Increased lymphocyte adenosine deaminase in typhoid fever. Scand. J. Infect. Dis., 13: 47-50.

Giusti, G., 1974. Adenosine deaminase. In: Bergmeyer, H. U. Methods In Enzymic Analysis. 1974. 2nd Ed. Wiehen: Verlag-Chemie.

Hassan, Y., N. Yeap, K.H. Kim and M.M. Shahimi, 1996. Effects of sea-cucumber water extract on systemic anaphylactic reaction. Malaysian J. Med. Sci. (Supp.)., 3: 37-38.

Kanagawa, H., H. Fujiwara, S. Shoji, T. Fujii, S. Kudoh, K. Hirata, N. Kurihara and J. Yoshikawa, 1997. Adenosine modulates endothelin-induced brobchoconstriction in guinea-pig airway. Int. Arch. Allergy & Immunol., 112: 83-87.

Kaswandi, M. A., B. Safran, M.B.O. Rozali and B.H. Ridzwan, 1993. Kesan-kesan fisiologi ekstrak kasar saponin daripada empat spesies timun-laut. Prosiding Simposium Sumber Alam Tropika. UNIMAS, 6-11.

Kuznetsova, T. A., M.M. Anisimov, A. M. Popov, S. I. Baranova, Sh. Afiyatullov, I. I. Kapustina, A. S. Antonov and G.B. Elyakov, 1982. A comparative study in vitro of physiological activity of triterpene glycosides of marine invertebrates of echinoderm type. Comp. Physiol., 73: 41-43.

Olah, M.E. and G. I. Stiles, 1995. Adenosine receptor subtypes: Characterization and therapeutic regulation. Ann. Rev. Pharmacol. Toxicol., 35: 581 – 606.

Ridzwan, B. H., M. A. Kaswandi, Y. Azman and M. Fuad, 1995. Screening for antibacterial agents in three species of sea-cucumbers from coastal areas of Sabah. Gen. Pharm., 26: 1539-1543.

Ridzwan, B. H., B. I. Mohd. Nor , B. I. Nor Hayati , M. A. Kaswandi and M.B.O. Rozali, 1990. Timun laut (holothuroid) Sabah: Jus kasar beberapa spesies sebagai pemudah penyembuh luka aruhan. 7th National Seminar on Natural Products. USM & Inst. Kimia Malaysia. (Utara), 27-28: 207-209.

Saleh, T.S.F., J.B. Calixto and Y.S. Medeiros, 1999. Effects of anti-inflammatory drugs upon nitrate and myeloperoxidase levels in the mouse pleurisy induced by carageenan. Peptides, 20: 949 – 956.

Shimada, S., 1969. Antifungal steroid glycoside from sea-cucumber. Sci., 163: 1462-1471.

Thomas, C.E. and D. J. Reed, 1989. Current status of calcium in hepatocellular injury. Hepatol., 10 : 375 – 384.

Van Der Velden, V.H.J., 1998. Glucocorticoids: mechanism of action and anti-inflammatory potential in asthma. Mediators Inflamm., 7: 229 – 237.

Van der Weyden, M. B. and W. N. Kelly, 1976. Human adenosine deaminase distribution and properties. J. Biol. Chem., 251: 5448-5456.

Vinegar,.R., J. F. Truax, J. L. Selph and F. A. Voelker, 1982. Pathway of onset, development and decay of carageenan pleurisy in the rat. Fed. Proc., 41: 2588-2595.

Yasumoto, T., K. Nakamura and Y. Hashimoto, 1967. A new saponin, holothurin B, isolated from sea-cucumber *Holothuria vagabonda* and *Holothuria lubrica*. Agric. Biol. Chem., 31: 7-10.

Zainuddin, M., Y. T. Chua, S. Z. Idid and M. Paden, 1986. Air gamat (sea-cucumber (holothurian extract) does not have β-adrenoceptor agonist or phosphodiesterase inhibiting properties. J. Perubatan UKM., 8: 41-48.