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CURCUMIN, GARCINOL AND DIETARY N-3 FATTY ACIDS, LOWER THE RELEASE OF LYSOSOMAL ENZYMES IN RAT PERITONEAL MACROPHAGES.

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ABSTRACT

Male Wistar rats (12rats/group) were fed a diet containing 8 wt % coconut oil or groundnut oil or cod-liver oil for a total period of 8 weeks. The diets were also supplemented with 2 wt % groundnut oil for providing essential fatty acids. During the last 2 weeks, 6 rats form each group were additionally given curcumin (30 mg/kg body wt/day) or garcinol (5 mg/kg body wt/day) in 1 ml groundnut oil. The peritoneal macrophages from rats fed cod-liver oil diet secreted lower levels of lysosomal enzymes collagenase, elastase and hyaluronidase as compared to those from rats fed coconut oil or groundnut oil diets. Curcumin and garcinol significantly lowered the secretion of these lysosomal enzymes from macrophages in animals given coconut oil or groundnut oil diet. These studies indicated that dietary cod-liver oil (rich in n-3 fatty acids), and spice principles curcumin and garcinol can lower the secretory functions of macrophages in a beneficial manner. (Mol Cell Biochem 203:153–161, 2000)

KEYWORDS: macrophages, cod-liver oil, curcumin, garcinol, lysosomal enzymes.

INTRODUCTION

The macrophages are unique type of mononuclear cells which respond to different stimuli encountered in their environment by multiple inductive mechanisms. They function as phagocytic cells, being mainly involved in the disposal of unwanted materials such as tissue debris, bacteria and immune complexes and also function as secretory cells, of inflammatory

mediators, accessory cells for processing and presentation of antigens to the immune system, regulatory cells in the proliferation of other cell types and lytic cells of neoplastic tumor cells. Macrophages play a pivotal role in the initiation as well as progression of inflammation through the release of mediators as well as influencing functions of other immune cells. Potent mediators released by the macrophages include ROS, metabolites of arachidonic acid, lysosomal enzymes and other biochemical molecules. Reactive oxygen species (ROS) possess microbial functions, whereas prostaglandins are hyperalgesic, pyrogenic and vasodilatory agents causing pain, heat and redness and leukotrienes are potent chemo tactic agents, also stimulating other phagocytes at the site of inflammation. The hydrolytic enzymes collagenase, elastase and hyaluronidase released also help in fighting infection. Thus, although it is evident that release of the inflammatory mediators is a helpful function of macrophages to fight infection, prolonged uncontrolled release can result in pathological conditions leading to auto inflammatory diseases such as Arthritis.

Rheumatoid arthritis is an autoimmune disorder characterized by chronic inflammation in the joints[1,2] caused by number of proinflammatroy molecules released by macrophages.[3] These include the reactive oxygen species, lysosomal enzymes such as collagenase, elastase, hyaluronidase.[4–6] The regulation of these mediators secreted by macrophages and other immune cells therefore may control the chronic inflammatory conditions.[6, 7] Nutritional factors are known to modulate inflammatory responses.[8] Dietary n-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid and docosahexaenoic acid are lowered iron induced toxicity and lipid peroxidation in rats.[12] We have also demonstrated that macrophages treated *in vitro* with curcumin and garcinol secreted lower amounts of lysosomal enzymes when the cells were activated with zymosan.[13] The present investigation was undertaken to evaluate the effects of dietary n-3 PUFA, curcumin and garcinol on the secretion of lysosomal enzymes.

Spice principles such as curcumin (from turmeric) and garcinol (from Garcinia indica) which are widely used in Asian diets as food additives are also reported to exhibit anti-inflammatory and anti-arthritic properties.[10] Dietary curcumin and eugenol (from cloves) have been shown to lower carrageenan induced paw edema in rats.[9] However the biochemical mechanisms underlying the observed anti-inflammatory effects of dietary curcumin and garcinol along with n-3 PUFA have not received much attention. Our earlier studies have shown that feeding

curcumin (30 mg/kg body wt/day) and garcinol (5mg/kg/body wt/day) to rats for 15 days lowered the generation of reactive oxygen species by macrophages.[11]

MATERIALS AND METHODS

Dulbecco's modified eagle's medium (DMEM), phorbol myristate acetate (PMA), calcium ionophore A23187, Zymosan A, fatty acid free bovine serum albumin (BSA), indomethacin, N- acetyl glucosamine, collagenase (Type VII from clostridium histolyticum), elastase (From bovine pancreas), hyaluronidase (from bovine testes), hyaluronic acid, Dextran T-70, were obtained from Shree Venkateshwara Chemical Co., Bangalore, India. Natural Garcinol was from Karwar,.India Curcumin (99% pure) was purchased from Flavours and Essences, Bangalore, India. All other chemicals and solvents used were of the analytical grade. Medicinal grade cod-liver oil.Refined coconut oil and groundnut oil were purchased from the local market.

Animals

Male Wistar rats weighing approximately 75–80 g were used in these studies.

Dietary study

Rats (75–80 g) were fed AIN-76 diets[14] containing 8 wt % coconut oil or groundnut oil or cod-liver oil.Two wt % groundnut oil was added to each of the diets to provide essential fatty acids. The basal composition of the diet was as follows: Sucrose [60%], Casein [20%]. Cellulose [5%], AIN 76 mineral mix [3.5%], AIN 76 vitamin mix [1%],methionine [0.3%] and choline chloride [0.2%]. Fresh diets [20 g/day] were fed to rats daily. The fatty acid compositions of dietary lipids are given in the Table 1. After 6 weeks, 6 rats from each group were additionally given curcumin (30 mg/kg body wt/day) or garcinol (5 mg/kg body wt/day) by gavage in 1 ml of groundnut oil for 15 days.[11] The control animals received 1 ml of the groundnut oil. Animals had free access to food and water all the times. Animals were housed in the approved, modern animal house facility at Bangalore University Institute, Bangalore, India

Isolation of peritoneal macrophages

Macrophages were isolated from the rat peritoneal exudates in Hank's balanced salt solution [HBSS] as described previously.[11]

Fatty acid analysis

Total lipids were extracted from macrophages by Bligh and Dyer's procedure.[15] Phospholipids were separated by thin layer chromatography using chloroform: methanol (8:1 v/v). Fatty acids from phospholipids were saponified and methylated with BF3/methanol.[16]

Lysosomal enzyme activities

Macrophage monolayers (2.5 ´ 106 cells) were incubated in 1 ml of HBSS containing 500 µg of zymosan A for 18–72 h. The hydrolytic enzymes collagenase, elastase and hyaluronidase released by macrophages in the culture supernatants were measured.

Collagenase activity

Collagenase activity was measured colorimetrically at 365 mm using the synthetic substrate DNP peptide III (DNP-pro-Gln-Gly-Ile-Ala-Gly-Gln-D-Arg) as described by Nagai *et al*.[17] The amount of DNP-peptide hydrolysed was quantitated using a molar extinction coefficient of 1.49. Collagenase activity is expressed as µM/mg protein.

Elastase activity

The activity of elastase was assayed at 405 nm as described by Kawabata *et al*. using the synthetic substrate Suc-Ala-Pro-Ala-pNA in 0.1 M Tris-HCl buffer (pH 5.0).[18] One unit of elastase activity was defined as the quantity of enzyme that liberates 1 µmol of p-nitroanilide in 60 min.

Hyaluronidase activity

Hyaluronidase activity in the culture supernatant was determined by the amount of N-acetyl glucosamine released from hyaluronic acid.[19]

Protein estimation

Total protein was estimated by the method of Sedmak and Grossberg using bovine serum albumin as reference standard.[23] Macrophage protein was quantified after digesting the cells in 1 ml of 1N NaOH overnight

Statistical analysis

The data were statistically analysed by one way analysis of variance (ANOVA) and multiple pair wise comparisons were done using Tukey test. Additionally, two ways ANOVA and multiple comparisons by Dunnetts method were also carried out using the SIGMASTAT (version 2.0) Software. A p value of less than 0.05 was considered significant.[24]

RESULTS

The amount of food consumed (18.2 ± 1.6 g/day/rat) and the net gain in body wt (236 ± 18 g, combined mean ± S.D., n = 6 rats/group) were comparable between rats given different dietary lipids and with or without spice principle supplements.

The release of lysosomal enzymes

Dietary lipids differentially influenced the secretion of lysosomal enzymes by macrophages (Table 3). Macrophages from rats fed coconut oil and groundnut oil secreted comparable levels of collagenase, and elastase at 18 h of incubation. However, the release of collagenase was significantly reduced by 34, 27 and 20% at 24, 48 and 72 h in macrophages from rats fed cod-liver oil compared to that released from rats fed coconut oil (Table 2). Similarly, elastase secretion was lower by 46, 17 and 11% in macrophages from rats fed cod-liver oil compared to that released by rats fed coconut oil at the end of 24, 48 h and 72 h of incubation periods respectively (Table 2). The secretion of hyaluronidase by macrophages from rats fed cod-liver oil was lower by 53 and 41% at 48 and 72 h of incubation as compared to that observed in macrophages from rats fed coconut oil diets (Table 2). However there was no significant difference in the secretion of hyaluronidase at 24, 48 and 72 h of incubation in macrophages from rats fed coconut oil and groundnut oil diets (Table 2).Feeding curcumin and garcinol lowered the release of hydrolytic enzymes for up to 24 h in macrophages (Table 2).Feeding curcumin to rats on coconut oil diets lowered the collagenase secretion in macrophages by 84% at 18 h and 36% at 24 h of incubation with zymosan as compared to that found in rats fed coconut oil alone. However, beyond 24 h, there was no effect of spice principles on the secretion of collagenase by macrophages. Feeding capsaicin also lowered the collagenase activity only at 18 and 24 h of incubation by 81 and 45% respectively. Similarly, curcumin or garcinol lowered collagenase secretion in rats fed groundnut oil. At 18 h of incubation macrophages from curcumin and capsaicin fed animals secreted 45 and 31% lower levels of collagenase compared to the macrophages from rats fed groundnut oil alone. The collagenase secretion at 24 h was reduced by 41 and 30% respectively in macrophages from rats fed curcumin and garcinol. Beyond 24 h, there were no differences in the secretion of collagenase by macrophages. Dietary curcumin or garcinol in cod-liver oil fed rats did not influence the release of collagenase, elastase and hyaluronidase compared to that released from rats fed cod-liver oil alone (Table 2).

Table 1. Fatty acid composition of dietary lipids

Fatty acid	Coconut oil diet (CO)	Groundnut oil diet(GNO)	Cod liver oil diet(CLO)
		Mol%	
10:00	10	ND	ND
12:00	34.41	ND	ND
14:00	21.9	1.04	1.85
16:00	10.19	18.15	17.29
16:01	ND	ND	14.92
18:00	3.95	3.19	3.85
18:01	10.2	41.43	29.61
18:2 n-6	9.15	34.65	10.19
18:03	ND	0.39	2.15
20:4 n-6	ND	1.15	0.59
20:5 n-3	ND	ND	8.59
22:01	ND	ND	3.04
22:6 n-3	ND	ND	7.92
Saturated fatty acids(S)	80.65	22.38	22.99
Polysaturated fatty acids(P)	9.15	35.8	32.48
P/S ratio	0.11	1.6	1.41

ND – Not detected; Addition of curcumin or garcinol did not alter fatty acid composition of the diet.

Table 2. Effect of dietary lipids and spice principles on the release of lysosomal enzymes by macrophages

Time of incubation (h)	Coconut oil (CO)	CO+Curcumin	CO+Garcinol	Groundnut oil (GNO)	GNO+Curcumin	GNO+Garcinol	Cod liver oil (CLO)	CLO+Curcumin	CLO+Garcinol
Collagenase (μM/mg protein)									
18	0.26a ± 0.020	0.04b ± 0.003	0.05b ± 0.009	0.26a ± 0.002	0.144c ± 0.05	0.18c ± 0.006	0.23a ± 0.002	0.22a ± 0.002	0.2a ± 0.003
24	0.47a ± 0.0009	0.30b ± 0.063	0.26b ± 0.009	0.46a ± 0.003	0.273b ± 0.010	0.32b ± 0.023	0.26b ± 0.030	0.26b ± 0.030	0.26b ± 0.260
48	0.93a ± 0.012	0.86a ± 0.096	0.87a ± 0.006	0.89a ± 0.009	0.92a ± 0.010	0.92a ± 0.010	0.69b ± 0.036	0.69b ± 0.036	0.76c ± 0.009
72	2.11a ± 0.360	2.10a ± 0.950	2.03a ± 0.093	2.08a ± 0.063	2.07a ± 0.054	2.11a ± 0.103	1.68b ± 0.139	1.44b ± 0.193	1.81b ± 0.101
Elastase (μM/mg protein)									
18	9.11a ± 1.230	3.13b ± 1.360	2.85b ± 0.113	8.92a ± 0.139	3.76b ± 0.960	4.89b ± 0.960	4.89b ± 0.960	4.84b ± 0.390	4.63b ± 0.930
24	12.84a ± 1.390	7.95b ± 1.950	7.67b ± 0.930	13.41a ± 1.390	8.63b ± 1.360	8.08b ± 1.390	10.47c ± 1.190	9.55b ± 2.930	9.23b ± 1.380
48	15.96a ± 2.360	16.20a ± 2.360	14.61a ± 2.940	16.14a ± 1.290	16.37a ± 1.280	15.62a ± 1.260	13.16b ± 2.390	13.13b ± 1.960	13.19b ± 1.280
72	31.90a ± 0.950	30.08a ± 3.390	30.70a ± 1.360	30.38a ± 2.930	30.79a ± 2.630	30.43a ± 2.140	28.39a ± 2.940	27.80a ± 2.940	27.98a ± 1.430
Hyaluronidase (U/mg protein)									
18	1.58a ± 0.090	1.14b ± 0.007	0.97c ± 0.006	2.02d ± 0.093	1.14b ± 0.043	1.17b ± 0.046	1.13b ± 0.059	0.81c ± 0.009	0.98c ± 0.003
24	4.55a ± 0.630	3.66b ± 0.036	3.10c ± 0.088	4.32a ± 0.077	2.78d ± 0.093	2.94d ± 0.006	2.33e ± 0.034	2.35e ± 0.039	2.56e ± 0.083
48	6.27a ± 0.943	6.01a ± 0.830	5.94a ± 1.360	6.54a ± 1.110	6.61a ± 1.390	6.50a ± 1.360	2.96b ± 10.930	2.71b ± 0.990	3.04b ± 0.110
72	6.44a ± 1.360	6.49a ± 1.390	6.54a ± 0.960	6.79a ± 0.840	7.12a ± 0.630	6.99a ± 1.630	3.83b ± 0.930	3.25b ± 0.960	3.50b ± 0.830

Values are mean ± S.D. of 6 rats. Values with the same superscript in each row are not significantly different from each other ($p > 0.05$).

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