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Zerumbone's Effect on Major Histocompatibility Complex Type II Cells in Synovial Membrane of OsteoArthritic Joint

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Abstract: Effect of zerumbone on the infiltration major histocompatibility complex class II cells were evaluated in rats with collagen-induced osteoarthritis. The changes in the number of these cells were compared between the corn oil and the zerumbone treated group. The results showed that the number of MHC class II-immunoreactivity was significantly reduced in zerumbone treated group compared to the corn oil group and only type A cells were positive for MHC II staining. Therefore, present results suggest that zerumbone has the property of inhibiting the antigen presenting cells (type A) which is present during inflammation, thus reducing the inflammatory process in osteoarthritis.

Key words: *Zingiber zerumbet*, zerumbone, MHC II, arthritis, synoviocytes, rats

INTRODUCTION

Current treatment for osteoarthritis includes NSAIDs and dexamethasine. Sites of action of these compounds range from inhibition of enzymes responsible for production of arachdonic acid metabolites to inhibition of cytokine expression. However, these compounds tend to have adverse site effects, which maybe ameliorated by the use of botanical alternatives. Extracts from medicinal plants have been reported to have effect as an anti-inflammatory, anti-oxidant, anti-thrombotic and anti-cancer agent (Tyler, 2000; Ojewole, 2006; Surh, 2002). Zerumbone is one such candidate.

Zerumbone is the crystalline sesquiterpene derived from a subtropical ginger, *Zingiber zerumbet* and has been found to exhibit antiproliferative and anti-inflammatory activities (Huang *et al*., 2005; Takada *et al*., 2005). However, the underlying molecular mechanism in these activities is still poorly understood. Therefore, many works are being done on zerumbone to further study the mechanism of its action. Zerumbone has recently been used successfully *in vitro* as an antitumour drug (Kirana *et al*., 2003) and it inhibits the synthesis of iNOS and cyclooxygenase 2 (Murakami and Ohigashi, 2006; Murakami *et al*., 2004). Due to zerumbone's anti-inflammatory and antioxidant properties the current work was conducted to evaluate the effect of zerumbone on major histocompatibility complex type II (MHC II) expression in the synovial membrane in rats with collagen-induced osteoarthritis.

Synoviocytes, in particular the fibroblast-like synoviocytes has been associated with joint inflammation and destruction (Mor *et al*., 2005; Meinecke *et al*., 2005). Studies in human and canine rheumatoid arthritis showed that the synoviocytes can function as Antigen Presenting Cells (APCs) [MHC positive cells] and are able to present peptides derived from autoantigens found within joint tissues (Tran *et al*., 2007; Trautwein *et al*., 1999). MHC II molecules recognize peptides that are derived from proteins degraded in intracellular vesicles. The MHC II and peptide form a complex that is exposed on the surface of antigen presenting cells, which is then recognized by T-helper cells.

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A number of studies have shown that MHC II positive cells increase in the synovial membrane in human arthritis, where they play a major role in arthritic changes by activating T-lymphocytes in the synovial membrane. Therefore, these APCs cells are found in patients with OA and RA and suppression of inflammation in OA reduces the infiltration of these cells. The purpose of this research is to study the effect of zerumbone on MHC II expression in the synovial membrane of rat OA.

MATERIALS AND METHODS

Animals and Treatment

Thirty eight adult Sprague Dawley rats were used in this study. All animals will have free access to water and pellet food. The study was conducted in April 2007 at the Faculty of Veterinary Medicine, Universiti Putra Malaysia. Osteoarthritis was induced by injecting collagenase into the right stifle joint at D1 and at D4 (approved by animal ethic committee animal utilization protocol No. 07R7). The rats were supplemented orally with either 0.5 mL corn oil (control group; n = 19) or 0.5 mL of 0.02% w/v zerumbone in corn oil (experimental group; n = 19) from D7-D28. At D29 the rats were sacrificed and synovial membrane samples from the right stifle joint and contralateral joint (left stifle joint) were processed for immunohistochemistry work.

Light Microcopy Immunohistochemistry

The synovial membrane samples were fixed in 4% paraformaldehyde overnight and then processed following a standard immunohistochemistry method. Briefly the samples were frozen in liquid nitrogen cooled in isopenthane and sectioned at 6 μm. The sections were dehydrated in ethanol, rinsed in 0.1 M Phosphate Buffered Saline (PBS) and then incubated in monoclonal antisera against MHC II (a kind gift from Dr. C. Vaillant, University of Liverpool), to mark the antigen presenting cells. Sections were then incubated in secondary antiserum and subsequently with avidin-biotinylated HRP complex. Samples were rinsed in PBS after each step. Finally, sections were immersed in glucose diaminobenzidine nickel substrate, washed in distilled water and stained with hematoxyline.

Preembedding Electron Microscopy Immunohistochemistry

Electron microscopic technique was used to study the type of synovial cells that express MHC II. A pre-embedding technique was used. Briefly samples were dehydrated in 50% ethanol for 30 min, then in PBS before immersing in 1% sodium borohydride ($NaBH_4$) in PBS for 40 min. Incubation in primary, secondary and tertiary were the same as the light microscopy staining technique but for a longer period of time. Samples were post-fixed in 1% osmium tetraoxide (OsO_4) in 0.1 M sodium cacodylate [$(CH_3)_2As(O)O.Na.xH_2O$)] buffer for 1 h, then dehydrated in a varying concentration of ethanol and then followed by acetone. The samples were then incubated in a mixture of 50% TAAB resin in acetone at room temperature, then in 100% TAAB resin for 5 h at room temperature for better penetration of the resin before polymerizing at 60°C overnight. Semithin sections were stained with toluidine blue and examined under the light microscope. Areas containing labelled cells were selected for ultrathin sectioning. These sections were examined under transmission electron microscope, stained with lead citrate ($C_{12}H_{10}O_{14}Pb_3$) for 5 min, rinsed in distilled water and then stained with uranyl acetate [$UO_2(CH_3COO)_2.2H_2O$)] for 5 min before rinsing again in distilled water. Finally sections were examined under transmission electron microscope.

Statistical Analysis

ANOVA was used to analyse the data.

RESULTS

The synovial membrane of control group (corn oil) joints exhibited varying degrees of mononuclear cell infiltration (Fig. 1-4). The synovial cell hyperplasia with 3-4 lining cells was observed. However, inflammatory changes in zerumbone treated group were less prominent than corn oil group.

Staining for MHC class II antigens, revealed that most of the synovial lining cells in the corn oil treated were positive for MHC II. In the zerumbone treated group, the numbers of MHC II + cells were significantly lower compared to the corn oil treated group (Table 1). When compared to the synovial membrane samples from the contralateral (left stifle) joint, the MHC II + cells were significantly lower for both corn oil and zerumbone treated groups (Table 1).

In the synovial membrane from zerumbone treated group, there was a marked decrease in the number of lining cells but in the corn oil treated joints, there was a marked increase in the number of synovial lining cells and virtually all the cells stained positive for MHC II (Fig. 1). In the zrumbone treated synovial membrane, the proportion of lining cells stained varied between different regions of the sections. In some regions the majority of lining cells showed positive labeling for MHC II, whereas in other regions only the outer layer of cells was positive. Furthermore in the subintimal layer of

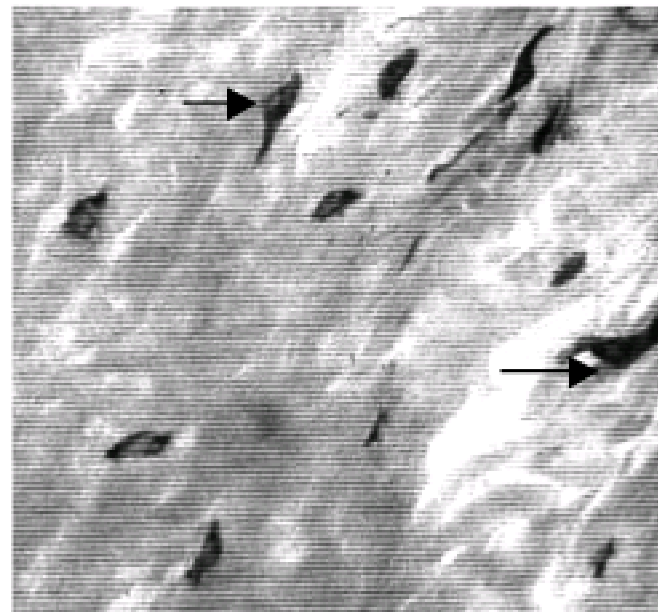

Fig. 1: Immunostaining for MHC class II in synovial membrane obtained from zerumbone (A) treated group. There were very few number MHC II + cells. The MHC II+ cells in the subintimal layer had dendritic cell morphology (→) (x670)

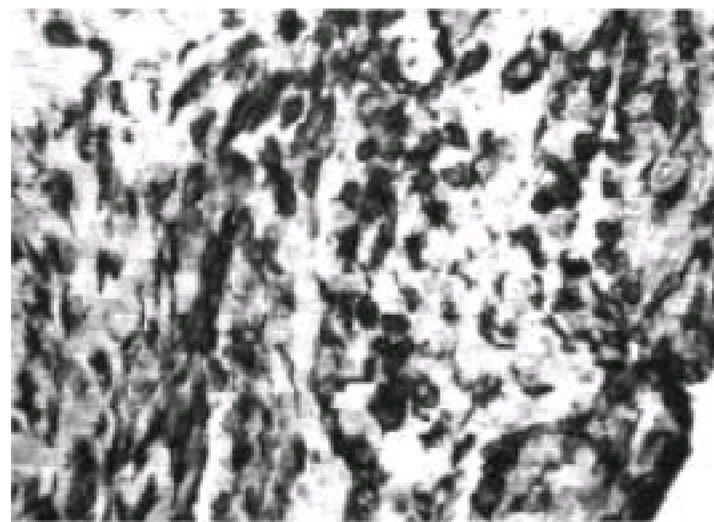

Fig. 2: Immunostaining of corn oil treated group. Hyperplasia of the lining cells is very clear and almost all of these cells are positive for MHC II (x670)

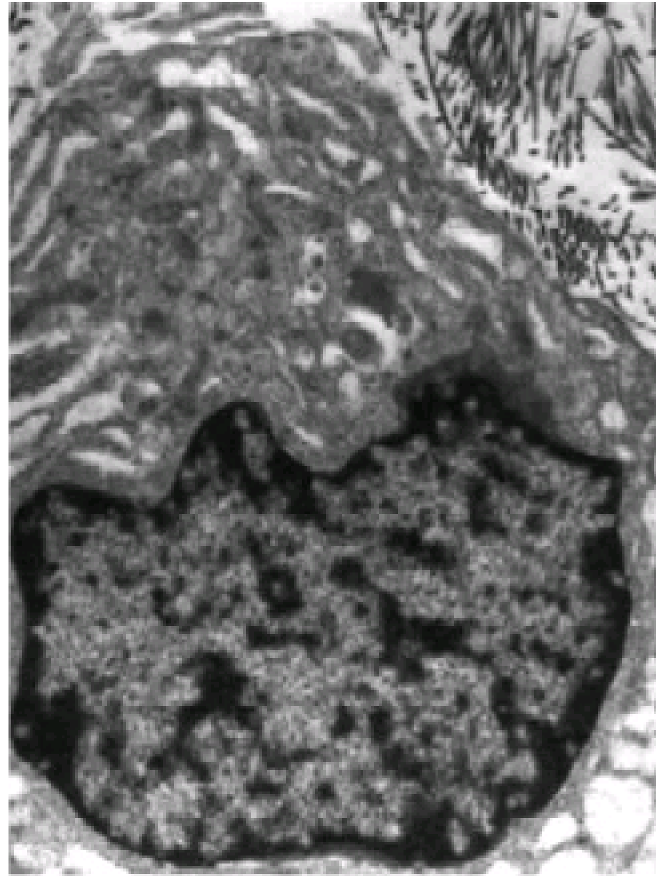

Fig. 3: Electron micrograph of rat type B cells with prominent rough endoplasmic reticulum (x25000)

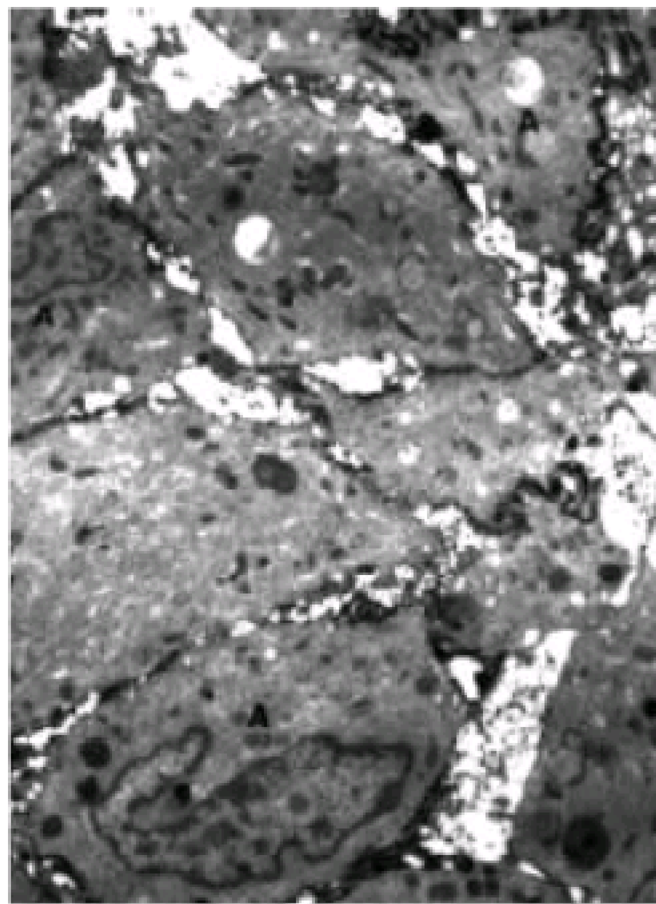

Fig. 4: MHC II labeling of rat synovial membrane from corn oil treated group. More type A cells are present. Only type A cells (A) are labelled for MHC II. Notice also that some of the type A cells are not labelled (x10000)

zerumbone and corn oil treated group, a small number of scattered cells were also positively stained (Fig. 2). The MHC II + cells decreased in number compared to the corn oil group. Most of the MHC II + scattered cells in the subintimal layers of both corn oil and zerumbone samples had dendritic cell morphology with spine-like projections.

Immunoperoxidase staining at the electron microscopy level, revealed two types of synovial cells: the type A cell which is phagocytic cell and type B cell which is a fibroblast-like cells (Fig. 3, 4).

Table 1: The number of MHC II+ cells in the synovial membrane from the corn oil (control) and zerumbone treated group

Treatments	Right stifle (induced)	Left stifle
Corn oil	218.05 ± 12.625^{ax}	18.79 ± 0.763^{bx}
Zerumbone	25.58 ± 1.0921^{ay}	16.21 ± 0.935^{by}

[ab]Mean with different superscript within the row are significantly different ($p<0.01$); [xy]Mean with different superscript within the column are significantly different ($p<0.01$)

The type A cell contained a prominent Golgi apparatus and some lysosomes but little rough endoplasmic reticulum, whereas the type B cells contained abundant rough endoplasmic reticulum (Fig. 3, 4).

In zerumbone treated samples, there were more type B than type A cells, whereas in the corn oil samples type A cells predominated. In this type A cells, there is an increase in the number of lysosomes compared to type A cells in zerumbone treated synovial membrane. More type A cells were observed in corn oil treated group compared to the zerumbone treated group. In both corn oil and zerumbone treated synovial membrane, only type A cells were MHC II positive. Type B cells did not show any positive labeling for MHC II (Fig. 3, 4).

DISCUSSION

The results of the current study showed that supplementation of zerumbone reduce the percentage of inflammatory cells in the synovial membrane of OA. This was clearly demonstrated by the reduction of MHC class II expressing cells in the synovial membrane of zerumbone treated group. The labelled cells in this study had the morphology of type A cells. Thus, type B cells were not induced to express MHC II. In man, type A cells are known to be antigen presenting cells (Burmester *et al*., 1983). The present study reports that rat type A cells express MHC II and are, therefore antigen presenting cells in this species. The number of MHC II expressing cells increased in synovial membrane from corn oil treated samples. In humans, an increase in MHC II (HLA-DR) expressing cells has been reported in synovial membrane from cases of osteoarthritis and rheumatoid arthritis (Thomas *et al*., 1994; Athanasou *et al*., 1988). A number of studies have shown that MHC II + cells increase in the synovial membrane in human rheumatoid arthritis where they play a major role in arthritic changes by activating T- lymphocytes in the synovial membrane (Trautwein *et al*., 1999; Kumar Bhardwaj, 1995; Klareskog, 1991; Athanasou *et al*., 1988). The factors stimulating this increase are not known but the sensory neuropeptides, substance P is known to modulate synoviocyte function and exert chemotactic effects on antigen presenting cells (Shanthi *et al*., 2004; Matucci Cerinic *et al*., 1995).

The current study also shows that zerumbone treatment reduces the number of MHC II expressing cells in the synovial membrane and thus, reduces the number of type A cells which are present during arthritis. The reduction of these cells clearly demonstrates that zerumbone does have some anti-inflammatory effect that acts on these cells that are present during inflammation.

The anti-inflammatory action of zerumbone has been claimed to be the basis of a positive effect on symptoms in rheumatic disorders (Srivasta and Mustafa, 1992). However, the anti-inflammatory mechanism of action of zerumbone on the OA joint is beyond the scope of the current study but it may be due to high content of HMP which is a dual inhibitor of cyclooxygenase on 5-lipoxygenase which makes zerumbone even more interesting in the field of rheumatology (Murakami *et al*., 2002, 2005; Sharma *et al*., 1994).

The current research also showed a positive correlation between MHC class II cells and arthritis. This finding can be used to indicate inflammation in osteoarthritis. However, the 28 days periods therapy in this study might not have been sufficient for all effects of zerumbone to be discovered. Future studies might look into dose-response and duration of therapy.

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