近畿大学工学部研究報告　No.44, 2010年, pp.1-7
Research Reports of the Faculty of Engineering,
Kinki University No.44 2010, pp.1-7

Anti- inflammatory effects of Volatile Compound of Macrophage Migration Inhibitory Factor (MIF) Induction of Inflammatory in Macrophage Cell

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SUMMARY

Macrophage migration inhibitory factor (MIF) plays an important role in systemic local inflammatory and immune responses. Tumor necrosis factor-α (TNF-α)are major proinflammatory cytokines and is mainly products by monocytes and macrophages, and inducing the synthesis and release of many inflammatory mediators. It is involved in immune regulation, autoimmune diseases, and inflammation. Moreover, MIF is a counter regulator against corticosteroids. We previously assay that synthetic surfactant to inhibition of releases of proinflammatroy cytokines and then found the important characterization of releases of MIF. In this study, we tested to proinflammatory cytokines suppression of volatile compound to macrophage cells inducing MIF. As a result, carvone, eucarvone, karahanaenone, hinokitiol and α-camphorenaldhyde were inhibition to release of TNF-α from MIF-stimulated RAW 264.7 cells at concentration of 10μg/ml, respectively. Moreover, these compounds did not inhibit the release of TNF-α in THP-1 cells inducing LPS at tested concentrations. This result suggested that a specific pharmacological action should be involved in the inhibitory activity of these compounds. It seems that the compounds are direct acting MIF signal in cell.

Keyword : proinflammatory cytokines, suppression, volatile compounds, macrophage cell, macrophage migration inhibitory factor

INTRODUCTION

Macrophage migration inhibitory factor (MIF) also plays an important role in systemic as well as local inflammatory and immune responses[1-3]. MIF was first identified as T cell-drived factor that inhibited the random migration of macrophages *in vitro*[4),5)]. MIF was promoted to produce cytokine such as tumor necrosis factor (TNF)-α that induced a various inflammatory diseases. Tumor necrosis factor-α (TNF-α) was

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major proinflammatory cytokines that induce the synthesis and release of many inflammatory mediators. Recently, it is regarded that anti-MIF antibody therapy would be a possible tool for treatment of these immune diseases. However, there is no report of the compound which controls the inflammation caused by MIF.

Terpenoids extracted from plants are a valuable source of a vast array of bioactive lead structures from more potent and less toxic drugs[6]. For these reasons, many researchers have assayed to screen novel biological compounds from various plant sources including medicinal compounds. It was previously[7] reported that synthetic surfactant inhibited TNF-α production without MIF effect in lipopolysacadide (LPS)-stimulated human monocytes. In this study, we investigated the anti-inflammatory activity of terpenoid and assayed the reaction of the proinflammatory cytokines TNF-α which they secretes in response to MIF, using RAW264.7 cells in vitro.

Materials and Methods

Murine macrophage cell line RA-W264.7 cell and human monocyte cell line THP-1 cells were obtained from the American Type Culture Collection. Dulbecc's modified Eagle's minimal essential medium (DMEM) and RPMI-1640 medium were purchased from GIBCO (NY, USA), fetal calf serum (FCS) was obtained from GIBCO (NY, USA). FCS used was heat-inactivated. Phorbol myristate acetate (PMA) and lipo-polysaccharide (LPS) (*E.coli*.055:55) were purchased from Sigma (MO, USA). Recombinant mouse MIF (rmMIF) was obtained by the met-hod described. TNF-α ELISA kit was purchased from Genzyme Techne (MA,USA). All other chemicals were of reagent grade.

Carvone [1] was purchased from Sigma Aldrich. Eucarvone [2], was prepared to previous report[8]. Karahanaenone [3] and eucarvone alcohol [8] was gift from Taiyo koryo Co., (Japan), and their derivatives amine [4], [5], [6], diol [7] were prepared as described in detail pre-viously[9),10]. Hinokitiol [9] was purched from Wako Chemical Co., (Japan). α-Camphorene aldhyde [10] and sesquikarahanaenone [11] were prepared to previous reported[11-14].

Cell Culture

RAW246.7 cells were culture in complete DMEM medium containing 10% heat − inactivated FCS, 1mM L-glutamine, 50unit/ml penicillin, 50μg/ml Streptomycin and 50μM 2-mercaptoethanol at 37°C in a moist 5% CO_2 incubator.

THP-1 cells were cultured in complete RPMI-1640 medium supplemented with 10% heat−assay inactivated FCS, 1mM L-glutamine, 50unit/ml penicillin, 50μg/ml streptomycin and 50μM 2-mercaptoethanol at 37°C in a moist 5% CO_2 incubator.

Assays were performed at a density of 10^7 cells/ml.

Cytokine Assays

RAW264.7 cells were suspended in complete DMEM mediume.

In vitro cultivation was done in triplicate over 24h at 37°C in a humidified atmosphere containing 5% CO_2, under the following culture conditions: Unstimulated RAW264.7 cells were

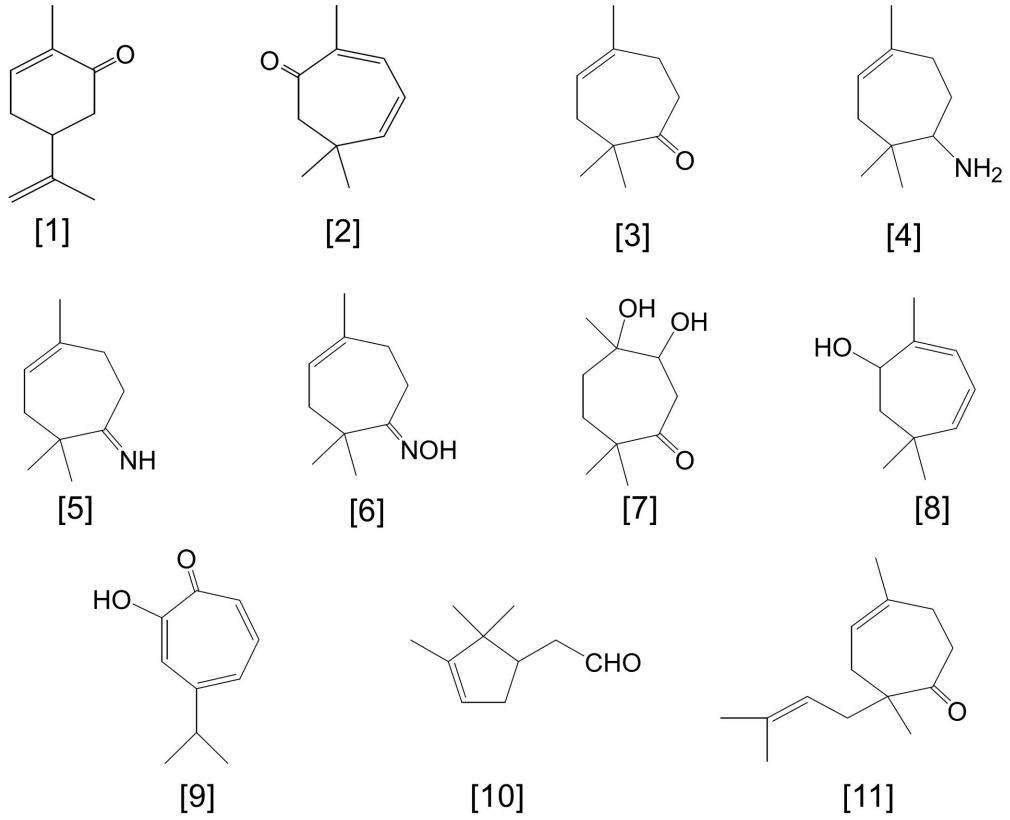

Fig.1 Chemical stracture of various compounds

cultivated in complete DMEM. In contrast, RAW264.7 cells were treated with rmMIF (100-1000ng/ml) for 48h. THP-1 cells were suspended in complete RPMI-1640 medium. In vitro cultivation was done in triplicate over 24h at 37°C in a hum idified atmosphere containing 5% CO_2, under the following culture conditions: Unstimulated THP-1 cells were cultivated in complete RPMI-1640. In contrast, THP-1 cells were pretreated with PMA (10nM) for 6h. After incubation, culture medium was removed and cells were washed three times with RPMI-1640 without serum. Pretreated cells were stimulated with either LPS at a concentration of 10ng/ml, or by LPS combined with tested compounds at 100ng/ml. Cultures were incubated for 24h at 37°C.

The compounds solubilized with vechicle (dimethyl sulfoxide) were diluted with complete DMEM or RPMI-1640. The final concentration of vechicle never exceeded 0.1% in the culture mediume, respectively. After incubation, supernatants were collected and assayed for TNF- α by commercial enzymed linked immuneosorbent assay (ELISA) kits.

Results

Release of TNF- α in MIF stimulated RAW264.7 cell

The results of release condition are shown in Fig. 2 and 3. MIF made TNF- α secretion of RAW

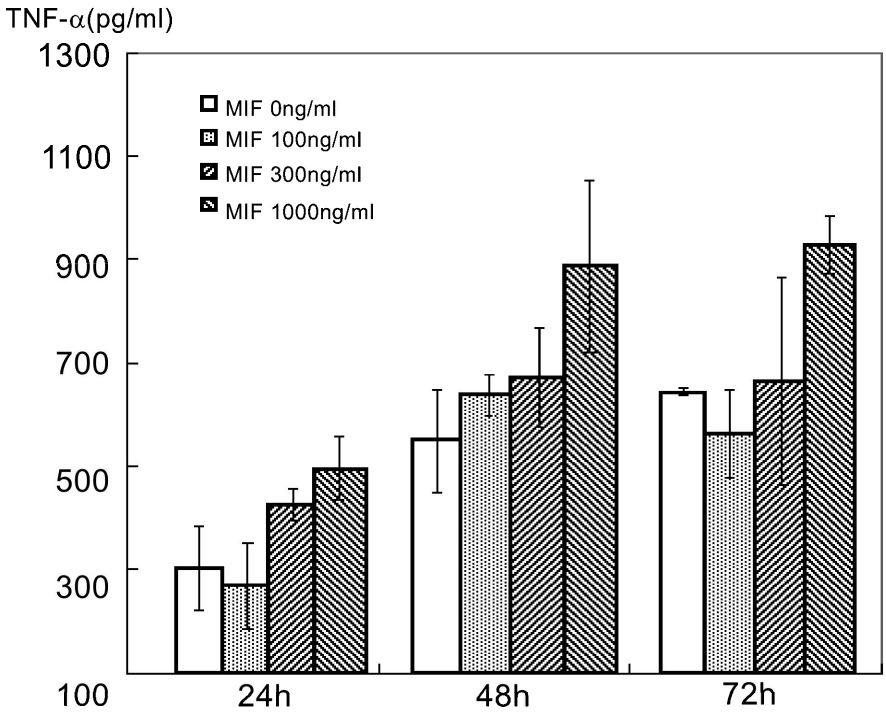

Fig.2 Effects of the production of MIF-stimulated TNF-α by RAW264.7cells. rmMIF was tested at concentration of 100-1000 ng/ml. Conditions for cultures are described under Materials and Methods. Data are given as mean ±SD(n = 3).

264.7 cell increase in dose dependent manner (100-1000ng/ml). The release of TNF-α increased in time dependence (24-72h). The production RAW 264.7 cells were suppression by anti MIF antibody at 1000ng/ml in cultured for 48h.

Effect of TNF-α release in MIF-stimulated RAW264.7 cell.

The chemical structures of a various compounds are shown in Fig. 1 and the results of inhibition are shown in Table 1. The compounds controlled the TNF-α release by MIF stimulus which used RAW264.7 cell.

Namely, [4], [7], [10] and [11] were suppression of a percent of inhibition to 51.2, 70.5, 68.9 and 67.8%, respectability.

However, [6] was did not effect to release of TNF-α in MIF-stimulated RAW264.7 cell. The compound which activity was the highest was [7] and a percent of inhibition was 70.5%. On the other hand, the compound which activity was the weakest was [8] and the value was 19.8%. In addition, the six member of carvon [1] was suppression of a percent inhibition to 37.1%.

Effect of TNF-α released in LPS-stimulated THP-1 cell

Each compounds did not affect the TNF-α release by LPS stimulus which used THP-1 cell. Compound [7], [8] and [11] were suppression of a percent of inhibition to 28.1, 18.1 and 16.9%. Compound [1], [3], [4] and [10] were also weak activity. The percent of inhibition to 7.5, 8.4, 4.6 and 9.5%, respectability.

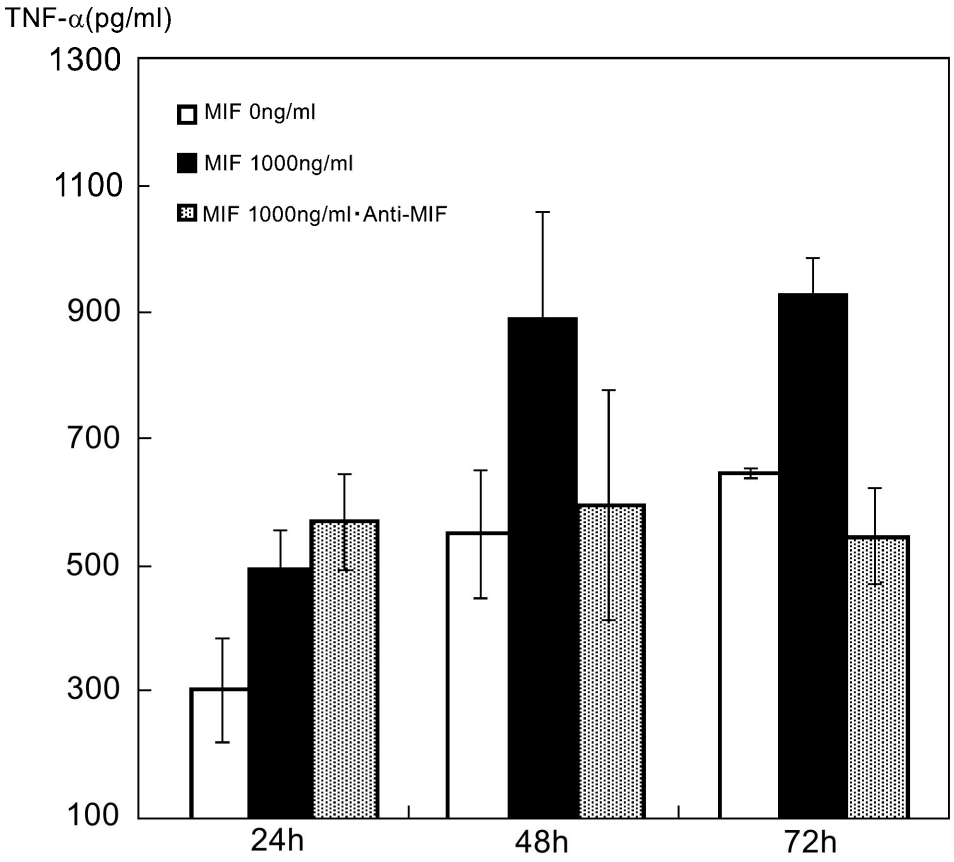

Fig.3 Effects of Anti-MIF antibody on the production of MIF-Stimulated TNF-α by RAW264.7 cells. Anti-MIF antibody was tested at concentrateons of 10 μg/ml. Conditions for cultures are described under Materials and Methods. Data are given as mean ± SD(n = 3).

Discussion

The focus of this study was to research of MIF interaction of anti-inflammatory activity. The purpose of present study was to research of anti-inflammatory activity of compounds from natural product. In this experiment, we researched to release condition of cytokines in both of RAW264.7 and THP-1 cell by a various stimuli. In an attempt to research that the parameter of anti-inflammation activity constructs as TNF- α the evaluation, which used RAW264.7 cell and stimulus used MIF. Moreover, it was assay to release of TNF- α inducing LPS in THP-1 cells. We first examined experiment conditions and confirmed the increase in TNF- α in RAW264.7 cell by MIF stimulus. In THP-1 cell, according to modified previously reported.

In these compounds, the release of TNF-α increased by MIF stimulus as compared with control. Eucarvon, karahanaenone and hinokitiol are having a unique seven member skeleton. α-Camphlene aldhyde is having a five member skeleton. There are no reports of the anti-inflammation activity that examined of TNF-α of these compounds. The compound which suppression of TNF-α release was the strongest was compound [7] in seven member.

Table 1 Effect of TNF-α products from MIF stimulated RAW264.7 cell and LPS-stimulated THP-1 cell

Compound	MIF-stimulation[a)] TNF-α (pg / mL)*	% of inhibition	LPS-stimulation[b)] TNF-α(pg / mL)*	% of inhibition
Control	1333.2 ± 256.4	0.0	258.9 ± 60.1	0.0
[1]	1034.0 ± 346.3	37.1	240.0 ± 14.9	7.5
[2]	1074.3 ± 202.5	32.1	286.1 ± 56	-10.7
[3]	1009.0 ± 210.9	29.1	237.6 ± 35.7	8.4
[4]	920.6 ± 91.3	51.2	247.3 ± 25.8	4.6
[5]	1013.0 ± 129.4	39.7	241.1 ± 23.0	7.0
[6]	1519.5 ± 378.9	-23.1	242.6 ± 37.4	6.4
[7]	765.4 ± 107.9	70.5	187.6 ± 56.4	28.1
[8]	1173.9 ± 610.4	19.8	212.9 ± 33.3	18.1
[9]	1038.4 ± 256.3	36.6	338.9 ± 84.1	-31.6
[10]	778.0 ± 163.6	68.9	234.9 ± 27.6	9.5
[11]	786.7 ± 130.9	67.8	216.1 ± 35.6	16.9

a) RAW264.7 cells were precultured for 6h at 37°C. After incubation, the mediume was removed and pretreated cells were cultured at 1×106 cell / mL with rmMIF (10 μg /mL) in the absence or presence of test compound (10 μM) for 48h. After that, supernatants were assayed for TNF-α rereased by unstimulated RAW264.7 cells was 527.6±109.6 (mean±S.D.) at 48h.

b) THP-1 cells were precultured for 6h at 37°C with PMA (10nM). After incubation, the mediume was removed and PMA-treated cells were cultured at 1×106 cell / mL with LPS (100 ng / mL) in the absence or presence of test compound (10μM) for 24h. After that, supernatants were assayed for TNF-α released ELISA. TNF-α released unstimulated THP-1 cells was 5.4±1.3 (mean±S.D.) at 24h.

* Value are expressed as mean±S.D.(n=3)

In compound [7] that introduced OH group into the $C_{3,4}$ position of karahanaenone [3], activity increased. The percent of inhibition value was 70.5%. Compound [4] derived to amine, activity was strong. The percent of inhibition value was 51.2%. It is very interesting that high OH group of activity of a seven member compound was the strongest.

We first demonstrated that derivatives of karahanaenone and eucarvon were inhibited to release of TNF- α in RAW264.7 cells by MIF stimulus. We have also examined the effect of these compounds on a cellular inflamemation in THP-1 cell caused by LPS. It was previous reported that monoterpene of seven member skeleton was inhibited to expression of proin-

flammatory using the macrophage cell. These data suggest that seven member of skeleton would be lead structure in medicinal chemistry.

With four compounds, karahanaenone diol was the most activity. This compound is oxidized of karahanaenone. Moreover, the activity increased when it substituted to the amino group from the carbonyl group. However, the activity of dehydrogenated compound was decreased. Hinokitiol was activity to suggest anti-inflammatory. It was previously reported that natural product was inhibited platelet type 12-lipoxygenase enzyme activity.[15] Hinokitiol of this data corresponds with the postulate from our experimental findings that an anti-inflammatory activity is shown.

It is not known to these compounds of anti-inflammatory activity that suppressed inducing exogenous MIF in cell. In the present study, we found that the anti-inflammation compound is inhibits to produce in macrophage cell caused by MIF. The tested compounds suppressed to production of TNF- α from RAW264.7 cells in MIF stimulus and were not inhibited to production of LPS stimulation THP-1 cell at the concentration of 10μM. These data suggested that a specific MIF signal action should be involved in the inhibitory activity of these compounds.

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