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Proinflammatory Cytokine (TNF-α) Suppression of Various Terpenoids to Human Monocytic Cell

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Synopsis

In the present study, the structure and the TNF-α inhibitory effect of terpenoids were determined. Among these compounds, aromadendren showed a potent inhibitory effect on TNF-α release induced by LPS. In addition, these compounds were not cytotoxic at the tested concentrations. These results suggested that terpenoids may be effective in alleviating inflammatory diseases in clinical trials.

Key words: TNF-α suppression, sesquiterpene, diterpene, triterpene, human monocyte cell

1. Introduction

Terpenoids are a class of molecules chemically derived from isopreneunits assembled and modified in thousands of different ways that commonly occur in plants. These natural substances have historically provided the compounds used most successfully as leads for pharmaceutical, agricultural, and other commercial applications[1,2]. For example, some terpenoids have been found to possess anti-inflammatory and antitumor effects[3,4].

There are also reports of terpenoids inhibiting the expression of enzymes including cyclooxygenase and iNOS and the release of an inflammatory mediator from leukocytes[5,6].

Recently, it was reported that monoterpenoids inhibited tumor necrosis factor-α (TNF-α) production from human monocyte cells induced by a lipopolysaccharide (LPS) without a cytotoxic effect[6,7].

TNF-α is a major pro-inflammatory cytokine and is mainly produced by monocytes and macrophages. It is involved in immune regulation, autoimmune regulation, and inflamemation[8-10].

Therefore, it is regarded that a TNF-α inhibitor would be possible tool for treatment of these inflammatory diseases.

We have been studying the various biological activities of terpenoids[11-12]. In the present study, terpenoids have been found to inhibit TNF-α production in the LPS-stimulated human monocyte cell line, THP-1. Furthermore, a cytotoxic effect was performed using the Alamar Blue™ method.

2. Materials and Methods

2.1 Materials

Aromadendren, ursolic acid, uvaol, betulin and betulinic acid were purchased from Fluka. Aromadendren oxide, globulol and carnosol were prepared in detail previously as described [11,12]. Human monocyte THP-1 cells were obtained from the

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American Type Culture Collection. RPMI-1640 medium and fetal calf serum (FCS) were purchased from GIBCO (NY, USA). Phorbol myristate acetate (PMA) and lipopolysacchalide (LPS) (*E.Coli.* 055:55) were purchased from Sigma (MO, USA). The TNF-α ELISA kit was purchased from Genzyme Techne (MA, USA). All other chemicals were of reagent grade.

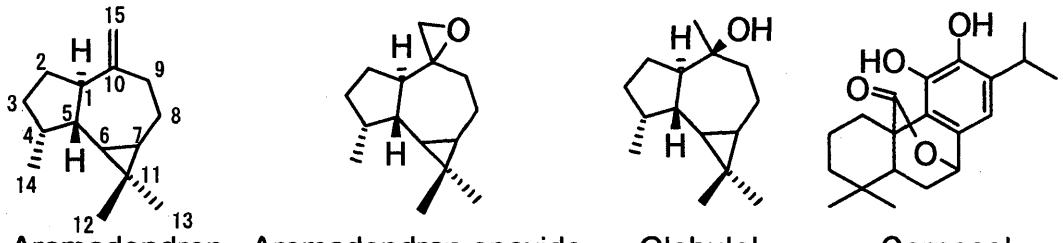
2.2 Cell Culture

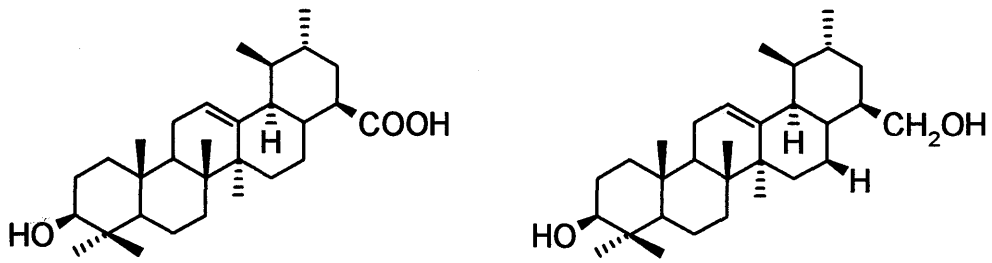
THP-1 cells were cultured in complete RPMI-1640 medium supplemented with 10% heat-inactivated FCS, 1mM L-glutamine, 50U/ml Penicillin, 50μg/ml Streptomycin and 50μM 2-maercaptoethanol under the conditions of 5% CO_2 / 95% air and 37℃.

Assays were performed at a density of 1×10^7 cells / ml.

2.3 Assays for TNF-α

For experiments using LPS as the stimulus TNF-α, THP-1 cells were suspended in complete RPMI-1640 medium. The compounds solubilized with solvent (dimethyl sulfoxide) were diluted with complete RPMI-1640. The final concentration of solvent never exceeded 0.1% in the culture medium. In vitro cultivation was done in triplicate over 24h at 37℃ in a humidified atmosphere containing 5% CO_2, by employing the following culture conditions. The control cells were cultivated in complete RPMI-1640. In contrast, THP-1 cells (1×10^6





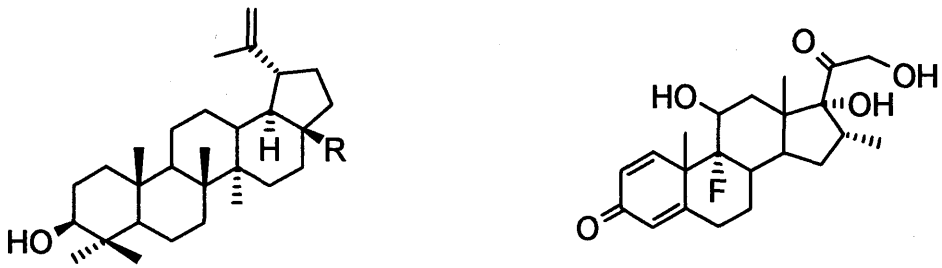


Fig. 1 Chemical structure of various terpenoids

cells/ml) were pretreated with PMA (10nM) for 6h.

After incubation, the culture medium was removed and washed three times with RPMI-1640 without serum. The PMA-pretreated cells were either stimulated by LPS at a concentration of 10μg/ml or by LPS containing the sesqui, di and triterpenoids at 10μg/ml. The cultures were incubated for 24h at 37℃. The incubated supernatants were collected and assayed for TNF-α using a commercial ELISA kit.

2.4 Cell Viability Assay

Cell viability was examined using Alamar Blue methods with minor modifications, as previously reported [13]. THP-1 cells were seeded onto 96-well plates at a cell concentration of 1×10^6 cells/ml and then preincubated for 19h. After preincubation, the test compounds (10μM) were added to each well for a 5h incubation. The fluorescence was measured with a micro-plate reader at 570nm (fluorescence $_{570\text{-}630}$). The mean value of the fluorescence for 3 wells was used for calculating the viability (% of control).

2.5 Statistical analysis

The data are presented as means ± S.E.M. Statistical significance was tested by Student's *t* -test. A probability value of less than 0.05 was considered significant.

3. Results

3.1 LPS induced TNF-α release from human monocyte cells

The chemical structures of the various terpenoids are shown in Fig. 1. To determine the anti-inflammatory properties of these compounds, the study of TNF-α release from the THP-1 cells induced by LPS-stimulation was used. In the preliminary experiments, the concentration and incubation time of LPS to obtain the appropriate TNF-α release were examined. Consequently, an incubation in the absence of LPS and 10μg/ml of LPS for 24h resulted in the release of 5.4 ± 1.3 and 231.7 ± 22.3 pg/ml, respectively. As shown in Table 1, aromadendrene, aromadendrene epoxide and globulol belonging the sesquiterpene inhibited the TNF-α release and the values were 27.3, 12.7

Table 1. Inhibition of LPS-stimulated TNF-α products from human monocyte THP-1 cells in various terpenoids

Compound	TNF-α (pg/ml)	Inhibition(%)
Spontaneous	5.4± 1.3	—
Control	231.7±22.3##	100.0
Aromadendren	168.3± 1.8*	27.3
Aromadendren oxide	202.3±12.8	12.7
Globulol	185.9±15.8	19.7
Carnosol	255.3±21.2	-10.2
Ursolic acid	187.1±18.2	19.2
Uvaol	221.0±18.3	4.6
Betulin	244.5±36.1	-5.6
Betulic acid	256.5±27.8	-10.7
Dexamethazone	103.0± 5.2**	55.5

Each value represents the mean±S.E.M. for 3-4 experiments.
##; $p<0.01$ compared with the spontaneous group.
*; $p<0.05$, **; $p<0.01$ compared with the control group.

and 19.7%, respectively. Among these compounds, only aromadendrene showed a significant inhibition of the TNF-α release. In the triterpene, ursolic acid and uvaol had an inhibitory effect of this response, although these compounds had no significant effect on the TNF-α release. On the other hand, carnosol (diterpene), betulin and betulinic acid (triterpene) had no effect on the TNF-α release from THP-1 cells induced by LPS stimulus. In addition, dexamethsone, which showed an anti-inflammatory effect, significantly inhibited the TNF-α release and its value was 55.5%.

3.2 Effects of various terpenoids on the viability

The viabilities of the THP-1 cells are shown in Table 2. Each compound was not cytotoxic at the concentration of 10μM. The value of the viability did not change when compared with the control.

4. Discussion

In the present study, human monocytes were used as an *in vitro* model to evaluate the anti-inflammatory effect of various terpenoids. THP-1 cells activated by a stimulant released various mediators including TNF-α, cytokines and prostaglandines.[14] Hart et al. described that TNF-α is known as the most important cytokine for the development and maintenance of chronic inflammation; this was evidenced by the ability of anti-TNF-α antibodies to reduce inflammatory diseases such as rheumatoid arthritis.[6] Therefore, the study of TNF-α released from THP-1 cells induced by LPS- stimulation was used.

In this study, we demonstrated that aromadendren and its derivatives inhibited the TNF-α release from human monocyte cells by LPS stimulus. The common feature of these compounds is that they possess a tricyclic skeleton. Hwang et al. and Jae et al. reported that sesquiterpene lactones inhibited the release of TNF-α from macrophages induced by LPS, however, we found for the first time that a sesquiterpene hydrocarbon inhibits TNF-α secretion.[7,15] On the other hand, among the tested sesquiterpene hydrocarbons, modification of C-10 as seen in the aromadendren epoxide and globulol resulted in loss of the inhibitory effect. These results suggest that the C-10 moiety participates in the inhibitory ability of these tricyclic sesquiterpenes.

We also revealed the inhibitory effect of triterpenoids on the TNF-α release from THP-1 cells. Among the triterpenes tested, ursolic acid and uvaol (ursane skeleton) showed inhibitory activity. However, betulin and betulic acid (lupane skeleton) had no inhibitory effect. Therefore, it seems that the six membered ring E of the pentacyclic structure (the ursane skeleton) is necessary for the activity against

Table 2. Effects of various terpenoids on the viability of THP-1 cells

Compound	Fluorescence$_{(570\text{-}630nm)}$	Inhibition(%)
Control	55431±1864	100.0
Aromadendren	54284±1567	28.6
Aromadendren oxide	51705±1849	14.1
Globulol	54173± 501	21.1
Carnosol	58038±1406	-8.4
Ursolic acid	63350±1038	20.6
Uvaol	54872±3038	6.2
Betulin	61819± 801	-3.8
Betulic acid	60140±1164	-8.9
Dexamethazone	55319±2608	56.3

Each value represents the mean±S.E.M. for 3 experiments.

TNF-α release from THP-1 cells induced by LPS.

Moreover, as compared with ursolic acid, uvaol was less active. These results suggest that among the ursane skeleton based compounds, a substituent group at C-28 has a remarkable influence. Huguet et al. have found that as most of the triterpenoids are inactive against the inflammation induced by arachidonic acid and in the neurogenic inflammatory model, their effects may depend on the *in vivo* inhibition of PKC.[16] On the other hand, Lior et al. reported that TNF-α secretion after LPS stimulation of human monocytes requires the activation of protein tyrosine kinase and PKC.[17] Therefore, our results suggest that the tested triterpenoids may depend on the inhibitory effect of PKC.

In conclusion, terpenoids showed an inhibitory effect on TNF-α release induced by LPS without any cytotoxic effects. These results suggested that terpenoids might be effective in alleviating inflammatory diseases in clinical trials. However, each inhibitory effect of the terpenoids was relatively weak when compared with dexamethazone. Further studies are needed.

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