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 isoprene units 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 applications1,2. For example, some terpenoids have been found to possess anti-inflammatory and antitumor effects3,4.

There are also reports of terpenoids inhibiting the expression of enzymes including cyclooxygenase and iNOS and the release of an inflammatory mediator from leukocytes5,6.

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 effect6,7.

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 inflammation8-10.

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

We have been studying the various biological activities of terpenoids11,12. 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 described11,12. Human monocyte THP-1 cells were obtained from the...
American Type Culture Collection. RPMI-1640 medium and fetal calf serum (FCS) were purchased from GIBCO (NY, USA). Phorbol myristate acetate (PMA) and lipopolysaccharide (LPS) (*E Coli. 055:55*) were purchased from Sigma (MO, USA). The TNF-α ELISA kit was purchased from Genzyme Technne (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′mercaptoethanol under the conditions of 5% CO2 / 95% air and 37°C.

Assays were performed at a density of $1 \times 10^7$ 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°C in a humidified atmosphere containing 5% CO2, by employing the following culture conditions. The control cells were cultivated in complete RPMI-1640. In contrast, THP-1 cells ($1 \times 10^6$)

\[ \text{Aromadendren} \quad \text{Aromadendren epoxide} \quad \text{Globulol} \quad \text{Carnosol} \]

\[ \text{Ursolic acid} \quad \text{Uvaol} \]

\[ \text{R=CH}_2\text{OH : Betulin} \quad \text{R=COOH : Betulinic acid} \]

**Fig. 1 Chemical structure of various terpenoids**
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3. Results

3.1 LPS induced TNF-α release from human monocyte cells

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

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

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, dexamethasone, 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. 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. Therefore, the study of TNF-α released from THP-1 cells induced by LPS stimulation was used.

In this study, we demonstrated that aromadendrene 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. 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 (570-630nm) | 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.
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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. 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. 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.

REFERENCES