ESCELENTOSIDE A (EsA) is a saponin isolated from the roots of Phytolacca esculenta. Previous experiments have shown that it has strong anti-inflammatory effects. Tumour necrosis factor (TNF) is a very important inflammatory mediator. It is known that there are two types of TNF—TNFα is from macrophages/monocytes and TNFβ is from activated lymphocytes. In order to study the mechanism of the anti-inflammatory effect of EsA, it was determined whether TNFα production from human peripheral monocytes was altered by EsA under lipopolysaccharide (LPS)-stimulated conditions. EsA was found to decrease TNFα production in a dose-dependent manner at concentrations higher than 1 μmol/l EsA. Recent studies have shown that EsA has a curative effect on chocolate cyst and other inflammatory diseases. Our previous studies have shown that EsA could reduce the release of platelet activating factor (PAF) from rat macrophages, and inhibit interleukin-1 and interleukin-6 production from murine macrophages. The reducing effects of EsA on the release of TNFα, IL-1, IL-6 and PAF may explain its anti-inflammatory effect.

Key words: Esculentoside A, Human peripheral monocyte, Tumour necrosis factor α

Inhibitory effect of esculentoside A on tumour necrosis factor α production by human monocytes

H-B. Wang, CA J. Fang and Q-Y. Zheng

Department of Pharmacology, College of Pharmacy, Second Military Medical University, Shanghai 200433, People’s Republic of China

CA Corresponding Author

Introduction

Esculentoside A (EsA) is a saponin isolated from the root of Phytolacca esculenta, and is identified as: 3-O-[β-D-glucopyranosyl-(1-4)-β-D-xylo-pyranosyl] phytolaccagenin. The structure of this compound is shown in Fig. 1. Experiments have shown that it has strong anti-inflammatory effects.1 It is now known that tumour necrosis factor (TNF) possess a number of properties of inflammatory response.2-3 Platelet activating factor (PAF), interleukin-1 and interleukin-6 are also inflammatory mediators.9 It has been shown that EsA inhibited the production of PAF by A23187 stimulated rat macrophages,4-5 and the production of TNFα, IL-1, IL-6 of LPS stimulated murine peritoneal macrophages.6-8 The aim of this work was to evaluate the effects of EsA on the production of TNFα by LPS stimulated human peripheral monocytes.

Materials and Methods

Reagent: RPMI-1640, lipopolysaccharide (Escherichia coli 055:B5) and calcimycin (A23187) were purchased from Sigma (USA).

Human peripheral monocyte preparation: Human monocytes were isolated by a combina-

FIG. 1. The structure of esculentoside A.
Inhibitory effect of esculetoside A

**TNFα production**: Two hours later, the medium containing non-adherent cells was decanted and the non-adherent cells in supernatant were counted to measure the adherence ratio. TNFα activity was expressed as U/10⁶ monocytes. The adherent cells were rinsed twice with Hanks’s solution. Then the adherent cells were incubated with A₂₃₁₈₇ (1 μmol/l) for 6 h. After incubation, the medium was discarded, and the cells were washed three times with RPMI-1640 to remove A₂₃₁₈₇. Fresh culture medium without serum was added to every well with lipopolysaccharide (LPS, 10 μg/ml) in the presence or absence of EsA. The cultures were incubated in a humidified atmosphere of 5% CO₂ and at 37°C for another 6 h. The supernatants were harvested and centrifuged. The cell-free supernatants were collected, dialysed in phosphate-buffer solution for 24 h, and stored at -20°C prior to activity assay.

**TNFα production assay**: TNFα assay was performed essentially as described by Kunkel et al. with minor modifications. Briefly, L₉₂₉₀ cells (50 000/well) were dispensed into 96-well flat-bottomed microtitre plates in a volume of 0.1 ml/well. The following day, the cells were incubated for 18 h in the presence of 1 μg of actinomycin D and serial 1:2 dilutions of test sample. Media were then decanted, and the remaining cells in each well were stained with crystal violet for about 15 min, washed with tap water, and dried at 40°C. Absorbance of the cells in each well was read using a microenzyme linked immunosorbent autoreader. Units of TNFα activity were defined as described by Kunkel et al.

**Cell viability**: The trypan blue exclusion test was performed after 6 h incubation with or without EsA. Cell viability was also determined by measuring lactate dehydrogenase (LDH) activity in the cell-free supernatants according to the method of Beutle.

**Statistics**: Each experiment was carried out at least three times. Values were expressed as the mean ± standard error. Variation between parallel experiments was less than 30%. Probability values for statistical differences were determined by Student’s t-test and p values of less than 0.05 were considered significant.

**Results**

**Effects of EsA on production of TNFα from human monocytes**: TNFα in the supernatant after being dialysed was assessed by the killing of L₀₂₉₀ cells. One unit of TNFα was defined as the reciprocal of the dilution of a preparation that results in 50% survival of the cells. The results are presented in Table 1. It is observed that the decrease of TNFα production was significant at the concentrations of 1 and 10 μmol/l EsA.

**Effects of Es on the kinetics of TNFα production from human monocytes**: Monocytes were cultured in LPS (10 μg/ml) with or without EsA, and the supernatants were harvested at 2 h intervals. TNFα activity can be detected after 2 h exposure to LPS. Figure 2 shows that TNFα production by human monocytes were inhibited in a time dependent manner by EsA at a concentration of 5 μmol/l.

**Effects of EsA on cellular activity**: In order to eliminate the possibility that EsA was toxic for the cells tested, cell viability was monitored by measuring LDH activity in the supernatant and by the trypan blue exclusion test performed at the end of the macrophage culture. Results of the

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**Table 1. Effect of esculetoside A on tumour necrosis factor α production from 10⁶ human peripheral monocyte stimulated by LPS 10 μg/ml, mean ± SD, n = 3 samples**

<table>
<thead>
<tr>
<th>Concentration (μmol/l)</th>
<th>TNFα activity (U/10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>51.4 ± 4.6</td>
</tr>
<tr>
<td>LPS + EsA (μmol/l)</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>56.0 ± 13.5</td>
</tr>
<tr>
<td>0.1</td>
<td>45.2 ± 8.9</td>
</tr>
<tr>
<td>1.0</td>
<td>36.5 ± 3.6*</td>
</tr>
<tr>
<td>10.0</td>
<td>27.1 ± 6.1*</td>
</tr>
</tbody>
</table>

* p < 0.05 versus control group.
LDH in supernatant of adherent macrophages, cultured for 6h with various concentrations of EsA, was not different from that of the control, as is shown in Table 2. Cell viability was confirmed by the trypan blue exclusion test. The cell viability ratio in test samples was more than 99% (Table 2). The result was the same as previously reported.10

**Discussion**

In this study, EsA induced a dose-dependent decrease in the TNFα concentrations measured in the supernatant of LPS-stimulated human monocytes. The kinetics of TNFα production were also changed. TNF was initially identified as a factor that appeared in the circulation of animals following the injection of endotoxins. TNFα is a product of stimulated monocytes and macrophages, but it is also produced by keratinocytes.6 In addition to the cytotoxic activities of TNFα in some types of transformed cells, recent data have shown that TNFα mediated stimulation of collagenase synthesis and prostaglandin E2 (PGE2) production by synovial cells,7 and stimulated bone resorption and inhibition,9 suggesting that TNFα might be an important mediator of inflammation. Platelet activating factor (PAF) is a mediator of anaphylaxis and inflammation; it plays an important role in inflammation, and there is cooperation between PAF and TNFα in inflammatory reactions.11 It has been found that EsA reduced release of PAF from rat macrophages. A previous study also showed that EsA can inhibit the inflammatory reaction induced by carrageenan.1 Recent clinical trials showed that a Chinese herb containing EsA had a significant curative effect on chocolate cyst. Our recent test showed that EsA could significantly inhibit IL-1 and IL-6 production from murine macrophages.12 Thus, together with the present investigation it is suggested that the anti-inflammatory effects of EsA might be due to its reducing effects on the release of TNFα, PAF, IL-1, IL-6 and other inflammatory mediators.

**References**


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