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Short communication

# Effect of selected triterpenoids on chronic dermal inflammation

Salvador Máñez, M. Carmen Recio, Rosa M. Giner, José-Luis Ríos *

*Departament de Farmacologia, Universitat de València, Avda. Vicent Andrés Estellés s / n, 46100 Burjassot, Valencia, Spain*

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**Abstract**

The activity of four natural triterpenoids on a 12-*O*-tetradecanoylphorbol-13-acetate multiple-dose model of skin chronic inflammation was studied. Erythrodiol and ursolic acid were significantly effective. The most important features concerning structure–activity relationship and previous data on the effect of these triterpenoids on other inflammatory conditions are discussed. © 1997 Elsevier Science B.V.

*Keywords:* Inflammation, chronic; Skin; 12-*O*-Tetradecanoylphorbol-13-acetate; Triterpenoids

## 1. Introduction

Triterpenoids are almost ubiquitous in plants and have been long considered the anti-inflammatory principles of several important drugs, particularly in the form of triterpene glycosides. Most triterpenoids, at least those from angiosperms, belong to the oleanane, ursane or lupane classes. Considering their similarity to steroidal compounds, they have often been attributed a mechanism related to that of these anti-inflammatories, for example, in studies on the antiedematous effect of ursolic acid in the models of arachidonic acid or 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced skin inflammation (Huang et al., 1994). In relation to endogenous corticosteroid metabolism, it has been demonstrated that glycyrrhetinic acid inhibits cortisol inactivation by 11-$\beta$-hydroxysteroid dehydrogenase in mouse skin, thereby potentiating the effects of that hormone (Teelucksingh et al., 1990). Furthermore, we have reported that betulinic acid probably acts by a mechanism related to that of glucocorticoid because its activity is severely reduced by inhibitors of transcription and translation (Recio et al., 1995a).

Concerning the metabolism of arachidonic acid, it is well established that leukotrienes are involved in many inflammatory processes, especially in those characterized by a chronic course and damage, like bronchial asthma, arthritis and psoriasis (McMillan and Walker, 1992). Inhibition of 5-lipoxygenase has been assessed for some triterpenoids such as the ursanes from *Boswelia serrata* resin, a well-known antiinflammatory remedy in oriental medicine. Acetyl-11-keto-$\beta$-boswellic acid in particular, causes selective blockade of the enzyme through a pentacyclic structure-binding site which is different from the substrate binding site (Safayhi et al., 1995).

In connection with the above and as a continuation of our work on structure–activity relationships (Recio et al., 1995b), we have examined the effect of a group of selected anti-inflammatory triterpenoids on a system of chronic dermal edema and cellular proliferation caused by repeated application of TPA, to determine their value in skin inflammation. This method represents an advantage over the topical acute tests (arachidonic acid and TPA edemas) because a great many skin diseases are essentially chronic and their pharmacological treatment is often applied when the lesion has already occurred. Moreover, as it constitutes a model of considerable selectivity for corticosteroids and leukotriene synthesis inhibitors, it is useful for evaluating topical agents for the treatment of proliferative inflammatory conditions such as psoriasis (Stanley et al., 1991).

## 2. Material and methods

### 2.1. Animals

Groups of six female Swiss mice weighing 25–30 g were used. All animals were fed a standard diet ad libitum

* Corresponding author. Tel.: +34-96-3864973; fax: +34-96-3864943; e-mail: riosjl@uv.es.

and housed in a temperature controlled room with a 12 h light/dark schedule.

### 2.2. Chemicals

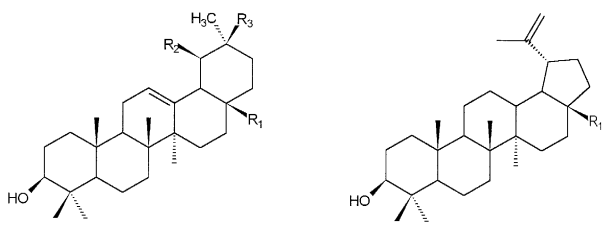
Ursolic acid was obtained from the aerial parts of *Helichrysum stoechas* (L.) Moench (Asteraceae) (Recio et al., 1991). Betulin, dexamethasone, 12-*O*-tetradecanoylphorbol-13-acetate, hydrogen peroxide, tetramethylbenzidine and hexadecyltrimethylammonium bromide were purchased from Sigma Chemical Co., St. Louis, betulinic acid and erythrodiol from Roth A.G., Karlsruhe and sodium acetate from Panreac, Barcelona.

### 2.3. Mouse ear edema induced by multiple topical applications of TPA (Stanley et al., 1991)

Chronic inflammation was induced by topical application of 10 $\mu$l of TPA (2.5 $\mu$g/ear) to both the inner and outer surface of both ears of each mouse with a micropipette on alternate days. Triterpenoids were dissolved in 70% aqueous ethanol and applied topically (0.5 mg/ear) twice daily for four days, in the morning immediately after TPA application and 6 h later. Dexamethasone was used as the reference drug (0.05 mg/ear). The mice were killed by cervical dislocation 6 h after the last TPA application. Circular biopsies of each ear were taken with a leather punch. Ear weight is presented as the mean of 12. After weighing, biopsies were frozen for future myeloperoxidase assay.

### 2.4. Myeloperoxidase assay (De Young et al., 1989)

Each biopsy was homogenized with 0.75 ml of 0.5% hexadecyltrimethylammonium bromide at pH 5.4. After adding a second 0.75 ml aliquot, the sample was centrifuged at 12000 *g* at 4°C for 20 min. The supernatant (30 $\mu$l) was mixed with 20 $\mu$l of tetramethylbenzidine 18.4 mM and 15 $\mu$l of $H_2O_2$ 0.017% in a 96-well microtiter plate. After 3 min at 37°C the enzyme activity was determined using a Labsystems Multiskan MCC/340 plate reader set at 620 nm. This activity reflects, in a specific mode, the accumulation of neutrophils in the inflamed tissue, which was maximal after 3–4 days of TPA application.




| | $R_1$ | $R_2$ | $R_3$ | | $R_1$ |
|---|---|---|---|---|---|
| Erythrodiol | $CH_2OH$ | H | $CH_3$ | Betulin | $CH_2OH$ |
| Ursolic Acid | COOH | $CH_3$ | H | Betulinic Acid | COOH |

Fig. 1. Structure of the triterpenoids.

Table 1
Anti-inflammatory effects of assayed products

| Product | Ear weight (mg) | I.R. [a,b] | MPO [c] | I.R. [a] | I.R. [f] |
|---|---|---|---|---|---|
| Control (TPA) | 23.4 ± 1.0 | — | 1473 ± 110 | — | — |
| Betulin | 21.5 ± 1.7 [ns] | 20 | 587 ± 127 [e] | 60 | 46 |
| Betulinic acid | 21.1 ± 2.4 [ns] | 24 | 1054 ± 216 [e] | 29 | 30 |
| Erythrodiol | 17.4 ± 0.9 [d] | 63 | 509 ± 72 [e] | 65 | 30 |
| Ursolic acid | 17.7 ± 0.3 [d] | 59 | 481 ± 127 [e] | 67 | 35 |
| Dexamethasone | 15.8 ± 0.6 [e] | 79 | 120 ± 12 [e] | 92 | 61 |

ns = not significant.
[a] Inhibition ratio percentage.
[b] Chronic inflammation inhibition relative to acetone-only control (13.8 ± 0.5).
[c] Myeloperoxidase assay; (mOD/biopsy, $n = 5$ animals).
[d] $P < 0.05$.
[e] $P < 0.01$ with respect to the control group (Dunnet's *t*-test).
[f] Ratio of percentage inhibition of ethyl phenylpropiolate-induced ear edema, data from Recio et al. (1995a,b).

### 2.5. Statistics

Percentages of edema reduction are expressed as the mean with S.E.M. Dunnet's *t*-test for unpaired data was used for statistical evaluation.

## 3. Results

The experimental protocol for chronic skin inflammation was applied to betulin, betulinic acid, erythrodiol and ursolic acid (Fig. 1), because they were the four triterpenoids that had given the best results in previous tests, such as the edemas induced by single applications of TPA and ethyl phenylpropiolate. As seen in Table 1, the most active compounds were erythrodiol, which reduced the inflammation, measured as the increase in ear weight, by 63% and ursolic acid, which produced a 59% reduction. These compounds also caused a parallel decrease in neutrophil infiltration, detected by myeloperoxidase activity of 65 and 67%, respectively.

## 4. Discussion

In order to distinguish between the effects of TPA and those of ethyl phenylpropiolate and consequently explore the possible mode of action of a given skin anti-inflammatory agent, some biochemical and histological features should be reviewed. Repeated doses of TPA cause edema, epidermal proliferation, leukocyte infiltration, the appearance of *dark cells* (basophilic keratinocytes) and dermal

fibrosis. TPA also decreases the number of pale dendritic cells, an important sign indicative of tumour promotion (Baxter et al., 1988). Ethyl phenylpropiolate displays a series of effects, some of which are similar to those of TPA, while others are different: it causes epidermal hyperplasia, appearance of dark cells, neutrophil infiltration, vascular leakage and, to a much lesser extent than TPA, induction of ornithine decarboxylase activity. Ethyl phenylpropiolate specifically augments eosinophil infiltration, causes focal epidermal destruction and does not affect protein kinase C, the main target for TPA activity. Ethyl phenylpropiolate was once considered a non-promoting or weakly promoting inflammatory irritant and later a stage 3-specific tumour promoter, thereby acting when applied after limited administration of stage 1 and 2 promoting agents (i.a. TPA and analogues) (Baxter et al., 1989). Nevertheless, a certain degree of promotion was observed in the two-stage model initiated with 7,12-dimethylbenz(a)anthracene in SENCAR mice (Cameron et al., 1991). For most of its effects, ethyl phenylpropiolate toxicity is largely, even dramatically, dependent on the dose.

In contrast with results of our previous experiments with this kind of triterpenoids, in which no relationship between basic structural skeleton and activity was found, we now observed that this relationship does exist for skin chronic antiinflammatory activity, being detrimental for the lupane derivatives, betulin and betulinic acid. Their appreciable effects in single-dose TPA (81 and 86%, respectively) and ethyl phenylpropiolate (see I.R.[f] at Table 1) tests were obviously of no predictive value. Furthermore, the fact that these compounds have a steroid-like mode of action, as seen with betulinic acid and partially with betulin (Recio et al., 1995a), was not related to positive effects in the chronic model. Nevertheless, it should be pointed out that betulin at least reduced efficiently the neutrophil influx. Alternatively, ursolic acid (ursane-based) and erythrodiol (oleanane-based), both lacking any steroidal-like mechanism of action, were effective in both acute and chronic tests, reducing ear weight and myeloperoxidase activity. Therefore, it seems that a six-membered ring E of the pentacyclic structure is necessary for the activity against multiple-dose TPA-caused inflammation. Erythrodiol, an oleanane compound possessing a primary hydroxyl group at C-28, deserves special attention. The influence of this substituent on the bioactivity, as already observed by ourselves (Recio et al., 1995b), is intriguing and could explain the antiinflammatory activity of that terpenoid in each of the methods applied.

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