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# In vitro and in vivo evaluation of terpenoid esters of indomethacin as dermal prodrugs

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**Abstract**

Indomethacin terpenoids' esters (1–4) were synthesized and assessed both in vitro and in vivo as indomethacin dermal prodrugs. Esters 1–4 showed high lipophilicity, poor water solubility, good stability in hydro-alcoholic medium (ethanol/water 1:1) and rapid enzymatic cleavage. Results from in vitro percutaneous absorption studies showed that esters 1 and 2 slightly increased the cumulative uptake of indomethacin through excised human skin compared with the parent drug. In vivo results, using methyl nicotinate (MN) induced erythema as an inflammatory model in human volunteers, showed an interesting delayed and sustained activity of ester 1 compared with the parent drugs. © 1997 Elsevier Science B.V.

*Keywords:* Dermal prodrugs; Indomethacin; In vivo; Sustained activity; Terpenoids

## 1. Introduction

The prodrug approach represents a very promising strategy to enhance drug skin permeability (Sloan, 1989). The prodrug concept involves the chemical modification of a drug into a bioreversible form in order to change its physicochemical characteristics and thus enhance its skin permeation. Generally, regeneration of the parent drug occurs into the skin by enzymatic processes. Since the skin is a highly metabolic organ (Bickers and Kappas, 1980), this approach has been increasingly used in the past few years to optimize the dermal and transdermal delivery of drugs (Chan and Li Wan Po, 1989; Sloan, 1992).

In a recent review, Guy and Hadgraft (1992) suggested that drug derivatization with a promoiety which possesses inherent enhancing ability

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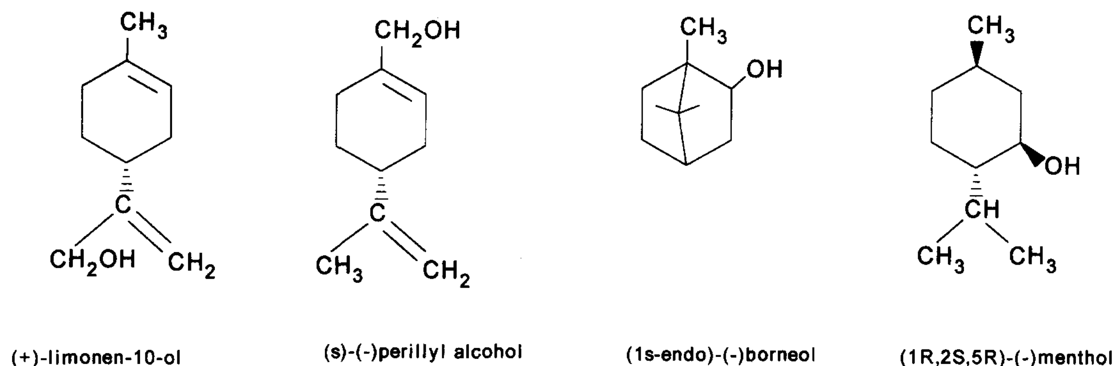



Fig. 1. Terpenoids used as promoieties.

would be a promising strategy to design dermal and transdermal prodrugs. In the last 5 years, we obtained several dermal prodrugs of non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin and naproxen, conjugating them by ester linkage to different penetration enhancers such as *N*-alkyllactames (Bonina et al., 1991, 1993), *N*-acyllactames (Bonina et al., 1995b), polyoxyethylene glycols (Bonina et al., 1995a). Some of these esters showed the main requirements to be regarded as interesting dermal prodrugs in in vitro and in vivo evaluations.

Recently, a novel series of skin penetration enhancers, namely terpenes and terpenoids, has been described (Williams and Barry, 1990) and several authors have demonstrated the promoting effects of terpenes and terpenoids on percutaneous absorption of anti-inflammatory drugs such as indomethacin (Kikuchi et al., 1992; Levison et al., 1994; Ogiso et al., 1995), ketoprofen (Ohara et al., 1994) and diclofenac (Obata et al., 1993).

In this paper, in order to evaluate the feasibility of using terpenoids (see Fig. 1) as promoieties in designing NSAIDs dermal prodrugs, we synthesized the indomethacin terpenoids' esters 1–4 (see Fig. 2) and assessed their chemical and enzymatic stability, solubility, lipophilicity and flux through excised human skin. Furthermore, in order to investigate the relationship between in vitro skin permeation data and in vivo topical anti-inflammatory activity, we evaluated the ability of compounds 1–2, which showed the best in vitro profile, to inhibit methyl nicotinate (MN)-induced skin erythema on healthy human volunteers.

## 2. Materials and methods

### 2.1. Apparatus

Precoated silica gel Merck 60 F254 plates were used for thin layer chromatography; detection of components was made by ultraviolet light (254 nm) and/or treatment with iodine vapors. Chromatographic separations were performed in columns packed with silica gel 60 (Carlo Erba 70-230 mesh ASTM). Melting points were determined with a Kofler hot stage microscope and are uncorrected. Elemental analyses were performed on a Carlo Erba mod. 1108 elemental analyzer.

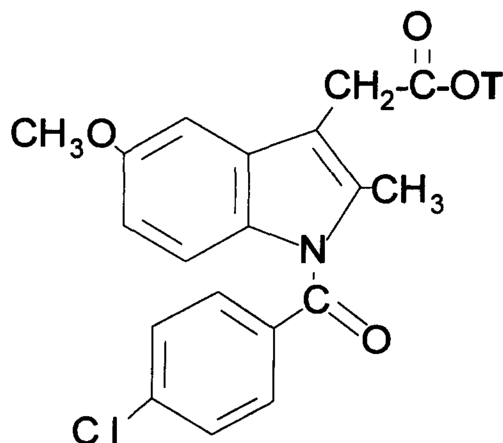


Fig. 2. Chemical structure of esters 1–4. Ester 1: T–OH = (+)-limonen-10-ol; ester 2: T–OH = (*s*)-(–)-perillyl alcohol; ester 3: T–OH = (1*s*-endo)-(–)borneol; ester 4: T–OH = (1*R*, 2*S*, 5*R*)-(–)-menthol.

The $^1$H- and $^{13}$C-NMR (nuclear magnetic resonance) measurements were performed on a Bruker WM 250 spectrometer and/or on a Bruker AMX 500 spectrometer equipped with a Bruker X-32 computer.

High-performance liquid chromatography (HPLC) was performed as follows. A Water Associates Model 600E liquid chromatograph equipped with a Model 7125 Rheodyne injection valve (fitted with a 20 $\mu$l loop) and a Water Associates Model 486 ultraviolet detector set at 254 nm was used. The chromatograms were recorded by a Data Module Model 746 (Millipore). The stainless steel column was a Nucleosil 5 $C_{18}$ (4 × 250 mm) (Macherey, Nagel-Duren, Germany).

### 2.2. Chemicals

Indomethacin was purchased from Sigma, St. Louis, MO. (+)Limonen-10-ol, (*s*)-(−)perillyl alcohol, (1*s*-endo)-(−)borneol, (1*R*, 2*S*, 5*R*)-(−)menthol and 1,1′-carbonyldiimidazole were obtained from Aldrich. Acetonitrile and water used in the HPLC procedures were of HPLC grade and were bought from Carlo Erba (Milan, Italy). All other chemicals and solvents were reagent grade.

### 2.3. Synthesis of indomethacin terpenoids' esters 1–4

The syntheses of esters 1–4 were performed following the method described to prepare a series of indomethacin oligoethylene esters (de Caprariis et al., 1994), with slight modifications.

Indomethacin (1.79 g, 5 mmol) was dissolved in dry ($CaCl_2$) alcohol-free chloroform (50 ml). 1,1′-Carbonyldiimidazole (0.97 g, 6 mmol) was added portion-wise, and the reaction mixture was stirred for 45 min at room temperature, until effervescence ceased. The intermediate imidazolide obtained was not isolated, but the appropriate terpenoid (5 mmol) was added to this solution. The reaction mixture was stirred under reflux for 12 h, cooled, washed with water (2 × 50 ml), aqueous 0.1 N HCl (2 × 50 ml), water (2 × 50 ml), aqueous 0.1 N NaOH and water (2 × 50 ml), dried (anhydrous $Na_2SO_4$), filtered and evaporated to dryness in vacuo. The product was then purified by chromatography on silica gel columns using ethyl acetate/*n*-hexane 2:8 for esters 1–3 and chloroform/*n*-hexane 9:1 for ester 4. All the products obtained were crystallized from *n*-hexane. Melting points and yields are listed in Table 1. Elemental analyses (C, H, N) were within ± 0.4% of theoretical values. The infrared (IR), $^1$H-NMR and $^{13}$C-NMR spectral data of esters 1–4 were consistent with their chemical structures.

### 2.4. HPLC analysis of indomethacin and esters 1–4

Esters 1–4 were quantified in isocratic conditions using as mobile phase a mixture of acetonitrile/0.1 M acetic acid, 85:15 (v/v), at a flow rate of 1 ml/min. A calibration graph of peak area against drug concentration was constructed for each compound and used to quantify the unknown samples.

For the simultaneous determination of indomethacin, esters 1–4 and their hydrolyzed products, a gradient method was developed: a 9-min linear gradient between 40:60 and 95:5 (v/v) acetonitrile/0.1 M acetic acid was run at 1 ml/min.

The compound's retention is expressed by the logarithm of capacity factor, $\log k'$, defined as $\log k' = \log[(t_r - t_0)/t_0]$, where $t_r$ and $t_0$ are the retention times of the drug and a non-retained compound, respectively.

### 2.5. Chemical and enzymatic hydrolysis

The hydrolysis rate of esters 1–4 was determined in hydro-alcoholic solution (water/ethanol 1:1) following the disappearance of the ester by the HPLC method reported above.

Enzymatic hydrolysis of esters 1–4 was determined as previously reported (Bonina et al., 1991). Porcine esterase was diluted 1000-fold with phosphate buffer, pH 7.4, prior to use. A total of 100 $\mu$l of aqueous ester solution ($10^{-4}$ M) containing 5% (w/v) Tween 80 was added to 10 ml of phosphate buffer (0.05 M, pH 7.4), thermostatically controlled at 37°C and then 100 $\mu$l of the esterase solution was added. The concentration of

Table 1
Molecular weights (MW), Melting points (M.p.), yields, half-lives ($t_{1/2}$) of chemical and enzymatic hydrolysis, water/ethanol (1:1) solubility ($S_{we}$), isopropyl myristate (IPM), solubility ($S_i$), lipophilicity index ($\log k'$), calculated partition coefficient (CLOGP)[a], indomethacin equivalent cumulative amount permeated through excised human skin after 24 h ($Q$) of indomethacin and esters 1–4

| Compound | MW | Yield (%) | M.p. (°C) | $t_{1/2}$ | | $S_{we}$ (mg/ml) | $S_i$ (mg/ml) | $\log k'$ | CLOGP | $Q \pm$ S.D.[b] ($\mu$g/cm$^2$) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ethanol/water (1:1) (days) | Esterase (1.3 U/ml) (h) | | | | | |
| Indomethacin | 357.8 | — | — | — | | 2.76 | 1.58 | −0.28 | — | 0.78 ± 0.09 |
| Ester 1 | 492.0 | 52 | 77–78 | 137 | 0.42 | 0.21 | 18.7 | 0.57 | 3.31 | 1.29 ± 0.14* |
| Ester 2 | 492.0 | 48 | 79–80 | 187 | 0.73 | 0.25 | 17.8 | 0.57 | 3.31 | 1.18 ± 0.12* |
| Ester 3 | 493.0 | 38 | 111–112 | 129 | 4.73 | 0.29 | 15.7 | 0.65 | 3.52 | 0.24 ± 0.04 |
| Ester 4 | 495.1 | 35 | 74–75 | 45 | 4.97 | 0.62 | 128.2 | 0.78 | 4.18 | N.d.[c] |

[a] Values calculated for the corresponding acetic esters of the terpenes used.
[b] All the experiments were run in duplicate on three different donors.
[c] Not detectable.
* Significant at $p < 0.01$ compared with control.

the ester in the solution was monitored by HPLC at different times. Pseudo-first-order rate constants for the hydrolysis (both chemical and enzymatic) were determined from the slopes of linear plots of the logarithm of residual indomethacin ester against time.

### 2.6. Solubility, lipophilicity indices and calculated partition coefficient of esters 1–4

The solubility of indomethacin and esters 1–4 was determined in both hydro-alcoholic solution (ethanol/water 1:1) and isopropyl myristate (IPM) in duplicate by stirring an excess of each compound in 2 ml of solvent for 24 h at room temperature. The mixtures were then filtered, suitably diluted with acetonitrile and analyzed by the HPLC method described above to determine the concentrations of indomethacin and esters 1–4.

Lipophilicity indices of esters 1–4 were obtained by the isocratic HPLC method described above, measuring compounds' retention times expressed as $\log k'$ (Lambert, 1993). Estimates of lipophilicity of terpenoids' moieties were also performed by calculating the calculated logarithm of P (CLOGP) of the corresponding acetic esters (C-QSAR program, version 1.83, Biobyte, Claremont, CA).

### 2.7. In vitro skin permeation experiments

Samples of adult human skin (mean age 38 ± 10 years) were obtained from breast reduction operations. Stratum corneum and epidermis membranes (SCE) were prepared, stored and rehydrated as previously described (Bonina and Montenegro, 1992). Skin permeation experiments were carried out using Franz diffusion cells (LGA, Berkeley, CA) whose exposed skin surface area and receptor volume were 0.75 $cm^2$ and 4.5 ml, respectively. The receiving compartment contained ethanol/water 1:1 for ensuring sink conditions (Mueller, 1988; Touitou and Fabin, 1988). The receiving solution was stirred and maintained at 35 ± 1°C throughout the experiments. Indomethacin and esters 1–4 were applied to the skin surface as IPM saturated solution (400 μl) and the experiment was run for 24 h. Samples of the receiving solution (50 μl) were withdrawn at 24 h and analyzed for indomethacin or ester 1–4 content by the HPLC method described above.

### 2.8. Preparation of aqueous gel

Carbopol gels, containing indomethacin or esters 1–2, were prepared by dispersing 1.5 g of Carbopol 934 in distilled water (75.4 ml for indomethacin gel and 75.0 ml for esters 1–2 gels) with constant stirring. Indomethacin (1.0 g, 2.7 mmol) or ester 1–2 (1.4 g, 2.7 mmol) were dissolved in ethanol (20.0 ml) together with methyl-*p*-hydroxybenzoate (0.1 g). The ethanolic solution was added to the carbopol dispersion and the mixture was then neutralized and made viscous by the addition of triethanolamine (2.0 g). The gels were stored at room temperature for 24 h under air-tight conditions prior to use.

### 2.9. In vivo anti-inflammatory activity of esters 1–2 on methyl nicotinate-induced erythema

#### 2.9.1. Instrument

The induced MN erythema was monitored by using a reflectance visible spectrophotometer X-Rite model 968, having 0° illumination and 45° viewing angle, as previously reported (Bonina et al., 1995a). The instrument was calibrated with a supplied white standard traceable to the National Bureau of Standards' perfect white diffuser. The spectrophotometer was controlled by an IBM PS2 50 computer, which performed all color calculations from the spectral data by means of a menu driven suite of programs (Spectrostart) supplied with the instrument. Reflectance spectra were obtained over the wavelength range 400–700 nm using illuminant C and 2° standard observer. From the spectral data obtained, the erythema index (EI) was calculated using an equation (Eq. (1)) similar to that reported by Dawson et al. (1980):

$$\mathrm{EI} = 100\left[\log\frac{1}{R_{560}} + 1.5\left(\log\frac{1}{R_{540}} + \log\frac{1}{R_{580}}\right) - 2\left(\log\frac{1}{R_{510}} + \log\frac{1}{R_{610}}\right)\right] \quad (1)$$

where $1/R$ is the inverse reflectance at a specific wavelength (560, 540, 580, 510 and 610 nm).

#### 2.9.2. Protocol

In vivo experiments were performed on six volunteers of both sexes in the age range 25–35 years. The volunteers were fully informed of the nature of the study and the procedures involved. The participants did not suffer from any ailment and were not on any medication at the time of the study. They were rested for 15 min prior to the experiments and room conditions were set at $22 \pm 2$°C and 40–50% relative humidity.

Eight sites on the ventral surface of each forearm were defined using a circular template (1 $cm^2$) and demarcated with permanent ink. For each volunteer, two of the eight sites of each forearm were used as a control applying 50 mg gel without active compounds and the other six sites were treated with 50 mg indomethacin, ester 1 or ester 2 gel. The preparations were spread uniformly on the site by means of a solid glass rod. The sites were then occluded for 3 h, using Hill Top Chambers (Hill Top Research, Cincinnati, OH). After the occlusion period, the chambers were removed and the skin surfaces were washed to remove the gel and allowed to dry for 15 min. On each pre-treated site, MN aqueous solution (0.5% w/v) was applied at different times after gel removal: immediately ($t = 0$), 3 and 6 h later ($t = 3$ and $t = 6$, respectively). MN was applied on the skin surface for 1 min using a Hill Top Chamber (1 $cm^2$) whose cotton pad was saturated with 200 $\mu$l of MN solution and the induced erythema was monitored for 100 min. EI baseline values were taken at each designated site before application of gel formulation and they were subtracted from the EI values obtained after MN application at each time point to obtain $\Delta$EI values. For each site, the area under the response ($\Delta$EI)–time curve (AUC) was computed using the trapezoidal rule.

## 3. Results and discussion

### 3.1. Chemical and enzymatic stability

The poor water solubility of esters 1–4 did not allow us to determine their stability in phosphate buffer medium. So the chemical stability of esters 1–4 was studied in hydro-alcoholic solution (water/ethanol 1:1) and in IPM, that were used as receiving and donor phases in in vitro skin permeation experiments, respectively. As shown in Table 1, esters 1–4 had a notable chemical stability in hydro-alcoholic solution with half-lives ranging from 45 to 187 days. Esters 1–4 were also stable in IPM since no significant hydrolysis was observed over 48 h.

Since an essential prerequisite for the successful use of dermal prodrugs is their reconversion into the parent drug within the skin, we assessed the enzymatic cleavage of esters 1–4 by using porcine liver esterases which are regarded as good models for skin esterase enzymatic activity (Cheung et al., 1985; Wong et al., 1989).

To perform in vitro enzymatic hydrolysis experiments in aqueous medium, a small amount of surfactant (Tween 80) was used to solubilize esters 1–4. A similar procedure has been described studying the enzymatic hydrolysis of other lipophilic esters of indomethacin (Mishima et al., 1990). As shown in Table 1, all the prodrugs were readily hydrolyzed by porcine esterase, especially esters 1 and 2 whose half-lives were less than 1 h. It is worth noting that no relationship could be observed between the chemical stability in hydro-alcoholic medium and the rate of enzymatic hydrolysis of esters 1–4.

### 3.2. Solubility and lipophilicity

As mentioned above, since esters 1–4 showed poor water solubility, in vitro skin permeation experiments were performed using vehicles different from water. Indomethacin and ester 1–4 solubilities in water/ethanol 1:1 (used as receiving solution) and in IPM (used as donor phase) are reported in Table 1. As may be noted, all the esters were less soluble than indomethacin in hydro-alcoholic medium but more soluble in IPM.

Since the horny layer is regarded as the main barrier in drug skin permeation and is basically a

lipophilic barrier, drug lipophilicity is considered as one of the key parameters controlling drug skin permeation. The esterification of the carboxylic function of indomethacin with the terpenoids' moieties is expected to strongly increase the lipophilic/hydrophilic balance. Two different parameters were considered for the evaluation of lipophilicity of esters 1–4: CLOGP data from theoretical calculation and $\log k'$ chromatographic indices. The determination of theoretical values of lipophilicity, CLOGP, for esters 1–4 was not possible as values greater than 8 were generated. This results from an inadequate capability of the CLOGP method in handling molecules possessing very high lipophilicity. Hence, as the considered esters are characterized from a variable (terpenoid) moiety, the lipophilicity of this portion can supply a relative scale of lipophilicity of the esters. Therefore, the CLOGP values of the acetic esters of terpenoids' moieties have been determined (Table 1). On the other hand, a direct measure of lipophilicity is represented from the chromatographic data ($\log k'$) isocratically determined by reversed phase HPLC (Table 1). It is worth noting that an 80% acetonitrile percentage was required to elute esters 1–4. At this very high organic modifier fraction, the increase in $\log k'$ of esters with respect to the parent drug was at least one order of magnitude, indicating a very strong increase in lipophilicity. Finally, the two systems yielded not only the same ranking order, but also strongly correlated scales. All the esters 1–4 showed a lipophilicity that was at least 10-fold higher than that of the parent drug.

### 3.3. *In vitro skin permeability*

In vitro skin permeation experiments were performed using SCE membranes, since other authors reported that the dermis in vitro can act as a significant additional barrier to the permeation of lipophilic compounds (Scheuplein and Blank, 1973; Bronaugh and Stewart, 1984). Since esters 1–4 were poorly soluble in water, we used IPM as a vehicle to apply esters 1–4 to the skin. Saturated IPM solutions of indomethacin and esters 1–4 were used in order to ensure the maximum thermodynamic activity, thus obtaining a constant driving force. At the end of the permeation experiments, no indomethacin was found in the receiving solution. The same lack of enzymatic activity was found in previous studies on other indomethacin ester prodrugs (Bonina et al., 1991) and was attributed to the use of SCE membranes obtained by a means of a thermal separation technique.

In vitro skin permeation results, expressed as cumulative amount of indomethacin or indomethacin equivalent permeated through human skin after 24 h, are reported in Table 1. As may be noted, esters 1–2 provided only a slight increase of indomethacin cumulative amounts permeated through the skin compared to indomethacin ($p < 0.05$). Ester 3 proved to permeate the skin less than indomethacin, and while applying ester 4 on the skin surface, no indomethacin or ester 4 could be detected in the receiving compartment.

The poor increase of in vitro skin permeability obtained for compounds 1–2 (and the lower fluxes observed for compounds 3–4) could be attributed to the very high lipophilic character of these derivatives, associated with lower water solubility, compared to indomethacin. As reported by Sloan (1989) and in some of our previous papers (Bonina et al., 1991, 1993), to obtain an enhanced dermal delivery by prodrug approach, prodrugs should possess both increased lipophilicity and water solubility compared with the parent drug. In the present study, esters 1–4 showed a notable increase in lipophilicity, but were less water soluble compared with the parent drug.

### 3.4. *In vivo anti-inflammatory activity of indomethacin, ester 1 and ester 2*

Esters 1–2, which showed the best in vitro profiles, were assessed for their topical anti-inflammatory activity in order to perform in vitro/in vivo comparisons.

Different models have been reported in the literature for evaluating topical anti-inflammatory activity of NSAIDs (Bouclier et al., 1989). Among these models, UV-B- and methyl nicotinate-induced erythema are the most used in humans

Table 2
$AUC_{0-100}$ values obtained pretreating skin sites with gel containing indomethacin, ester 1 or ester 2 and applying MN immediately after gel removal ($t = 0$), 3 h ($t = 3$) or 6 h ($t = 6$) later

| Subject | $AUC_{0-100}$ [a] | | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|
| | $t = 0$ | | | $t = 3$ | | | $t = 6$ | | | |
| | Indomethacin | Ester 1 | Ester 2 | Indomethacin | Ester 1 | Ester 2 | Indomethacin | Ester 1 | Ester 2 | |
| A | 484.5 | 1171.0 | 1113.1 | 811.3 | 1014.8 | 1094.5 | 810.9 | 650.1 | 1117.3 | 1158.0 |
| B | 530.0 | 1292.3 | 1415.6 | 775.2 | 1082.6 | 1215.0 | 702.4 | 726.4 | 1094.5 | 1325.8 |
| C | 390.3 | 824.5 | 942.4 | 635.4 | 670.4 | 824.3 | 754.2 | 530.8 | 769.7 | 984.4 |
| D | 492.5 | 1221.7 | 1212.1 | 785.2 | 1055.0 | 1034.2 | 978.6 | 784.3 | 1215.6 | 1167.3 |
| E | 505.4 | 1092.3 | 1354.3 | 725.2 | 1025.3 | 1178.5 | 985.4 | 822.5 | 1122.3 | 1275.6 |
| F | 687.5 | 1152.5 | 1272.5 | 887.4 | 1021.7 | 1125.7 | 955.8 | 875.4 | 985.2 | 1084.2 |
| Mean | 515.0* | 1125.7 | 1218.3 | 769.9* | 978.3** | 1078.7 | 864.5* | 639.9* | 1050.8 | 1165.9 |
| ±S.D. | 97.2 | 162.2 | 171.8 | 84.7 | 153.0 | 139.8 | 124.3 | 109.8 | 156.2 | 124.3 |

[a] Each value represents the mean of two different sites in the same subjects.
* $p < 0.01$ compared with control; ** $p < 0.05$ compared with control.

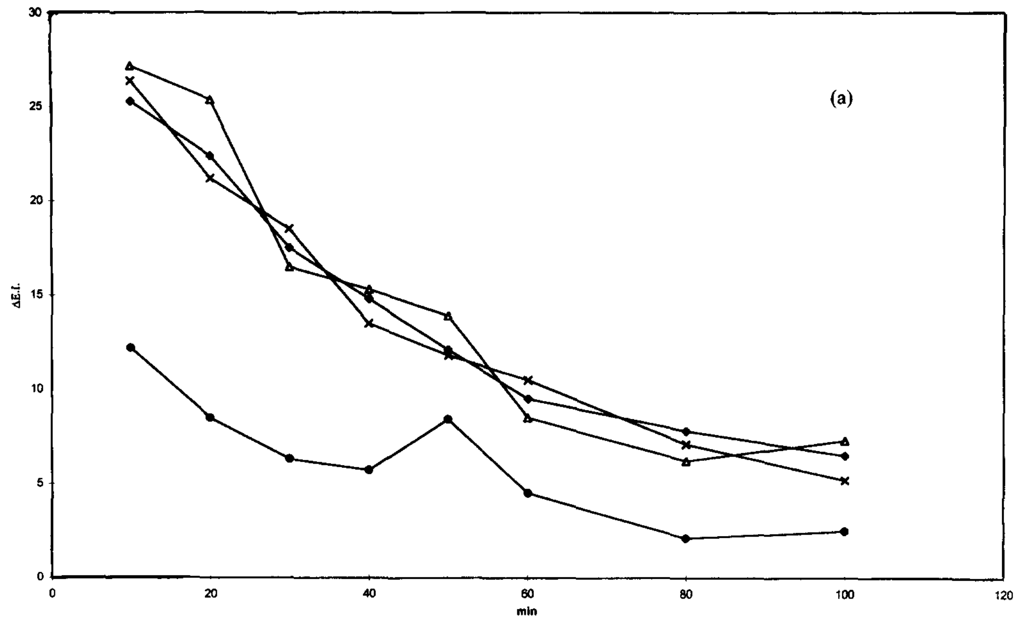
(Farr and Diffey, 1986; Chan and Li Wan Po, 1992). Recently, non-invasive techniques such as laser Doppler flowmetry, reflectance colorimetry or spectrophotometry have been used to obtain objective and more accurate evaluations of skin erythema (Seitz and Whitmore, 1988; Feather et al., 1989; Andersen et al., 1991). Reflectance filter colorimetry has been extensively used (Seitz and Whitmore, 1988; Westerhof et al., 1988) for designating the extent of erythema, by measuring the skin color surface in terms of CIE (Commission International d'Eclairage) $L^*a^*b^*$ color space parameters, since some authors (Braue et al., 1990) found significant correlation between $a^*$ values and visual grading of skin erythema. Unlike reflectance colorimetry, reflectance spectrophotometry provides skin reflectance spectra, generally in the range 400–700 nm, from which it is possible to obtain erythema index (EI) values (see Eq. (1)), providing very accurate and reliable evaluations of skin erythema (Dawson et al., 1980; Andersen and Bjerring, 1990).

The EI values were calculated, at each site and at different times, to monitor the extent of MN-induced skin erythema.

Since the bioavailability of MN in solution is about 10–100 times more rapid than that of NSAIDs such as indomethacin (Nowack et al., 1985), we pre-treated the skin with gel containing indomethacin or ester 1–2, before MN application. A 3-h pre-treatment time, under occlusion, was chosen since Nowack et al. (1985), studying the percutaneous bioavailability of 1% indomethacin gel applied under occlusion in humans, reported that drug maximal concentration was obtained at between 2 and 4 h. A typical time course of MN-induced erythema on skin sites pre-treated with indomethacin or ester 1–2 gels, for one subject, is reported in Fig. 3. Plotting ΔEI values versus time, AUC values were determined, for each subject, by calculating the areas between the response curve and the $x$-axis, and the mean AUC values are reported in Table 2. AUC values were inversely related to indomethacin and ester 1–2 ability to inhibit MN-induced skin erythema.

As may be noted in Table 2, ester 2 was not effective in inhibiting MN-induced erythema since no significant difference was observed compared with the control ($p > 0.05$).

Regarding indomethacin and ester 1, a time-dependent profile of activities was observed applying MN at different times ($t = 0$, 3 and 6 h) after active compound removal. Indomethacin showed a notable but decreasing efficacy, while ester 1 did not show any activity at $t = 0$ h, was slightly active at $t = 3$ h, and at $t = 6$ h was significantly more active than the parent drug ($p < 0.01$).



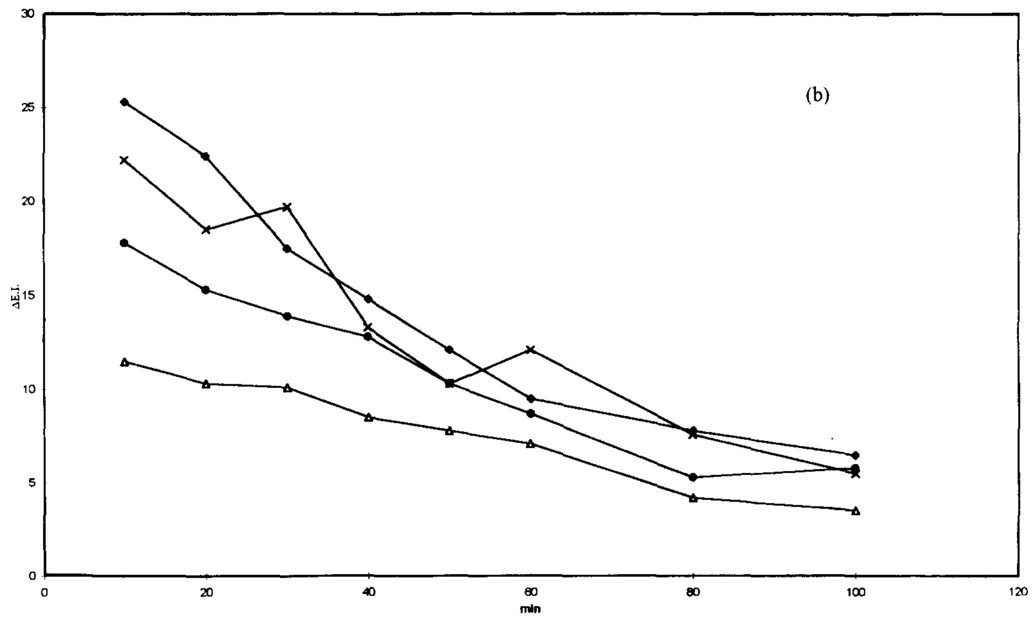




Fig. 3. ΔEI values vs. time for one subject. Indomethacin, ester 1 or ester 2 gels were applied for 3 h and MN was applied (a) immediately after their removal ($t = 0$), or (b) 6 h after their removal ($t = 6$). (●) Indomethacin; (△) ester 1; (×) ester 2; (◆) control.

As reported by other authors (Poelman et al., 1989), to better outline the results obtained, it is possible from AUC values to calculate the percentage inhibition of the inflammatory reaction induced by MN:

$$\text{Inhibition } (\%) = \frac{AUC_{(C)} - AUC_{(T)}}{AUC_{(C)}} \times 100 \quad (2)$$

where $AUC_{(C)}$ in the area under the response–time curve on the vehicle-treated site (control), and $AUC_{(T)}$ is the area under the response–time curve on the drug-treated site.

As may be noted in Fig. 4, the percentage of indomethacin inhibition was maximal (about 56%) when MN was applied immediately after gel removal, while it notably decreased (up to 26%) at $t = 6$ h. Since it is reasonable to suppose that the anti-inflammatory activity is related to indomethacin concentration in viable epidermis, these results suggest a rapid depletion of indomethacin in the viable tissue. A different trend was observed for ester 1, whose anti-inflammatory activity at $t = 0$ was negligible, increased at $t = 3$ h (about 16%) and increased again at $t = 6$ h (about 37%). This delayed topical anti-inflammatory activity of ester 1 could be explained on the basis of a greater SC reservoir, due to its increased lipophilicity compared with the parent drug, and a slow release from SC to underlying aqueous tissue, modulated by its enzymatic hydrolysis rate.

The same trend of ester 1 was observed in a series of polyoxyethylene indomethacin esters assessed as dermal prodrugs using the same experimental pattern (Bonina et al., 1995a).

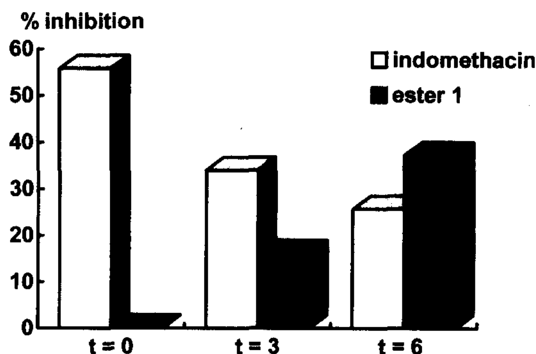



Fig. 4. Indomethacin and ester 1 percentage inhibitions of the inflammatory reaction induced by MN.

No relationship was observed between in vitro skin permeation of ester 1–2 and their anti-inflammatory topical activity. Both ester 1–2 showed a slightly higher skin permeability compared with indomethacin, but only ester 1 was more effective than the parent drug in reducing MN-induced skin erythema, and only at $t = 6$ h. The lack of correlation between in vitro permeability and in vivo efficacy at $t = 0$ h noted for ester 1 could be due to a slow prodrug hydrolysis within the skin. No explanation can be furnished for the ineffectiveness of ester 2.

The pharmacological results of ester 1 confirm that it could be important to evaluate in vivo dermal prodrugs efficacy at different times after their skin application, since a suitable time could be needed for regenerating and achieving skin therapeutic concentrations of parent drug, as we reported in a previous paper (Bonina et al., 1995a).

In conclusion, among esters 1–4, only esters 1–2 seem to increase indomethacin skin permeation, and only ester 1 showed a greater activity compared with the parent drug in inhibiting MN erythema induced 6 h after active compound removal. This interesting delayed anti-inflammatory activity has the same trend showed by some polyoxyethylene indomethacin esters (Bonina et al., 1995a) and suggests that the therapeutic efficacy of indomethacin as a topical drug could be prolonged by co-administering it with a prodrug that had such a pharmacological profile.

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