High-Performance Liquid Chromatography Determination of Acitretin in Plasma and Its Application to a Pharmacokinetic Study in Human Subjects

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INTRODUCTION

Etretinate (Tigason, Tegison), an aromatic retinoid, has now been used for over a decade in the treatment of dermatologic diseases such as psoriasis and congenital disorders of keratinization. Acitretin, previously etretin (Neotigason, Soriatane), is the main and active metabolite of etretinate, with clinical effects comparable to those of etretinate (Fig. 1) (1). In contrast to etretinate, acitretin is not sequestrated into fatty tissue and, as a consequence, is eliminated more rapidly from the body (2). Etretinate is extremely slowly eliminated, with a terminal plasma half-life of up to 120 days. This is possibly due to the lipophilicity of the drug causing accumulation in fatty tissues from where it is slowly released. Acitretin, the corresponding carboxylic acid, is less lipophilic and has been found to be eliminated much faster ($t_{1/2} = 2–4$ days) (2). The initial and major blood metabolite of acitretin (all-trans-acitretin) is the 13-cis isomer of acitretin (13-cis-acitretin) (Fig. 1) (3). Several methods for quantification of etretinate and acitretin in biological fluids by high-performance liquid chromatography (HPLC) with detection limits of 2–5 ng/ml have been described previously (4–6). These methods usually use all-trans-retinoic acid or 13-cis-retinoic acid as internal standard. However, retinoic acids are endogenous and can be detected in the range of 1 to 2 ng/ml in blank plasma samples (7–9). This may introduce errors measuring low acitretin concentrations when using low, adequately adjusted concentrations of internal standard. This paper describes a simple and sensitive isotropic HPLC method for the simultaneous and specific analysis of all-trans-acitretin and 13-cis-acitretin in plasma. The method was validated and subsequently applied to a pharmacokinetic study in subjects receiving multiple oral doses of acitretin.

MATERIALS AND METHODS

**Human Subjects.** Nine healthy subjects (all male; mean age 30.3 years; range, 26–33 years; mean weight, 71.4 kg; range 61–81 kg) took part in the study. A medical history was elicited from each subject and all underwent a complete physical and laboratory examination before, twice during, and once after the study. All subjects totally free of any preexisting dermatologic disease had to have normal blood values and had not to have been treated previously with any natural or synthetic retinoids. After the briefing, they were asked to sign a consent form. The trial was approved by the local Ethics Committee.

**Dosing and Sample Collection.** Capsules containing 25 mg acitretin were supplied by Roche Dermatologics, Nutley, NJ. The dose was 50 mg/day and the drug was taken with a standard breakfast once daily. Blood (10 ml) was collected by venipuncture into oxalated Vacutainers at appropriate points of time for 6 weeks. Group I ($n = 4$) was treated for 3 weeks. Blood was drawn on days 0, 7, and 14 before and 3 hr after dosing and on day 21 before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 24, 48, and 96 hr after dosing. Additional blood samples were taken 7 and 14 days after the last dose. Group II ($n = 5$) was treated for 4 weeks. Blood was drawn on days 7 and 14 before dosing and on days 21 and 28 before and 3 hr after dosing. Additional blood samples were drawn 7 and 14 days after the last dose.

**Photoprotection.** Because of photodegradation and photoisomerization of the retinoids, all manipulations with the samples were performed under minimal light exposure (e.g., yellow light, total light protection).

**Reagents and Standard Solutions.** All solvents were of analytical grade (Carlo Erba RS HPLC, France). HPLC-grade water was prepared from deionized water by purification with the Milli-Q Reagent Water System (Millipore Corp., France). All trans-acitretin (Ro 10-1670), 13-cis-acitretin (Ro 13-7652), and internal standard (aromatic ethyl sulfone; Ro 15-1570) (Fig. 1) were supplied by Hoffmann-La Roche Ltd. (Basel, Switzerland). Stock methanolic solutions (100 µg/ml) of all-trans-acitretin, 13-cis-acitretin, and the internal standard were prepared every 2 months, stored at 4°C in amber glass volumetric flasks, and screened from light. Working solutions of these compounds were freshly prepared every week by successive dilutions of the stock solutions in methanol.

**Extraction Procedure.** The plasma extraction procedure has been reported previously (8). Briefly, after the addition of a suitable volume of internal standard and pH 7 buffer, extraction from 0.3 to 1 ml plasma was performed by diethyl ether/ethyl acetate (1/1, v/v). The organic solvent layer was separated and evaporated under a stream of nitrogen. The residue was then dissolved in 30–50 µl methanol and transferred into an injection vial for HPLC analysis. This provided an extraction recovery better than 80%.

**Chromatography.** Analyses were performed with a Kontron liquid chromatograph equipped with an autosampler (Model 460) and a variable-wavelength UV detector.
Uvikon (Model 430). A 250 × 4.2-mm (inner diameter) reversed-phase column with Nucleosil C18, 5 μm, was used at ambient temperature. The following mobile phase was used: organic phase, methanol/acetonitrile (7/3, v/v); and aqueous phase, purified water with 98.5/1.5 (v/v) acetic acid. The components of the mobile phase were filtered before degassing through a 0.2-μm membrane (organic phase) and a 0.45-μm membrane (aqueous phase). The elution solvent was obtained by mixing the organic and aqueous phases (85/15, v/v). The flow rate was 1.2 ml/min and the detection wavelength was 350 nm. Under these conditions, the retention times were 6.0, 6.9, and 7.8 min for 13-cis-acitretin, all-trans-acitretin, and internal standard, respectively (Fig. 2). Peak-height ratios were computed by means of Kontron data system D450 and calibration curves for plasma were obtained from least-squares linear regression established daily from at least four calibration points.

**Data Analysis.** Pharmacokinetic parameters were calculated for group I. For all-trans-acitretin we used APIS software (10) on an IBM PS2 8555 microcomputer for data processing with a two-compartment model. For 13-cis-acitretin we used a noncompartmental calculation. The data from group II were included for the determination of the disappearance of drug from plasma after cessation of the treatment.

**Table 1. Intraassay Precision and Accuracy for (A) All-trans-Acitretin and (B) 13-cis-Acitretin in Plasma**

<table>
<thead>
<tr>
<th>ng added (X₀)</th>
<th>n</th>
<th>Xₘ</th>
<th>Sₓ</th>
<th>CV %</th>
<th>Bias %</th>
<th>Xₘ</th>
<th>Sₓ</th>
<th>CV %</th>
<th>Bias %</th>
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<tr>
<td>1</td>
<td>6</td>
<td>1.01</td>
<td>0.12</td>
<td>12</td>
<td>0.05</td>
<td>1.13</td>
<td>0.20</td>
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<tr>
<td>5</td>
<td>6</td>
<td>4.46</td>
<td>0.43</td>
<td>9</td>
<td>10.8</td>
<td>4.76</td>
<td>0.28</td>
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</tr>
<tr>
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<td>0.42</td>
<td>4</td>
<td>0.07</td>
<td>9.75</td>
<td>0.65</td>
<td>7</td>
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</tr>
<tr>
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<td>216.79</td>
<td>10.14</td>
<td>5</td>
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<td>216.77</td>
<td>9.34</td>
<td>4</td>
<td>8.3</td>
</tr>
</tbody>
</table>

* X₀, drug amount added; Xₘ, mean value; Sₓ, standard deviation (n − 1); CV %, coefficient of variation Sₓ × 100/X₀; bias %, (xₘ − X₀) × 100/X₀.