Oral Isotretinoin (13-cis-Retinoic Acid) Therapy in Severe Acne: Drug and Vitamin A Concentrations in Serum and Skin

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The disposition of oral isotretinoin to the skin and the effects of the drug on the vitamin A levels in serum and skin were studied in 17 patients with nodulocystic acne. All patients received 0.5 mg/kg/day for 3 months and 8 patients continued treatment with 0.75 mg/kg/day for another 3 months. The parent drug, the major metabolite (4-oxo-isotretinoin), and 2 natural retinoids (retinol and dehydroretinol) were monitored in serum and biopsies of uninvolved skin, using adsorption high-pressure liquid chromatography. During the initial 3 months of treatment the mean isotretinoin level in the serum was 145 ng/ml and in the epidermis 73 ng/g. The corresponding values for 4-oxo-isotretinoin were 615 and 113 ng/g, respectively. Even at the highest dosage there was no progressive accumulation of isotretinoin in serum, epidermis, or subcutis. After discontinuation of therapy the drug disappeared from both serum and skin within 2–4 weeks. The serum transport of vitamin A, monitored by the concentrations of retinol, retinol-binding protein, and prealbumin (transhyretin), was not affected by the treatment. By contrast, the retinol level in the epidermis increased by an average of 53% (p < 0.01) and the dehydroretinol level decreased by 79% (p < 0.001) as a result of 3 months of treatment. Both changes were reversible. The results suggest that isotretinoin therapy interferes with the endogenous vitamin A metabolism in the skin. J Invest Dermatol 86:384–389, 1986

MATERIALS AND METHODS

Patients The study comprised 17 patients (13 men, 4 women) aged 19–40 (mean 25.3) years with nodulocystic acne, participating in an open clinical trial of oral isotretinoin. The mean duration of the disease was 9.6 (0.5–26) years. Topical acne preparations were withdrawn at least 3 weeks prior to isotretinoin therapy. No concomitant systemic medication was allowed. Three females started with oral contraceptives 2 months before the trial and continued to take them throughout the study.

Two healthy male subjects served as placebo-treated controls. All patients were in good general health and, except for a staphylococcal carbuncle in 1 patient, no concurrent diseases developed. There was no dropout from the study. Our patients took part in a multicenter trial, the excellent clinical results of which will be presented elsewhere.

Written informed consent was obtained from the patients, and the study was approved by the Ethical Committee of Uppsala University.

Treatment Capsules containing 5, 10, or 20 mg of isotretinoin (Roaccutane) in oil were supplied by Hoffmann-La Roche & Co., AG, Basel, Switzerland. The initial oral dose was 0.5 mg/kg/day and the drug was taken with meals twice daily for 3 months. At the end of this period the dose was adjusted according to the clinical response. Thus, 8 patients with less than 66% improvement received 0.75 mg/kg/day for another 3 months, whereas the other 9 patients stopped treatment.

Placebo capsules, not containing isotretinoin, were provided by Hoffman-La Roche and administered (5 per day) to the 2 healthy controls.

Tissue and Blood Collection Epidermal shave biopsies (10–25 mg) and subcutaneous fat samples (5–15 mg) were obtained from
apparently normal skin on the buttock as described elsewhere [13, 14]. The biopsy was taken shortly before isotretinoin therapy and after 1, 3, and 6 months of therapy and again approximately 1, 3, and 6 months after discontinuation of the drug. The tissues were snap-frozen on dry ice and stored for 1–3 months at −70°C until analyzed. Fasting blood samples were drawn 8–18 h after a previous dose, using vacuum glass tubes wrapped in aluminum foil. Serum was separated by centrifugation and stored at −70°C until processed.

**Chemicals** Methanol, n-hexane, and ethyl acetate of HPLC grade were from Rathburn Chemicals Ltd., Walkerburn, U.K. Glacial acetic acid of analytical quality was from E. Merck, Darmstadt, West Germany. Ethanol of spectrographic grade was from Svensk Sprit AB, Sweden. Deionized water was passed through a Milli-Q water purification system (Millipore Corp., Bedford, Massachusetts) before use.

Crystaline all-trans retinol and tretinoin (all-trans-retinoic acid) were purchased from Sigma Chemical Co., St. Louis, Missouri. Isotretinoin, 4-oxo-isotretinoin, 4-oxo-tretinoin, the carboxylic acid of etretinate (Ro 10-1670), 13-cis isomer of Ro 10-1670 (Ro 13-7652), an aromatic analogue of all-trans retinol (Ro 12-0586), 11,13-di-cis-tretinoin, and 3,4-dehydroretinoin were gifts from Hoffmann-La Roche & Co., AG, Basel, Switzerland or Nutley, New Jersey.

**Internal Standard** The internal standard solution was prepared in a volumetric flask by dissolving 2.9 μg of aromatic analogue of retinol, 5.5 μg of carboxylic acid of etretinate, and 3.0 mg of butylated hydroxytoluene in 120 ml of ethanol. The solution was stored at −70°C under nitrogen.

**Saponification and Extraction** A previously described procedure [15] was used with some modifications. The tissue specimen or serum (100–500 μl) was added to a screw-capped glass tube containing 100 μl of 80% KOH and 250 μl of the standard solution. The tubes were flushed with nitrogen, sealed, and heated at 80°C for 15 min on a Multi-block heater (Lab-line Instruments Inc., Melrose Park, Illinois). After the tubes had been cooled in ice water, 0.5 ml of H₂O and 4 ml of hexane were added. The tubes were flushed with nitrogen and vigorously shaken for 5 min. The hexane layer was removed after centrifugation for 2 min at 600 g. The extraction was repeated once with a fresh portion of hexane. The combined supernatant (containing neutral retinoids) was evaporated to dryness at 50°C under a stream of nitrogen. A second extract (containing acidic retinoids) was obtained after adjustment of the remaining aqueous phase to pH 4–5 followed by extraction with 4 ml of hexane as above. Both the evaporated extracts (one containing neutral retinoids and the other containing acidic retinoids) were dissolved in 75 μl of the mobile phase (see below) by ultrasonic agitation for 3 min. Before injection onto the HPLC column, the sample was centrifuged for 5 min at 1300 g to remove particulate matters.

All samples were handled under yellow light to minimize photo-destruction of the retinoids.

**Chromatography** The HPLC equipment included an Altex Model 110 solvent metering pump with a Model 110–40 filter (Altex Scientific Inc., Berkeley, California), a Rheodyne Model 7120 sample injector valve equipped with an external 50 μl sample loop (Rheodyne Inc., Berkeley, California), and a Model 440 absorbance detector (Waters Assoc. Inc., Milford, Massachusetts) operating at fixed wavelengths (340 and 365 nm). Two dual-pen chart recorders (Linear Instruments, Irvine, California) were fitted with inputs of 1 and 10 mV, respectively, allowing a wide range of optimal detection (0.02–0.002 full-scale absorbance). Adsorption chromatography was performed at ambient temperature on a 150 × 4.6 mm (i.d.) column packed with 5-μm Nucleosil silica gel (Skandinaviska Genetec AB, Kungsbacka, Sweden). A Guard-PAK precolumn module (Waters Assoc. Inc.) containing a silica cartridge was used.

The first extract (containing neutral retinoids) was chromatographed isocratically (flow rate 1.2 ml/min) in a mixture of hexane and ethyl acetate (85:15 v/v) containing 0.5% ethanol and 0.05% acetic acid.

The second extract (containing acidic retinoids) was chromatographed in a discontinuous gradient changing the hexane to ethyl acetate ratio (v/v) from 93:7 to 85:15 at 6 min after injection of the sample (the solvents were otherwise identical, i.e., they contained 0.5% ethanol and 0.05% acetic acid). The flow rate was 1.4 ml/min and the time between injections (including re-equilibration) was 35 min.

**Calibration and Quantitation** Standard plots were prepared by adding increasing amounts of retinol, dehydroretinol, isotretinoin, and 4-oxo-isotretinoin to test tubes containing the internal standard solution (250 μl) and samples (20 mg) of vitamin A-depleted [16] skin (Figs 1, 2). As a result of saponification, the carboxylic acid of etretinate (internal standard) was partially

![Figure 1](image-url)

**Figure 1.** HPLC of (a) neutral and (b) acidic retinoids added to a vitamin A-depleted skin sample subsequently hydrolyzed and extracted with hexane (calibration runs). For details see Materials and Methods. The following retinoid standards were added: all-trans retinol (peak 1), dehydroretinol (peak 2), aromatic analogue of all-trans retinol (internal standard; peak 3), isotretinoin (peak 4), carboxylic acid of etretinate (internal standard; peak 5) and 4-oxo-isotretinoin (peak 12). Additional peaks appearing during analysis were: tretinoin (peak 5); 11,13-di-cis-tretinoin (peak 6), 13-cis carboxylic acid of etretinate (peak 7), and 4-oxo-tretinoin (peak 11). Peaks 9 and 10 were not identified. The base-line shifts in (b) at 10 and 18 min after sample injection (inj) were the results of a discontinuous change in solvent mixture gradients. Owing to the high extent of artifactual isomerization of 4-oxo-isotretinoin, the sum of the heights of peaks 11 and 12 was related to the sum of peaks 7 and 8 for quantitations.
(25–35%) converted to the 13- cis configuration (Fig 1b) and, conversely, isoretinoin and 4-oxo-isoretinoin isomerized to the all-trans configuration (25–35% and 50–61%, respectively). Since the artificial cis-trans isomerization of 4-oxo-isoretinoin was not invariably proportional to that of the internal standard, quantitation was accomplished by relating the sum of the peak heights of 4-oxo-retinoin and 4-oxo-isoretinoin to the sum of the cis and trans isomers of the internal standard. In contrast, retinol, dehydroretinol, and isoretinoin were quantitated by simply relating the peak height of the parent compound to that of the appropriate internal standard.

The coefficients of variation for analysis of retinol, isoretinoin, and 4-oxo-isoretinoin in a patient’s serum were 6.7, 7.7, and 8.7 (within-run; n = 10), and 4.4, 4.3, and 12.4 (between-run; n = 4), respectively. Dehydroretinol could not be assessed in this respect since it is not detectable in serum. By reverse-phase HPLC, however, the coefficients of variation for the analysis of epidermal retinol and dehydroretinol are similar [13]. Storage for 3 months reduced the retinoid concentrations by less than 5%.

Other Analyses Serum RBP and prealbumin concentrations were measured by radial immunodiffusion [17] using commercial rabbit antisera (Behringwerke AG, Marburg, West Germany).

Statistics For statistical evaluation a 2-way analysis of variance was employed [18].

RESULTS

Isoretinoin The serum and epidermal concentrations of isoretinoin and 4-oxo-isoretinoin during and after treatment with isoretinoin are shown in Table 1. In serum, the main metabolite usually predominated over isoretinoin by a factor of about 4.

The concentrations of both compounds remained fairly constant during the initial 3 months of therapy. Patients subsequently receiving 0.75 mg/kg/day, showed somewhat higher values at 6 months than at 3 months, although the difference was not statistically significant (all data not shown).

The epidermal levels of the drug were low. In order to obtain adequate measurements, acidic extracts of 3–5 skin samples were pooled prior to HPLC analysis. As seen in Table 1, the predominance of 4-oxo-isoretinoin over isoretinoin was less apparent in the skin than in the serum. In the limited series of samples, dose-related changes in the epidermal drug concentration were not assessable. However, 1 month after discontinuation of therapy the drug level in both epidermis and serum was below the detection limit.

Table 1. Serum and Epidermal Concentrations (Mean ± SEM) of Isoretinoin and 4-Oxo-isoretinoin During and After Isoretinoin Therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg/day)</th>
<th>No. of Patients</th>
<th>Serum</th>
<th>Epidermis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Isoretinoin</td>
<td>4-Oxo-isoretinoin</td>
</tr>
<tr>
<td>1 month</td>
<td>0.5</td>
<td>17</td>
<td>171 ± 23.2</td>
<td>594 ± 60.4</td>
</tr>
<tr>
<td>3 months</td>
<td>0.5</td>
<td>17</td>
<td>128 ± 40.7</td>
<td>634 ± 63.0</td>
</tr>
<tr>
<td>6 months</td>
<td>0.75</td>
<td>8</td>
<td>154 ± 36.0</td>
<td>642 ± 131</td>
</tr>
<tr>
<td>Posttreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>10</td>
<td></td>
<td>6.5 ± 1.6</td>
<td>12.8 ± 6.4</td>
</tr>
<tr>
<td>2 weeks</td>
<td>9</td>
<td></td>
<td>ND</td>
<td>6.7 ± 2.7</td>
</tr>
<tr>
<td>4 weeks</td>
<td>8</td>
<td></td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Samples were obtained 8–18 h after the last previous dose.
*The values do not distinguish 4-oxo-isoretinoin from 4-oxo-retinoin (see Materials and Methods).
*Each sample consisted of pooled extracts obtained from 3–5 identically treated patients.
*Not detected. The approximate detection limits were 5 ng/ml for serum and 20 ng/g for epidermis (50 mg).
*Value for patients treated for 6 months; the level in patients treated for only 3 months was below the detection limit.
Table II. Serum Concentrations (Mean ± SEM) of Retinol, RBP and Prealbumin in Patients Treated with Oral Isotretinoin

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Dose (mg/kg/day)</th>
<th>Retinol (µg/dl)</th>
<th>RBP (µg/ml)</th>
<th>Prealbumin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td></td>
<td>64.5 ± 5.43</td>
<td>40.9 ± 1.60</td>
<td>260 ± 7.8</td>
</tr>
<tr>
<td>Treatment 1 month</td>
<td>17</td>
<td>62.6 ± 6.16</td>
<td>39.2 ± 1.98</td>
<td>267 ± 11.1</td>
</tr>
<tr>
<td>Treatment 3 months</td>
<td>17</td>
<td>63.4 ± 6.78</td>
<td>39.4 ± 1.75</td>
<td>280 ± 7.2</td>
</tr>
<tr>
<td>Treatment 6 months</td>
<td>8</td>
<td>65.8 ± 6.71</td>
<td>41.1 ± 2.61</td>
<td>287 ± 10.9</td>
</tr>
<tr>
<td>Posttreatment (1-3 months)</td>
<td>17</td>
<td>69.5 ± 6.39</td>
<td>45.0 ± 2.73</td>
<td>301 ± 10.1</td>
</tr>
</tbody>
</table>

*None of the values observed during and after treatment differed significantly from the pretreatment values (p > 0.05).

In the subcutis no detectable amounts of 4-oxo-isotretinoin or isotretinoin were found during the first 3 months of treatment. In patients treated with the higher dose of the drug, the mean concentration of 4-oxo-isotretinoin was low (51 ng/g wet tissue) and isotretinoin was not detected. At the first follow-up, 1 month after withdrawal of therapy, both compounds were undetectable (<40 ng/g) in the subcutis.

Vitamin A. The mean serum concentrations of retinol and its transport proteins, RBP and prealbumin, did not alter significantly in association with isotretinoin treatment (Table II). No dehydroretinol was detected in the serum.

The epidermal contents of retinol and dehydroretinol changed markedly during the treatment (Fig 3). The mean concentration of retinol increased from 185 ng/g before therapy to 293 ng/g after 3 months of therapy (p < 0.01). Conversely, the mean dehydroretinol level decreased from 78 ng/g to 18 ng/g (p < 0.001) and in 4 of the patients the level of this compound actually fell below the detection limit of the assay (10 ng/g). As seen in Fig 3, in patients who continued to take isotretinoin for an additional 3 months, the altered retinol and dehydroretinol values persisted until the drug was discontinued. Pretreatment values were usually regained within 1 month after cessation of therapy. In 2 placebo-treated controls the epidermal values changed by less than 20% over a 6-week period (mean concentrations of retinol/dehydroretinol before and during placebo: 162/52 ng/g and 189/46 ng/g, respectively).

The subcutaneous concentration of retinol did not change significantly during isotretinoin treatment (mean values 2107, 2063, and 1733 ng/g, before, during, and after therapy). Since the concentration of dehydroretinol in the subcutis was usually below the threshold of detection (30 ng/g), the effect of isotretinoin on this substance could not be evaluated.

**DISCUSSION**

Methodology. Several HPLC methods have been described for measuring isotretinoin in blood [19-23]. Unfortunately, none of the methods could be applied directly to the determination of isotretinoin and 4-oxo-isotretinoin in epidermal shave biopsies. Initial experiments showed that in order to permit detection of small amounts of isotretinoin in the skin, this compound had to be an early eluent from the HPLC column. Adsorption (normal-phase) chromatography on silica gel, performed with a discontinuous gradient, allowed simultaneous analysis of isotretinoin and 4-oxo-isotretinoin, which were eluted in that order. A similar HPLC system, but without a gradient, was used for measurement of retinol and dehydroretinol. This assay yielded essentially the same results as reverse-phase HPLC, but gave somewhat higher serum retinol values than a fluorometric assay used previously [11]. The main disadvantages of the normal-phase HPLC system used were the long run times and the difficulties in achieving constant retention times during gradient elution, particularly when retinoids were assayed in fat-rich samples.

In order to obtain a quantitative recovery of retinoids from skin samples a hydrolytic extraction was used. For congruence, the sera were analyzed with the same hydrolytic procedure. By this procedure the retinoid esters were saponified. Furthermore, some artificial isomerizations occurred that were accounted for in the standardization (see Materials and Methods).

The shave-biopsy technique deserves special comments. For an experienced operator it is quite possible to adjust the cutting depth so that the skin sample will be uniformly composed of about 60-70% epidermis [13]. Scarring is not a problem with this technique, a matter of importance when up to 5 biopsies are required from one and the same patient. Unfortunately, the papillary dermis contaminating the epidermal sheets cannot be removed without exposing the sample to conditions harmful to the unstable

![Figure 3. Epidermal concentrations (mean ± SEM) of (a) retinol and (b) dehydroretinol in relation to isotretinoin therapy. All patients received a dose of 0.5 mg/kg/day for the first 3 months. At the end of this period the mean retinol and dehydroretinol values did not differ between patients who subsequently received treatment and those who did not.](image_url)
retinoids. However, this "contamination" does not appear to contribute significantly to the variability of epidermal retinoid values [13] and should certainly not result in spurious changes in retinoid mean levels observed at different times of therapy.

Pharmacodynamics Our results for the serum concentrations of isotretinoin 8–18 h after an oral dose of approximately 25 mg b.i.d. are in accordance with those of Brazzell et al [22], who used a different analytical procedure. Thus, during chronic dosing, 4:4:4-isotretinoin predominated 4- to 5-fold over the parent drug. Other metabolites, such as tretinoin, 11,13-di-cis-tretinoin, and 4-oxo-tretinoin [23], accounted for less than 10% of the drug concentration in a few sera extracted without hydrolysis (Rollman and Vahlquists unpublished observations), but were not assessable in hydrolyzed samples. The elimination of isotretinoin from the blood was rapid, especially compared with that of the aromatic retinoid etretinate [24].

To the best of our knowledge, no previous investigation on the distribution of isotretinoin in human skin has been undertaken. We found that the drug concentrations were generally lower in the epidermis than in the serum. This difference was especially evident for 4:4:4-isotretinoin, a compound of unknown biologic significance.

The total epidermal concentration (parent compound plus main metabolite) of isotretinoin is similar to that of etretinate, but in the subcutis these concentrations differ markedly [14]. Thus, etretinate accumulates in fat tissue, resulting in a very long biologic half-life of the drug [24], whereas isotretinoin seems to be devoid of affinity to this tissue compartment. The question of whether isotretinoin accumulates in the sebaceous glands, essential targets in the treatment of acne, remains to be elucidated.

Interaction with Vitamin A The effect of some synthetic retinoids on the transport and metabolism of vitamin A has been investigated previously in both humans and experimental animals. For example, in rats fed with isotretinoin [25] or tretinoin [26,27], the plasma level of retinol decreased, presumably by feedback inhibition of its release from the liver [26,27]. By contrast, increased serum vitamin A levels were observed in 3 patients treated with tretinoin for follicular keratoses [28]. In our series of isotretinoin-treated patients there were no significant changes in the serum levels of retinol, BDP, or prealbumin (transhytrenin).

Analogously, 2 previous studies [29,30] showed no effect of etretinate on the plasma transport of vitamin A in humans.

On the other hand, we recently reported that etretinate therapy reduced the epidermal retinoid level and slightly increased the level of dehydroretinol in the normal skin of patients with keratinizing disorders [14]. Isotretinoin, which induces similar structural changes in epidermis (e.g., cornification, thickening, and dermalis) as etretinate [3], had quite a different effect on the epidermal vitamin A content. The concentration of retinol in the epidermis almost doubled and the dehydroretinol concentration decreased to one-fifth of the original value. These effects, which were reversible after cessation of therapy and did not appear during placebo treatment, could for obvious reasons not be the result of a changed plasma transport of retinol to the skin. We find it more likely that alterations in the local vitamin A metabolism are involved. Since isotretinoin cannot be converted to retinol in the tissue [31], the increase in epidermal retinol induced by the treatment is probably due either to stimulated uptake of retinol to keratinocytes or to inhibition of the retinol metabolism in the cells.

The factors regulating the epidermal dehydroretinol level are virtually unknown. Dehydroretinol (or its conjugate in unhydrolyzed skin) is an in situ metabolite of retinol which accumulates in connection with epidermal hyperproliferation [11,32–34]. A situation in which dehydroretinol is decreased has not previously been encountered. Hopefully, the interactions between isotretinoin and endogenous retinol and dehydroretinol, may be instrumental in future investigations of the factors regulating epidermal vitamin A metabolism.

It is not known whether the change in the epidermal vitamin A composition induced by isotretinoin therapy is specific for acne patients. In the present series the treatment raised the decreased epidermal retinol concentrations found in untreated acne patients [11], but there was no apparent correlation between the clinical response to isotretinoin and the change in epidermal vitamin A composition during treatment (results not shown). However, this does not exclude the possibility that the effects on epithelial vitamin A are involved in the mechanism of action of isotretinoin.

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