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Tissue Distribution of Aromatic Retinoid (Etretinate) in Three Autopsy Cases: Drug Accumulation in Adrenals and Fat

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The problematic storage of etretinate in fat during chronic etretinate therapy prompted us to search for other "high-affinity" tissues in 3 patients studied at autopsy. Specimens from eleven organs were analysed for etretinate and its main metabolite, etretin, by high-performance liquid chromatography. The results confirmed an accumulation of etretinate in fat and to a lesser degree in liver. High levels of etretinate were also found in the adrenals and, in one case, this value exceeded that of the fat tissue. Low levels were observed in several other organs, notably the kidneys, brain and testis. With the possible exception of liver and gut, the metabolite did not accumulate in any particular organ. Although the available data is still limited, the risk that the accumulation of etretinate in the adrenals may adversely affect adrenal function must be examined. (Received March 20, 1986.)

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For several years, etretinate (Tigason®), an aromatic analogue of the ethylester of retinoic acid, has been marketed in most European countries for oral treatment of psoriasis and severe disorders of keratinisation. Unfortunately, the use of the drug in clinical practice is complicated by several untoward effects, including teratogenicity. Furthermore, the biological half-life of etretinate was found in patients receiving chronic treatment to be excessively long (1) and studies of human biopsy material have shown that the compound accumulates in subcutaneous fat (2). The detailed tissue distribution of etretinate in man is not known, however. We report, herein, the concentrations of etretinate and its main metabolite, etretin, in a variety of organs from three men who died from intercurrent diseases during etretinate therapy of psoriasis or ichthyosis. The results of one of the autopsies have been preliminary reported elsewhere (3).

MATERIALS AND METHODS

Organ specimens from three men undergoing etretinate treatment until time of death were collected at autopsy. The samples were immediately frozen, transported to the laboratory in boxes with dry ice and stored at −70°C until analysed. The patients' diagnoses and details of their therapies are listed in Table I. All patients died as a result of intercurrent diseases unrelated to etretinate therapy.

The tissue concentrations of etretinate (Ro 10-9359) and etretin (Ro 10-1670; the free carboxylic acid of etretinate) were analysed by reversed-phase high-performance liquid chromatography (HPLC) using a combination of two different procedures: in the first, the tissue sample is hydrolysed in ethanolic KOH whereby the drug may be quantitatively extracted (2). This procedure does not provide any information about the relative abundance of etretinate and etretin. In the second procedure the hydrolysis is omitted and the tissue is extracted with chloroform-methanol (2 : 1) after adjusting the homogenized sample to pH 4–5. This procedure, which does not quantitatively extract the drug, permits the relative amounts of etretinate and etretin in the sample to be estimated (2, 3). By combining data from the two analyses, the tissue concentrations of etretinate and etretin can be determined. All-trans retinoic acid is used as internal standard in both assays and HPLC is run identically using a Nucleosil (5 C_{18}) column and acetonitrile– H_2O-acetic acid (86 : 14 : 0.05) as mobile phase. The equipment and experimental details have been previously described (4).

RESULTS AND DISCUSSION

Table II shows the concentrations of the parent compound (etretinate) and the main metabolite (etretin) in various organs. Data for all eleven tissues were only available in case 2. Since the dosage and duration of etretinate treatment also differed considerably between the cases (see Table I), the data (means of duplicate analyses) are presented on an individual basis.

The accumulation of etretinate in the fat of all three patients corroborates previous findings (2). The lower etretinate value in case 3 is presumably due to the fact that this patient had received the drug only for 11 days prior to death. In one of the cases, the drug was shown to be uniformly distributed between various types of fat (see footnote to Table II); hence, the region from which the fat sample is taken does not appear to influence the results. Since elimination of the drug from fat is exceedingly slow (2), the variations in the

Table I. *Clinical data*

	Case 1	Case 2	Case 3
Age (yrs) and sex	23/♂	68/♂	85/♂
Indication for etretinate therapy	Mb Sjögren-Larsson	Pustular psoriasis	Pustular psoriasis
Other diseases	Cachexia	Ulcus duodeni Alcoholism (?)	Cancer prostata Chronic renal failure
Immediate cause of death	Pneumonia	Septicaemia Acute renal failure	Pneumonia
Duration of etretinate therapy (wks)	120	36	2
Average dose last week (mg/kg/day)	0.5	0.8	0.4
Approximate interval between last dose and death (h)	?	5	15
Approximate interval between death and autopsy (days)	0.5	3.5	3

Table II. *Concentrations of etretinate and its main metabolite (etretin) in various tissues.* The etretin values are in parentheses

Tissue	Case 1 (ng/g ww)	Case 2 (ng/g ww)	Case 3 (ng/g ww)
Fat	8 150 (92)	25 245[a] (275)	2 236 (213)
Liver	571 (1 429)	1 195 (865)	1 530 (249)
Kidney	627 (323)	441 (208)	144 (68)
Spleen	580 (221)	611 (204)	–
Brain	214 (436)	272 (232)	–
Adrenals	–	17 460 (727)	4 798 (148)
Jejunal mucosa	–	13 802 (2 629)	468 (470)
Tracheal mucosa	–	1 128 (470)	550 (ND)[b]
Urinary bladder mucosa	–	534 (ND)[b]	524 (ND)[b]
Testis	–	681 (130)	–
Epidermis	–	836 (231)	46 (51)

[a] Mean of values in subcutis (28 μg/g), omentum (26 μg/g) and perirenal fat (20 μg/g).
[b] Not determined. The drug concentration was assessed by the hydrolytic procedure: the etretinate value represents the sum of etretinate and etretin.

interval between the time of the last dose and fat sampling should not affect the values either.

The concentration of the drug in the liver was intermediate to that in other tissues. The level of etretinate exceeded that of etretin in two of the cases, and the reverse was seen in one case. This difference could relate to variations in the degree of fat infiltration of the liver (steatosis seems to promote etretinate accumulation (5)). The extent of post mortem degradation is not known. However, in liver specimens kept for 2 days at +6°C in the dark the levels of both etretinate and etretin were unchanged (unpubl observation).

The level of the drug in the kidney was similar in case 1 and 2 despite the acute renal failure in the latter case. Whether the low value in case 3 was related to his chronic renal failure or whether it resulted from the shorter period of etretinate therapy is uncertain. The total drug concentrations and relative abundances of etretinate and etretin were virtually identical in the renal cortex and medulla (data not shown). The values recorded in the spleen were similar to those in the kidneys.

The concentrations of etretinate and etretin were low in brain and did not differ significantly between the white and the gray matter. Since the metabolism of the drug in the brain is unknown, the extent to which etretinate and etretin pass the blood-brain barrier remains unresolved.

Unexpectedly high values were observed in the adrenals, available from two of the patients. In both cases, etretinate markedly preponderates over etretin, analogous to the situation in fat tissue. Since periadrenal fat was carefully removed before sampling and virtually identical etretinate values were observed in samples from the medulla or cortex of the adrenals, we disregard fat contamination as a cause of the high drug values. Further evidence for an accumulation of etretinate in the adrenals is accrued from the fact that in case 3 the etretinate concentration in the adrenals was twice that in fat tissue. The identification of etretinate in the adrenals was based on (i) a quantitative conversion to etretin after hydrolysis, (ii) a typical absorption ratio (340/365 nm) on HPLC and (iii) the absence of corresponding material in samples from control autopsies. We are not aware of any previous report implicating the adrenals as a storage site for etretinate in man. However, in rats 6 h following a single oral dose of ^{3}H etretinate the concentration of radioactivity in the adrenals was 3–10 times higher than in most other tissues except the liver which contained the highest activity (1). The analogy with naturally occurring retinoids (vitamin A) is obvious. Adrenals contain vitamin A-storing cells (6) and a regulatory role of retinol on corticosteroid synthesis has been proposed (7). Whether or not etretinate is located in the same cells as vitamin A and affects adrenal hormone production does not seem to have been examined.

Five types of epithelial tissues, important targets for retinoid action, were available for examination. In one of the patients, the jejunal mucosa contained high levels of both etretinate and etretin indicating recent intestinal absorption of the drug. It is uncertain whether etretin was derived from intestinal hydrolysis of etretinate or was part of an entero-hepatic recirculation. In the other patient, the drug concentration in jejunal mucosa was low, possibly reflecting a longer interval between last intake of drug and death. Tracheal and urinary bladder mucosa contained intermediate amounts of the drug, as did testis available from one of the patients.

The occurrence of etretinate in the skin has been analysed repeatedly in the past. The epidermal values found in the present study were higher and lower, respectively, than those observed previously in patients receiving 20–50 mg etretinate on a daily basis (2). The 13-cis isomer of etretin, which has previously been identified in human plasma during multiple dosing of etretinate (1), did not amount to more than 10% of the trans isomer concentration in any of the samples (non-hydrolytic procedure).

In conclusion, our data show that during treatment with etretinate the drug concentrates in at least three compartments, namely fat, adrenals and liver. Although the storage in fat sufficiently explains the long biological half-life of the drug, the accumulation of etretinate in the adrenals may have other important implications. Etretin, the less lipophilic metabolite of etretinate, did not accumulate in any particular organ, except possibly the liver and gut. The findings are reassuring in view of the recent efforts to replace etretinate with etretin as a therapeutic agent.

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