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Plasma Fat Elimination Tissue Lipoprotein Lipase Activity and Plasma Fatty Acid Composition during Sequential Treatment with Etretinate and Isotretinoin

C. VAHLQUIST,[1] H. LITHELL,[2] G. MICHAËLSSON,[1] I. SELINUS,[2] A. VAHLQUIST[1] and B. VESSBY[2]

Departments of [1]Dermatology and [2]Geriatrics, University of Uppsala, Uppsala, Sweden

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In an attempt to elucidate the mechanisms underlying retinoid-induced hyperlipidemia, the effects of etretinate (Tigason®) and isotretinoin (Roaccutane®) on two different plasma fat elimination variables and on the plasma fatty acid composition were studied. Twelve patients with various hyperkeratotic disorders participated in a double-blind cross-over study of etretinate and isotretinoin. Each drug was given for 8 weeks with an 8-week intermission. On five occasions an intravenous fat tolerance test (IVFTT) was performed and the lipoprotein lipase activity (LPLA) in adipose tissue and skeletal muscle was measured. Isotretinoin significantly reduced the fat elimination rate as measured by IVFTT ($p<0.001$) and also decreased the muscle LPLA ($p<0.05$). The etretinate-induced depression of these variables was not statistically significant. The LPLA of adipose tissue and the plasma fatty acid composition were not markedly altered by any of the drugs. The observed changes are probably not sufficient to entirely explain retinoid-induced hyperlipidemia but the results strengthen the opinion that plasma lipid metabolism is more unfavourably affected by isotretinoin than etretinate. *Key words: Retinoids; Lipid metabolism; Skin diseases.* (Received August 29, 1986.)

C. Vahlquist, Department of Dermatology, Uppsala University, Akademiska Sjukhuset, S–751 85 Uppsala, Sweden.

The lipoprotein changes observed during long-term treatment with etretinate and isotretinoin probably imply an increased risk of developing atherosclerosis (1–3). Although the mechanisms underlying the lipid changes are still largely unknown, the background is to be sought in some disturbances of the complex lipoprotein metabolism, which has recently been reviewed by Brown & Goldstein (4).

In short, dietary fat is transported from the intestine by the chylomicrons. Before entering the liver, the chylomicrons are exposed to tissue lipoprotein lipases, which remove some fatty acids. The cholesterol-rich chylomicron remnants then enter the liver, where the very-low-density lipoproteins (VLDL), packed with triglycerides and cholesterol, are synthesized. The VLDL particles are secreted into the circulation and the triglycerides are again exposed to the lipoprotein lipases of adipose tissue and skeletal muscle. The resulting process leaves cholesterol-rich low-density lipoproteins (LDL), which are taken up by the liver and other tissues. The high-density lipoproteins (HDL) are involved in the reverse transport of excess cholesterol from the peripheral tissues to the liver. Both etretinate (Tigason) and isotretinoin (Roaccutane) treatment is associated with increased VLDL and LDL and decreased HDL concentrations (5, 6).

* A preliminary report was presented at the Retinoid Symposium, Geneva, 1984.

From a comparative study of isotretinoin and etretinate we recently reported that the former drug had the more unfavourable effect on serum lipoprotein levels (7). As part of the same study we now report the effects on the results of an intravenous fat tolerance test (IVFTT), which measures the capacity to remove chylomicrons from the blood stream, and on the lipoprotein lipase activities (LPLA) of skeletal muscle and adipose tissue. In additon, the plasma fatty acid pattern was investigated at the start of the study and after the treatment periods.

PATIENTS AND METHODS

Patients

The study was performed on 12 out of earlier reported 16 patients (7). Four had palmoplantar psoriasis, six palmoplantar pustulosis, one hereditary palmoplantar keratodermia and one Darier's disease. The mean age of the patiens was 49 years (range 30–70). Ten were women and two were men. The mean body weight was 75 kg (58–96 kg). The mean weight was similar in the two treatment groups (73 kg in group A, 76 kg in group B).

Design of the study

A double-blind cross-over design with 2 patient groups, 5 time points and 2 drugs was used (7). Initially samples were taken, then each drug was given for 8 weeks, with an 8-week wash-out period in between. Group A started with etretinate, and group B with isotretinoin. The daily doses were 25 mg × 2 of etretinate and 20 mg × 2 of isotretinoin; these doses are equivalent in molar terms.

The plasma fatty acid composition was determined at the start of the trial and after the first and second treatment periods. The other analyses were performed at the start and after 8, 16, 24 and 32 weeks.

The study was approved by the Ethical committee of Uppsala University.

Methods

The IVFTT was performed according to the procedure of Carlson & Rössner (8). The 'fat tolerance' is expressed as the fractional elimination rate of Intralipid® (K_2, % per minute). According to Rössner (9) the K_2 value is individually highly reproducible when the test is repeated in control subjects at intervals of up to six months. A K_2 value above 4 % per min is considered normal.

Tissue LPLA was determined in biopsies from abdominal subcutaneous fat and skeletal muscle as described earlier (10). One milliunit of enzyme activity represents the release of 1 nmol of fatty acid per min from a defined triglyceride emulsion.

The procedure for determining the fatty acid composition of the plasma lipid esters has been described elsewhere (11). The following fatty acids were identified: 16 : 0, 16 : 1w7, 18 : 0, 18 : 1w9, 18 : 2w6, 18 : 3w6, 18 : 3w3, 20 : 3w6, 20 : 4w6, 20 : 5w3, 22 : 4w6, 22 : 5w3 and 22 : 6w3. The relative concentrations of the individual fatty acids were expressed in per cent of the sum of the concentrations of these fatty acids. Other fatty acids were present in only minute amounts (less than 1 %) and are not reported.

Statistics

The change-over design has been analysed in a 3-way analysis of variance model with main factors for patient, treatment and timepoints. The treatment–timepoint interaction was also included. The effects of the drugs were estimated and tested with the linear model above. All means presented are so-called least-square means which are means adjusted for the unbalance in the outcome of the experimental design. Effects versus baseline were estimated using all untreated time-points (start and washout occasions) as baseline. Mean square error (MSE), presented in the tables, is the variation not explained by the model. MSE is used when testing the effects. It is comparable to the use of the standard error of the *t*-test.

RESULTS

Intravenous fat tolerance test (IVFTT)

Not all patients underwent the IVFTT on all five test occasions: in group A two patients did not undergo the IVFTT on the last washout occasion; in group B one patient refused all IVFTTs and two patients did not undergo all the tests.

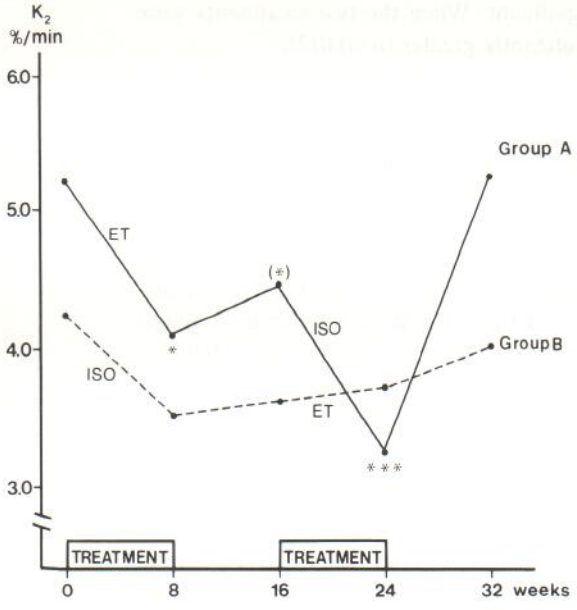

Fig. 1 a. Intravenous fat tolerance test (IVFTT). Adjusted mean values of fractional removal rate (K_2). Group A (solid line): patients starting treatment with etretinate (ET); group B (broken line): patients starting treatment with isotretinoin (ISO). Abbreviations for *p*-values as in Table I.

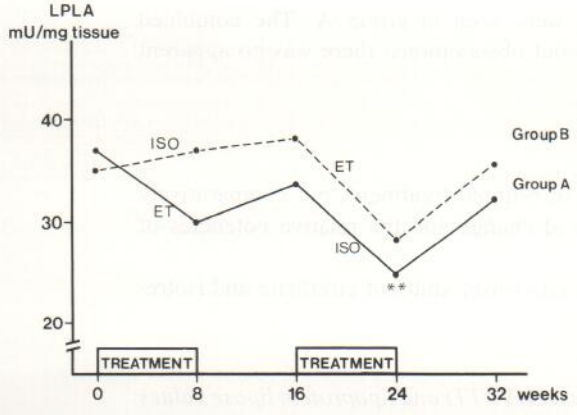

Fig. 1 b. Lipoprotein lipase activity (LPLA) in skelatal muscle. Adjusted mean values. Symbols and abbreviations as in Fig. 1 *a*.

Table I and Fig. 1 *a* show the fractional removal rate (K_2) in the two study groups. In group A the mean K_2 value decreased significantly during treatment with etretinate ($p \sim 0.02$) and even more so during treatment with isotretinoin ($p < 0.001$) as compared to the start of the study. The K_2 value increased after the first washout period (8–16 weeks) and fully reverted to pretreatment levels after the last washout period. A less consistent pattern was observed in group B. Isotretinoin decreased the K_2 value, but not significantly, and etretinate did not seem to lower the K_2 value at all in these patients. One reason for the somewhat contradictory results between the groups could be that group B contained a patient with Darier's disease, in whom the patterns of lipoprotein and K_2 changes differed considerably from those in the other patients. In general the K_2 values showed less tendency to regain the pretreatment levels after etretinate than after isotretinoin treatment.

Combined evaluation of the two treatment groups showed that the difference in effect of isotretinoin and baseline (see Statistics) was –1.88 %/min ($p < 0.001$). The lowering etretin-

ate effect (–0.42%/min) was not statistically significant. When the two treatments were compared, the isotretinoin effect on K_2 was significantly greater ($p \sim 0.012$).

Lipoprotein lipase activities

Muscle biopsies were not available from all patients for all five occasions: in group A one biopsy was not performed after the first washout and in group B two biopsies were not taken—one after the first washout and one during etretinate treatment. The muscle LPLAs in the two groups of patients are presented in Table I and Fig. 1 *b*. In group A there was a slight, but non-significant, decrease in LPLA after etretinate treatment, whereas isotretionoin markedly reduced the activity compared with the initial value ($p<0.001$). In group B neither of the drugs significantly decreased the LPLA. Combined evaluation showed that the lowering effects of isotretinoin and etretinate were –8 and –7 mU/mg ($p \sim 0.046$ and $p \sim 0.09$) respectively; there was no significant difference between the drugs.

The LPLA in adipose tissue did not change consistently during the treatment periods in either of the two groups (Table I) and no significant effects were found for either of the drugs.

Plasma fatty acid composition

The fatty acid patterns of the different plasma lipid esters did not alter greatly during retinoid treatment. Table II gives the values for eight of the 13 investigated phospholipid fatty acids which were considered of particular interest. In group B there was a small decrease in 16:0 and in 22:4w6 after treatment with etretinate and of 22:4w6 after treatment with isotretinoin. No such changes were seen in group A. The combined evaluation was complicated by the lack of wash-out observations; there was no apparent difference between the drugs, however.

DISCUSSION

Hyperlipidemia is a well recognized side-effect of retinoid treatment, but comparatively little is known about the causes of this undesired change and the relative potencies of different retinoids in this respect.

As previously reported from this double-blind cross-over study of etretinate and isotre-

Table I. *K_2 (%/min) of intravenous fat tolerance test (IVFTT) and lipoprotein lipase values (LPLA; mU/mg) of adipose tissue and skeletal muscle for groups A and B*

Mean values (adjusted for the unbalance in the outcome of the study) and MSE. MSE=mean square error—see under Statistics

		Time					
	Group	0	8	16	24	32 weeks	MSE
K_2	A	5.20	4.10*	4.43(*)	3.23***	5.21	
	B	4.23	3.56	3.62	3.73	4.02	0.74
LPLA muscle	A	37	30	34	25**	32	
	B	35	37	38	28	36	8
LPLA adipose tissue	A	153	200	198	175	194	
	B	167	135	136	130	160	47

(*) $0.05<p0.10$, * $0.01<p<0.05$, ** $0.001<p<0.01$, *** $p<0.001$, when testing versus initial values.

Table II. *Some fatty acids in plasma phospholipids*

Mean values (adjusted for the unbalance in the study) and MSE. MSE = mean square error. The values are expressed in percent of the sum of the concentrations of measured fatty acids

Fatty acid	Group	Time			MSE
		0	8	24 weeks	
16:0	A	31.6	31.5	31.3	
	B	32.2	31.4	30.8**	0.7
18:2w6	A	22.4	23.1	22.1	
	B	22.8	23.5	23.2	1.3
18:3w3	A	0.1	0.2	0.1	
	B	0.1	0.1	0.1	0.1
20:3w6	A	3.0	3.1	3.0	
	B	3.1	3.3	3.2	0.3
20:4w6	A	7.5	7.4	7.3	
	B	7.1	7.0	7.3	0.4
20:5w3	A	1.5	1.2	1.5	
	B	1.2	1.2	1.4	0.4
22:4w6	A	0.3	0.4	0.4	
	B	0.4	0.3*	0.4*	0.1
22:5w3	A	0.9	0.9	0.9	
	B	0.9	0.9	1.0	0.1

Symbols for significance as in Table I, when testing versus initials values.

tinoin (7), the latter drug significantly increased the triglyceride and cholesterol concentrations in both the VLDL and LDL fractions, and decreased the cholesterol in HDL. Etretinate induced similar, but less marked changes in the lipoprotein levels. Virtually all lipoprotein values returned to the pretreatment level within 8 weeks after discontinuation of the drugs.

Theoretically, the retinoid-induced lipoprotein changes could be the result of either enhancement of endogenous synthesis of VLDL and LDL or impairment of the fat elimination capacity (or both). We used the intravenous fat tolerance test in an attempt to mimic the chylomicron removal capacity as earlier described by Gollnick et al. (12) in their study of isotretinoin-induced hyperlipidemia. They found reduced removal capacity in retinoid-treated acne patients. In our study of adult patients with keratinizing disorders the K_2 values of the IVFTT were reduced by both drugs, but significantly so only by isotretinoin. The finding that isotretinoin was more potent than etretinate in this respect, corroborates previous data on the drugs' relative effects on serum lipoprotein levels (7).

During the washout periods the K_2 values of the etretinate-treated patients showed less tendency to return to pretreatment levels (Fig. 1 *a*). This finding may be related to the long biological half-life of etretinate which might cause a slight carry-over effect, although this was not apparent in the study of the lipoprotein levels (7).

There are several possible ways in which the retinoids could retard the fat elimination rate. Suppression of the tissue lipoprotein lipases, which are responsible for the hydrolysis of the circulating triglycerides, is perhaps the most likely explanation. Accordingly, we mostly found slight decreases in skeletal muscle lipoprotein lipase activity both after etretinate and after isotretinoin treatment (Fig. 1 *b*). However, only the decreasing isotretinoin effect was significant and none of the drugs significantly reduced the LPLA in adipose tissue. Although the relative potency of isotretinoin and etretinate to affect LPLA

seemingly correlated the drugs's effects on K_2 (see above), the modest decline in muscle LPLA is probably not sufficient to wholly account for the decreased fat elimination capacity observed during the treatment periods. Further studies are needed to elucidate this and related matters, such as the mechanism of action of retinoid-induced inhibition of LPLA.

It is known that lipoprotein disturbances resembling those observed during retinoid treatment, are sometimes associated with a change in the composition of plasma fatty acids. The relative amount of some polyunsaturated fatty acids has been reported to be decreased in patients prone to develop atherosclerosis (13). It was consequently of interest to see whether the fatty acid pattern of the different plasma lipid esters altered during retinoid treatment. Some changes were observed in the fatty acid composition of phospholipids, one of the major plasma lipid esters. However, the changes were small and did not conform to those observed in other groups of patients with hyperlipidemia (13).

In conclusion, we have found that retinoid-induced hyperlipidemia is associated with a decrease in the capacity for eliminating intravenously injected fat, which is more pronounced after isotretinoin than after etretinate treatment. The reduction of K_2 may to some extent be due to reduced LPLA. However, most probably a combination of several mechanisms of action is responsible for the altered lipoprotein metabolism during retinoid therapy.

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