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Retinoids and Fibrinolysis

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Vitamin A and its analogues have been reported to increase the release of tissue plasminogen activator *in vitro*. The aim of the present study was to reevaluate these findings and to investigate whether retinoids in doses used in dermatological therapy could enhance the release of endothelial fibrinolytic factors. Our results showed that endothelial cells incubated *in vitro* with retinoic acid increased the release of tissue plasminogen activator to the supernatant without concomitant secretion of plasminogen activator inhibitor-1. In patients treated with isotretinoin or etretinate these findings were confirmed, showing enhanced baseline tissue plasminogen activator concentrations in plasma in association with unchanged levels of plasminogen activator inhibitor-1 and von Willebrand factor. These findings are consistent with chronically augmented tissue plasminogen activator secretion without evidence of endothelial cell damage and may be of importance for the interpretation of the safety of long-term therapy with regard to retinoid-induced hyperlipemia and the development of cardiovascular disease. ***Key words: endothelial cells; tissue plasminogen activator; plasminogen activator inhibitor-1; von Willebrand factor; hyperlipemia; cardiovascular disease.***

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Vitamin A is essential to functions such as vision, growth and reproduction. Furthermore, it supports proliferation and differentiation of various cells and tissues, in particular epithelial structures such as those of the skin. Synthetic analogues of vitamin A or retinoids have been used in dermatology during the last decade for the treatment of cutaneous disorders characterized by disturbances of keratinization.

Long-term treatment with retinoids is associated with metabolic changes, including skeletal hyperostosis, hepatotoxicity and blood lipid disturbances, i.e. increase of triglycerides and cholesterol with a reduction of high-density lipoproteins (1). This type of hyperlipemia is associated with increased morbidity in cardiovascular disease (2).

Over the past years there have been some *in vitro* studies claiming that vitamin A and retinoids can increase the synthesis and release of tissue plasminogen activator (tPA) (3–5). The dual aim of this study was to reevaluate these findings and to investigate whether retinoids, given in therapeutic doses to patients with dermatological diseases, could promote fibrinolysis in the same way *in vivo* as described *in vitro*.

PATIENTS AND METHODS

The clinical study comprised 18 patients, 9 females aged 18 to 54 years (median 23) and 9 males aged 18 to 61 years (median 31). Fifteen patients with cystic or conglobate acne were treated with isotretinoin (Mw 300) in a dose of 40 mg per day, and 3 patients with ichthyosis or Darier's disease received etretinate (Mw 354) in a dose of 25 mg per day. Five of the young female patients were also taking oral contraceptives, but no other drugs were allowed. Before treatment and after 5–8 weeks of retinoid therapy the patients were evaluated for haemostatic and lipid metabolism parameters.

Blood samples were drawn in the early morning after a 12–14-h overnight fast and a 10-min bed rest, into siliconized Venoject® tubes containing 1/10 volume of 0.13 mol/l sodium citrate, and into Stabilyte® tubes (Biopool, Umeå, Sweden) containing 1/10 volume of 0.5 mol/l sodium citrate, pH 5.0. The blood was drawn before and after venous occlusion (VO), performed by inflating a sphygomanometer cuff on the arm at 100 mmHg for 10 min. The plasma was separated by centrifugation at 1,800 × g for 10 min and snap-frozen in aliquots, kept at −70°C and analysed within 1 month.

tPA mass concentration was measured with an ELISA, as described (6). Plasminogen activator inhibitor type 1 (PAI-1) activity and tPA activity were determined with a chromogenic substrate assay (7). The reagents used, Imulyse tPA and Spectrolyse/Fibrin, respectively, were purchased as kits from Biopool, Umeå, Sweden. Von Willebrand factor (vWF) was measured with an ELISA (8) using antibodies from Dakopatts, Copenhagen, Denmark. All other assays were performed according to the practices of our routine laboratory, as described previously (9).

Endothelial cells were obtained from human umbilical cord veins and isolated within 2 h of delivery by enzymatic treatment with thermolysin, as previously described (10). The cells were cultured in Medium 199 (Gibco, Paisley, U.K.) supplemented with 20% calf serum (Hyclone, Logan, USA), endothelial cell growth factor (Sigma) 30 mg/l, glutamine 2 mmol/l, penicillin 100 U/ml, gentamicin 5 mg/l and fungizone 2.5 mg/l. Cells from passage 2–4 grown to confluency in 24-well tissue culture plates (Nunc, Denmark) were incubated with retinoic acid in medium 199 supplemented with ascorbic acid (Sigma) 0.1 mmol/l for 24 h, when the supernatant was analysed for the concentration of tPA antigen and PAI-1 activity. All-*trans*-retinoic acid (Sigma) was dissolved in stock solutions of 1×10^{-2} M and stored at −20°C in glass tubes covered with aluminium foil. Immediately before use the stock solution was dissolved in incubation medium at final concentrations of 10^{-5}–10^{-9} M. The concentration of ethanol did not exceed 0.1% (v/v).

RESULTS

Incubation of endothelial cells *in vitro* with retinoic acid in the possible therapeutic dose range (10^{-9}–10^{-5} M) increased the

Table I. *The release of tissue plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) to the supernatant from cultured human umbilical vein endothelial cells incubated in vitro for 24 h in the presence of all-*trans*-retinoic acid (RA)*

RA conc (mol/l)	tPA antigen (μg/l)[a]	PAI-1 activity (U/ml)[a]
0, $n=6$	1.5 ± 0.4	84 ± 23
10^{-9}, $n=6$	1.1 ± 0.4	65 ± 26
10^{-7}, $n=6$	2.3 ± 0.5*	78 ± 11
10^{-5}, $n=6$	3.5 ± 0.7***	94 ± 19

a = mean ± SD; * = $p<0.05$; *** = $p<0.001$; Student's *t*-test.

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Table II. *Hemostatic and lipid variables before and during retinoid therapy in 18 patients*

Mean values (± SD) are given. Wilcoxon's rank sum test was used to compare pretreatment and treatment groups. tPA = tissue plasminogen activator; PAI-1 = plasminogen activator inhibitor type 1; VO = venous occlusion; vWF = von Willebrand factor.

Variable	Mean		*p*-value
	Baseline	Retinoid	
tPA mass conc. (μg/l)	6.4 ± 3.5	8.9 ± 7.0	0.0196
tPA mass conc./VO	24.9 ± 13.7	25.4 ± 15.6	
tPA activity (U/ml)	0.23 ± 0.20	0.19 ± 0.19	
tPA activity/VO	3.4 ± 2.5	4.0 ± 5.2	
PAI-1 activity (U/ml)	9.0 ± 5.6	9.9 ± 7.5	
vWF (% of normal)	97 ± 31	101 ± 33	
Antithrombin (% of normal)	92 ± 10	96 ± 15	
Cholesterol (mmol/l)	4.93 ± 1.10	5.45 ± 1.26	0.00185
Triglycerides (mmol/l)	1.19 ± 0.47	1.65 ± 0.89	0.0106

release of tPA without simultaneous change in PAI-1 secretion (Table I). In the patients treated with retinoids for periods of 5–8 weeks these findings were confirmed (Table II). Thus tPA antigen before venous occlusion was significantly increased in patients during retinoid treatment, compared to pretreatment levels. When blood was collected after the venous occlusion this effect of retinoid therapy was statistically insignificant. However, a scatter plot analysis of the influence of gender and therapy with oral contraceptives suggested that tPA secretion in females on oral contraceptives was not enhanced by retinoids (Fig. 1). As seen in Table II, the retinoid effect on tPA activity was also negligible in samples drawn both before and after venous occlusion.

There seemed to be no obvious influence on other haemostatic variables of this treatment, except for the impact on plasma lipids. Thus in our study the plasma concentration of both cholesterol and triglycerides was increased by long-term retinoid therapy (Table II).

DISCUSSION

This study confirms previous findings that vitamin A and analogues increase the release of tPA from cultured endothelial cells (4, 5). More important though, we were able to demonstrate that moderate therapeutic doses of retinoids augment the mean plasma mass concentration of tPA in patients. This is in agreement with the results obtained by Kooistra et al. (5) in rats, where vitamin A-starved animals had low activity and antigen levels for tPA in plasma and tissues, while retinoid-fed rats showed enhanced levels.

Our patients were given isotretinoin or etretinate. Although both drugs are highly active as therapeutic agents, neither molecule is able to bind to any of the known families of retinoid

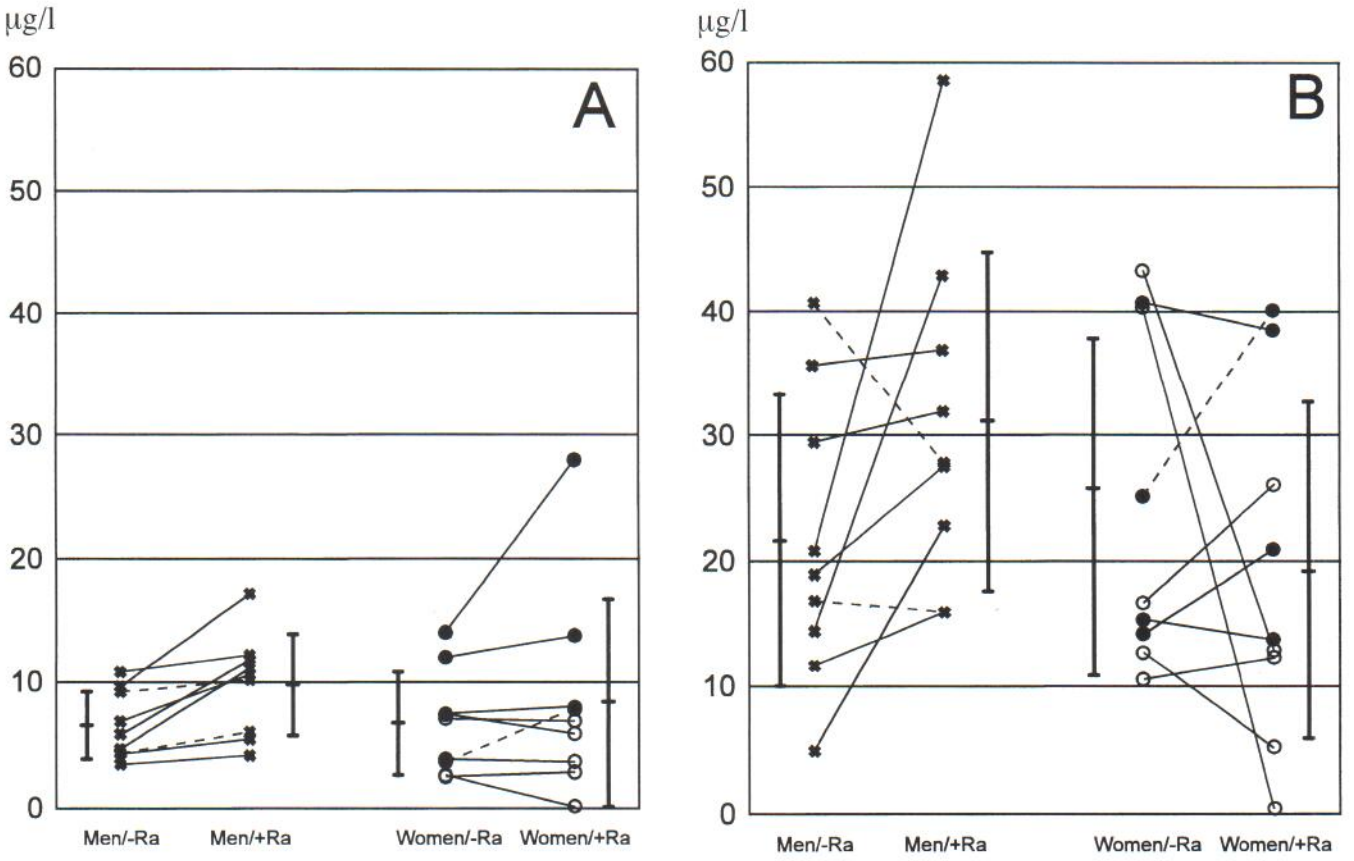

Fig. 1. tPA mass concentration in plasma. Panel A, before venous occlusion and panel B, after venous occlusion for 10 min. *Open circles* represent female patients on oral contraceptives and *filled circles* females without oral contraceptives. *Solid lines* denote patients treated with isotretinoin and *dashed lines* patients given etretinate. – Ra *before* and + Ra *during* retinoid treatment.

receptors without metabolic transformation. It cannot be excluded that the particular effect of retinoids on endothelial cell release of tPA may be induced through pathways disconnected from retinoid receptors. In fact, there is evidence suggesting that the induction of tPA synthesis in endothelial cells by retinoids may involve protein kinase C (5).

In our patients the retinoid effect on plasma tPA mass concentration was evident after 5–8 weeks of treatment, while Bounameaux et al. (11) did not find any change in plasma tPA levels 3 h after oral administration of 100 mg etretinate or acitretin. This may indicate that metabolic changes caused by vitamin A analogues are induced slowly and may not appear until after prolonged retinoid therapy, in analogy with the hypertriglyceridemia induced by isotretinoin treatment. Here the maximum level is reached by 4 weeks in men but by 12 weeks in women (12).

Low-dose oral contraceptives in young women are reported to decrease basal tPA antigen concentration (13) but to enhance the release of tPA after venous occlusion and to decrease the levels of PAI-1 (14, 15). In this study we were able to demonstrate that retinoids induce a significant increase of basal tPA release, but this effect was not sustained after venous occlusion. The reason may be that female patients on oral contraceptives are less prone to retinoid-induced tPA release (cf. Fig. 1), and thus our results could indicate an unfavourable interaction between oral contraceptives and retinoids. This issue should be clarified in studies on larger female patient populations stratified for age. There are, however, no studies suggesting that the post-occlusion values are predictive of future cardiovascular events. Such a predictive value has been demonstrated only for the baseline tPA levels (16, 17).

Within the dermatological community there has been some concern about the use of retinoids since they induce increased plasma triglycerides, as this could theoretically lead to problems with the safety of long-term treatment with regard to atherosclerotic vascular disease (2). Such a development would include reactive endothelial mechanisms, i.e. increased plasma levels of tPA (17), PAI-1 (18) and vWF (19, 20). Our findings, however, were an isolated augmented baseline release of tPA antigen associated with unchanged levels of both PAI-1 and vWF. This effect of retinoid therapy is in agreement with the results of the *in vitro* experiments and should not be interpreted as an indication of endothelial injury. The tPA activity remained unchanged, and so far this variable has not been related to cardiovascular end points (18, 19).

Our findings also raise the question of whether it may be reasonable to extend the studies of retinoids to other patient groups, especially those with cardiovascular problems characterized by impaired fibrinolysis (high PAI-1 activity) and thus having a higher risk for cardiovascular disease (21).

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