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 hyperlipidemia and the development of cardiovascular disease. Key words: endothelial cells; tissue plasminogen activator; plasminogen activator inhibitor-1; von Willebrand factor; hyperlipidemia; 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 hyperlipidemia is associated with increased morbidity in cardiovascular disease (2).

Over the past years there have been some in vitro studies claiming that vitamin A or 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.

RESULTS

Incubation of endothelial cells in vitro with retinoic acid in the possible therapeutic dose range (10⁻⁹–10⁻³ M) increased the release of tissue plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) from the supernatant from cultured human umbilical vein endothelial cells incubated in vitro for 24 h in the presence of all-trans-retinoic acid (RA).

Table 1. 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)

<table>
<thead>
<tr>
<th>RA conc (mol/l)</th>
<th>tPA antigen (µg/l)</th>
<th>PAI-1 activity (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0, n = 6</td>
<td>1.5 ± 0.4</td>
<td>84 ± 23</td>
</tr>
<tr>
<td>10⁻⁹, n = 6</td>
<td>1.1 ± 0.4</td>
<td>65 ± 26</td>
</tr>
<tr>
<td>10⁻⁷, n = 6</td>
<td>2.3 ± 0.5*</td>
<td>78 ± 11</td>
</tr>
<tr>
<td>10⁻⁵, n = 6</td>
<td>3.5 ± 0.7***</td>
<td>94 ± 19</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Retinoid</td>
</tr>
<tr>
<td>tPA mass conc. (µg/l)</td>
<td>6.4 ± 3.5</td>
<td>8.9 ± 7.0</td>
</tr>
<tr>
<td>tPA mass conc./VO</td>
<td>24.9 ± 13.7</td>
<td>25.4 ± 15.6</td>
</tr>
<tr>
<td>tPA activity (U/ml)</td>
<td>0.23 ± 0.20</td>
<td>0.19 ± 0.19</td>
</tr>
<tr>
<td>PAI-1 activity (U/ml)</td>
<td>3.4 ± 2.5</td>
<td>4.0 ± 5.2</td>
</tr>
<tr>
<td>vWF (% of normal)</td>
<td>90 ± 5.6</td>
<td>9.9 ± 7.5</td>
</tr>
<tr>
<td>Antithrombin (% of normal)</td>
<td>92 ± 10</td>
<td>96 ± 15</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.93 ± 1.10</td>
<td>5.45 ± 1.26</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.19 ± 0.47</td>
<td>1.65 ± 0.89</td>
</tr>
</tbody>
</table>

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
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 Bouma-
meaux 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).

REFERENCES

2. Inter-society commission for heart disease resources. Optimal re-