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All-trans retinoic acid modulates the balance of ADAMTS13 and VWF in human microvascular endothelial cells

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ABSTRACT

Purpose: To better understand the antithrombotic property of All-trans retinoic acid (ATRA), we investigated whether ATRA may affect the balance between ADAMTS13 and von Willebrand factor (VWF) in human microvascular endothelial cell.
Methods: Compared to tumor necrosis factor-alpha (TNF-α), we observed the effects of ATRA on the expression of ADAMTS13 and VWF. ADAMTS13mRNA in human microvascular endothelial cell (HMEC-1 cell line) were detected by real-time polymerase chain reaction amplification (RT-PCR). The levels of ADAMTS13 and VWF antigen were detected by western blot or enzyme-linked immunosorbent assay (ELISA), and the proteolytic activity of ADAMTS13 was also determined by using GST-VWF73-His peptide as a specific substrate.
Results: ATRA significantly upregulated the expression of ADAMTS13mRNA in HMEC-1, while TNF-α inhibited ADAMTS13mRNA expression. ATRA could reverse the inhibition expression of ADAMTS13 by TNF-α. The results were confirmed from the levels of ADAMTS13 protein and its activity, while ATRA had no significant affection on triggering release of VWF.
Conclusions: This study provides the evidence that ATRA modulates the balance of ADAMTS13 and VWF in human microvascular endothelial cell, which might be a very relevant compartment for the antithrombotic property of ATRA.

Introduction

VWF, a glycoprotein mainly secreted from endothelial cells, can capture circulating platelets to adhere to the sites of vascular damage (López and Dong, 1997; Sadler, 1998; Von, 1996). ADAMTS13, as a specific VWF cleaving protease, cleaves plasma VWF in A2 domain by a shear dependent manner. ADAMTS13 can prevent the microthrombus formation caused by ultra-large VWF multimers (UL-VWF) (Dong et al., 2002). On the one hand, ADAMTS13 deficiency could cause excessive accumulation of prothrombotic UL-VWF. On the other hand, even with normal level of ADAMTS13, an increased release of UL-VWF by vascular endothelial cells can also cause the hypercoagulable state. The liver has to be confirmed as a source of plasma ADAMTS13 (Levy et al., 2001; Uemura et al., 2005; Zhou et al., 2005a), while ADAMTS13 is also synthesized in human vascular endothelial cells. The vast number of vascular endothelial cells in the body could be an important source of ADAMTS13 (Turner et al., 2006, 2009; Shang et al., 2006). VWF are mainly stored in the endothelial Weibel–Palade bodies. The balance of ADAMTS13 and VWF in vascular endothelial cells is very important for the stable blood circulation.

Some study demonstrated that histamine might stimulate the UL-VWF rapid release from endothelial Weibel–Palade bodies (López and Dong, 2004), while other study confirmed that inflammatory cytokines (such as TNF-α) inhibited ADAMTS13 synthesis in endothelial cells (Cao et al., 2008). The balance of ADAMTS13 and VWF in vascular endothelial cells may be interrupted, which result in the accumulation of hyper-reactive UL-VWF and then induce platelet aggregation and adhesion.

All-trans retinoic acid (ATRA), as an antithrombotic drug, has been reported that could modulate the hypercoagulable state of human microvascular endothelial cell by upregulation the procoagulant tissue factor and downregulation the anticoagulant thrombomodulin (Marchetti et al., 2003). Considering that the balance between ADAMTS13 and VWF of microvascular endothelial cell, which play an important role on the controlling the hypercoagulable state of microvascular endothelial cell. To better understand the antithrombotic property of ATRA, we investigated whether ATRA could affect the balance.

Abbreviations: ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 repeats motif. 13; VWF, von Willebrand factor; ATRA, all-trans retinoic acid; HMEC-1, human microvascular endothelial cell; RT-PCR, real-time polymerase chain reaction amplification; ELISA, enzyme-linked immunosorbent assay; UL-VWF, ultra-large vWF multimers; TNF-α, tumor necrosis factor alpha.

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Table 1
Specific primers, PCR products, and GenBank Accession Nos. of genes for real-time PCR.

Gene	Primers		Size (bp)
ADAMTS13	Sense	5′-CAGGTTTACAGGCGGTATGG-3′	20
	Antisense	5′-CGTGGCTTAGGCTGGAAGTAG-3′	21
	Product		83
	Gene Bank Accession No	Human NM_139025	
b-Actin	Sense	5′-TGCGTGACATTAAGGAGAAGCTGTG-3′	25
	Antisense	5′-CAGCGGAACCGCTCATTGCCAATGG-3′	25
	Product		146
	Gene Bank Accession No	Human NM_001101	

Materials and methods

Cell culture

HMEC-1 was cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, epidermal growth factor (10 ng/mL), and antibiotics as described before (Ades et al., 1992). Passage was performed with 0.25% trypsin, and cells grown on 6-well plates were washed with PBS and incubated with different concentrations of ATRA/TNF-α (obtained from Sigma, U.S.A.) in serum-free-DMEM for 24 h. Then the cells and conditioned medium were collected.

Real-time polymerase chain reaction, RT-PCR

Confluent monolayer of HMEC-1 grown on 6-well plate (purchased from Nunc, Roskilde, Denmark) was stimulated with different concentrations of ATRA/TNF-α for 24 h, and then the cells were collected.

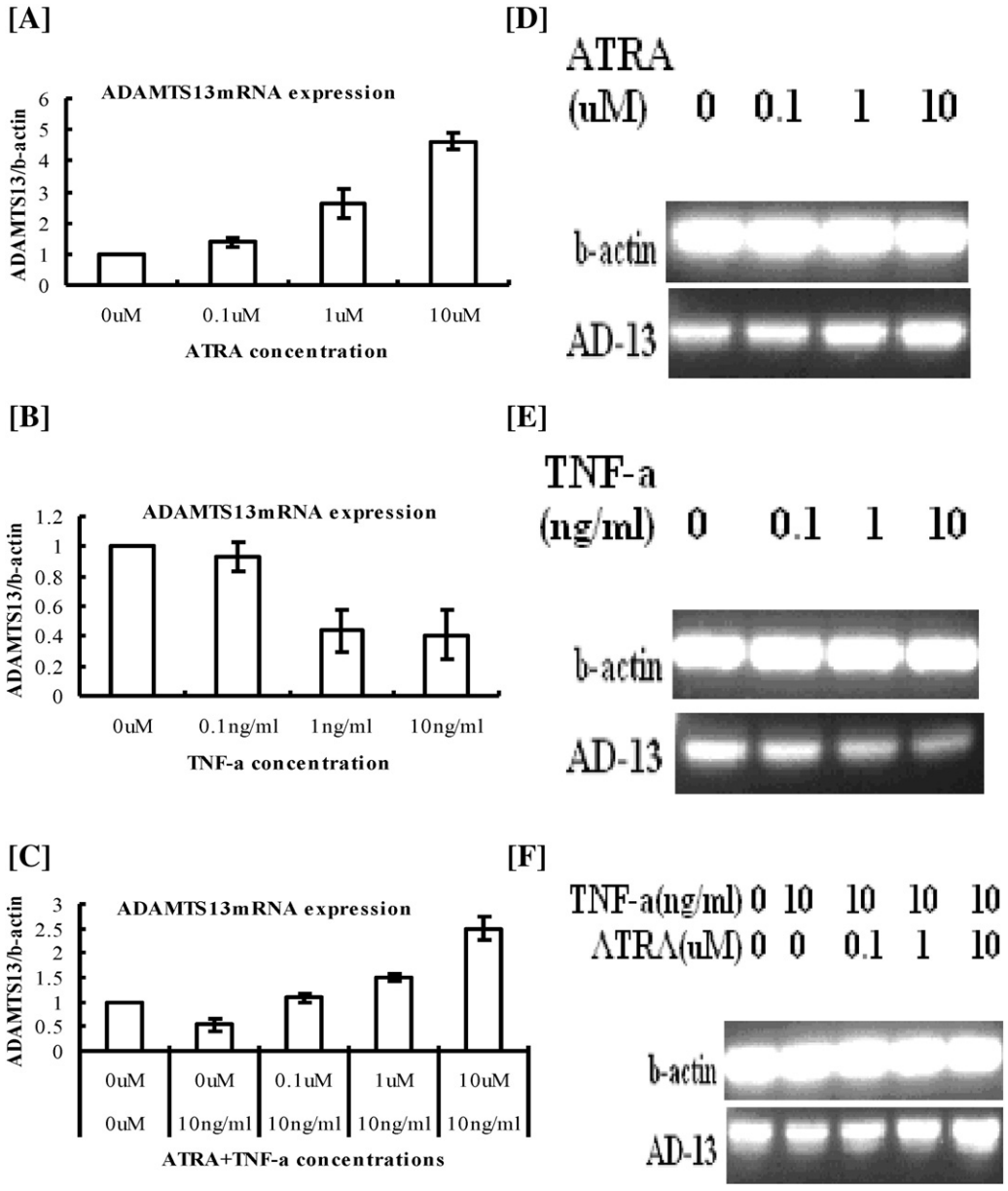

Fig. 1. Effects of ATRA or/and TNF-α on ADAMTS13mRNA expression in HMEC-1. HMEC-1 were incubated with different concentrations of ATRA (A, D), TNF-α (B, E), or ATRA + TNF-α (C, F) for 24 h. Gene expression are calculated and analyzed as (A, B, C, D, E, F). The data (in A, B, C) represent the mean ± SD of six independent experiments, (D, E, F) is a typical experiment of six independent experiments.