Differential modulation of adhesion molecule expression by hydroxycarbamide in human endothelial cells from the micro- and macrocirculation: potential implications in sickle cell disease vasoocclusive events

Sandrine Laurance,[1,2] Pauline Lansiaux,[1,2] François-Xavier Pellay,[3,4] Michelle Hauchecorne,[1] Arndt Benecke,[3,4] Jacques Elion,[1,2] and Claudine Lapoumeroulie[1,2]

[1]INSERM, UMR_S763, Hôpital Robert Debré, Paris; [2]Université Paris Diderot, Faculté de Médecine, Paris; [3]Institut des Hautes Etudes Scientifiques, Bures sur Yvette; and [4]CNRS, USR3078, Institut de Recherche Interdisciplinaire; Université de Lille 1, Lille, Cedex, France

ABSTRACT

Background
All the cellular partners of the vascular system and especially endothelial cells are involved in the pathophysiology of the vasoocclusive crises associated with sickle cell disease. In sickle cell disease, circulating cells adhere abnormally to endothelial cells in a chronic pro-inflammatory context. Hydroxycarbamide is the only drug with demonstrated efficacy to reduce the frequency of vasoocclusive crises. Here, we investigated the effects of hydroxycarbamide and/or cytokines on the expression of genes related to adhesion events in endothelial cells from three different vascular sites.

Design and Methods
Endothelial cells representative of the macro- (HUVEC) or microcirculation (TrHBMEC and HPMEC) were grown in the presence or absence of hydroxycarbamide and/or cytokines (TNFα and IFNγ). Expression of genes encoding adhesion proteins was analyzed by RQ-PCR, ELISA, flow cytometry, *in situ* ELISA for extracellular matrix proteins, and Western blot.

Results
In cells from the microcirculation, expression of *TSP-1*, *vWF*, and *PECAM-1* genes was decreased by hydroxycarbamide and/or cytokine treatment at the mRNA level. In the macrocirculation their expression was unaffected or increased. Hydroxycarbamide significantly decreased vWF incorporated in the TrHBMEC extracellular matrix. *CD36* mRNA was strongly down-regulated by cytokines in HPMEC, the only cell type in which it is expressed. Hydroxycarbamide decreased soluble PECAM-1 in HUVEC supernatants.

Conclusions
Our results highlight the heterogeneity of vascular endothelial cell responses to hydroxycarbamide and/or cytokines depending upon their origin. They also suggest that hydroxycarbamide has an anti-adhesogenic effect on endothelial cells, but by mechanisms which could vary according to their macro- or microcirculation and organ origin.

Key words: sickle cell disease, adhesion, hydroxycarbamide, vascular endothelial cells.

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Correspondence:
Claudine Lapouméroulie, INSERM, UMR_S763, Hôpital Robert Debré, 48 boulevard Sérurier, 75019 Paris, France.
Phone: international +33.140031924.
Fax: international +33.140031903
E-mail: claudine.lapoumeroulie@inserm.fr

The online version of this article has a Supplementary Appendix.

Introduction

Vasoocclusion is the major clinical feature of sickle cell disease (SCD). It results in acute painful vasoocclusive crises (VOC) and progresses to multiple organ failure. Vasoocclusion results from the polymerization of deoxyhemoglobin S. However, it is precipitated by complex processes involving multiple cellular and molecular partners. It mostly occurs in the microcirculation (bone marrow, lung, and kidney), although some complications, particularly strokes, involve large vessels. All the cellular components of the blood system are activated in SCD and the endothelium is damaged. This is attested by the presence of circulating endothelial cells, activated monocytes, neutrophils, and platelets, and circulating cytokines in a pro-inflammatory context.[1-4] Furthermore, sickle red blood cells (RBC) are more adhesive than normal.[5] Adhesion events are mediated by: (i) membrane-bound receptors at the circulating-cell surface including α4β1 integrin (VLA-4), CD36 (thrombospondin receptor), CD47 (integrin-associated protein), Lu/BCAM (CD239), ICAM-4 and phosphatidylserine (PS) at the RBC surface, L-selectin (CD62L), CD44, α4 and β2 integrins, PSGL-1 (P-selectin glycoprotein ligand-1, CD162) and PECAM-1 (CD31) at the leukocyte surface, PSGL-1, P-selectin (CD62P), and the $\alpha_{IIb}\beta_3$ and GP1bα glycoproteins at the platelet surface; (ii) membrane-bound counter receptors at the vascular endothelial cell (VEC) surface, including VCAM-1 (CD106), ICAM-1 (CD54), P- and E-selectins (CD62E), $\alpha_v\beta_3$ integrin, Lu/BCAM, CD36 and PECAM-1; (iii) subendothelial matrix elements exposed after VEC injury, including laminin, von Willebrand Factor (vWF), thrombospondin-1 (TSP-1 or THBS1), and fibronectin; and finally (iv) soluble proteins in the plasma, including TSP-1, vWF, fribrinogen, and fibronectin acting as adhesogenic molecules, and also sVCAM-1, sICAM-1, and sP-selectin that act as anti-adhesogens. Taken together, these interactions underlying adhesion events highlight the central role of VEC in the pathogenesis of VOC. Several observations reveal the heterogeneity of VEC.[6-8] In particular, Chi *et al.* established the transcriptomic profiles of 53 cultured VEC types and classified VEC as: (i) micro- or macro-VEC; (ii) arterial or venous VEC; and (iii) according to anatomical localization.[9] Expression of some markers is limited to particular VEC types, e.g. CD36 and vWF are preferentially expressed by VEC of the micro- and the macrocirculation, respectively.[10,11]

Despite the increasingly detailed description of SCD pathophysiology, only one effective drug is presently available, i.e. hydroxycarbamide (HC or hydroxyurea).[12] HC was initially given to SCD patients because it was seen to increase fetal hemoglobin (HbF) in HC-treated patients with myeloproliferative disorders. Increased HbF was expected to be beneficial because HbF interferes with deoxyhemoglobin S polymerization. Indeed, HC-treatment drastically reduces VOC incidence[13] and, in the long term, increased HbF level and decreased VOC frequencies are associated with reduced mortality.[14] However, no short-term correlation could be established between the clinical benefit observed and HbF increase. Indeed, HbF response is highly variable from patient to patient whereas the clinical benefit is almost constant and precedes laboratory changes including HbF increase.[15-17] Guided by this observation, our group and others decided to define more precisely the cellular and molecular targets of HC. In particular, the effects of HC on RBC adhesion and adhesion molecules have been studied. HC decreases the strength of RBC adhesion to VEC, TSP-1 and laminin matrices and also decreases the expression of CD36 and $\alpha_4\beta_1$ integrin at the RBC surface.[18-20] In comparison, data concerning the effects of HC on VEC, the other cellular partner of cell adhesion, are scarce. Adragna *et al.* reported that HC decreases the adhesion of normal RBC to treated primary VEC from the bovine aorta.[21] As the most frequent site of VOC is bone marrow, we studied the effects of HC on a VEC line derived from the human bone marrow microcirculation, TrHBMEC. Thus, we previously reported that HC modulates VCAM-1 and ICAM-1 expression in TrHBMEC.[22] We also found that HC down-regulates the expression of endothelin-1 (ET-1), a powerful vasoconstrictor, by TrHBMEC and that ET-1 was very rapidly and dramatically decreased in HC treated SCD children, providing an *in vivo* correlate to our *in vitro* observation.[22,23] These data definitely designate VEC as an HC cellular target.

More recently, we used a transcriptomic approach involving micro-array analysis to exhaustively list HC-target genes in TrHBMEC both at the basal state and in pro-inflammatory conditions and described the effect of HC on the production of pro-inflammatory cytokines by VEC.[24] The aim of the present study was to focus our analysis of the micro-array data on the expression of genes related to adhesion events and to extend our investigation to other human VEC types in order to appreciate how HC and/or cytokines (CY) may modify the adhesogenic patterns of vascular endothelia in different sites. Thus, in addition to TrHBMEC, we studied and compared the effect of HC and/or CY treatments on primary VEC from the lung microcirculation (HPMEC) and from the umbilical vein (HUVEC), the most widely utilized macrocirculation model.

Design and Methods

Endothelial cell culture

TrHBMEC were cultured as previously described and used between passage 19 and 23.[25] Human umbilical vein endothelial cells (HUVEC), primary cells from the macrocirculation, were isolated from umbilical cords as previously described.[26] HUVEC were cultured in Endothelial Cell Growth Medium (PromoCell, Heidelberg, Germany) and used between passage 3 and 7. Human Pulmonary Microcirculation Endothelial Cells (HPMEC), primary cells, were cultured according to the manufacturer's instructions (PromoCell) and used between passage 3 and 7. Cells were grown until 90% of confluence and then exposed to HC treatment with or without TNFα and IFNγ treatment[27] for 24 and 48 h for microarray experiments and for 5 h, 10 h, 16 h, 24 h and 48 h for RQ-PCR experiments and protein assays. HC (Sigma-Aldrich, St Louis, USA) was used at a final concentration of 250 μM and TNFα and IFNγ (R&D systems, Abingdon, UK) were used at 100U/mL each.

Microarray experiments

Microarray experiments with TrHBMEC treated with HC and/or CY for 24 h or 48 h and data analysis were performed as previously described using an Applied Biosystems 1700 Genome Survey Microarray platform (Applied Biosystems, Forster City, USA).[24] Data were generated using the Human Genome Survey (HGS) arrays (version 1 and 2) which measure gene expression levels of 29,918 validated human genes. Logarithmic fold-changes were calculated as previously described in an "everyone-against-everyone" scheme. Each subtraction profile (HC *vs.* NT and

HC+CY *vs*. CY both at 24 and 48 h) was normalized by the NeONORM method with k=0.2 and *P* values determined according to a normal distribution hypothesis of signal intensities using standard ANOVA methods. Among the genes identified as being modulated, analysis was focused on the 1,275 genes that GO, Kegg, and PANTHER annotations indicated to be related to adhesion events (*Online Supplementary Table S1*).

RNA isolation and retro-transcription

Total RNA was extracted from cultured cells using the NucleospinRNAII kit according to the manufacturer's instructions (Macherey-Nagel, Düren, Germany). The RNA samples were reverse transcribed as recommended by the manufacturer (Abgene, Epsom, UK). The mix was incubated for 10 min at room temperature and then 15 min at 42°C. The synthesized cDNA was kept at -20°C until real-time PCR experiments.

Real-time quantitative PCR experiments (RQ-PCR)

RQ-PCR experiments were carried out on an ABI 7300 (Applied Biosystems). The sybrgreen intercalant was used to detect amplification and the Sybrgreen Core Reagent was utilized according to the manufacturer's instructions (Abgene). The final concentration of cDNA was approximately 5ng/μL. The primers were used at 300 nM and designed using the Primer Express Software (Applied Biosystems) (*Online Supplementary Table S2*). The TATA box binding protein (TBP) gene was used for normalization of the quantitative data because its expression appears stable in our cell systems and TBP primers designed by Bièche *et al.* were used.[28] All RQ-PCR results are expressed as means±SD of percent of expression of the control value for 5 independent experiments each in duplicate. Values for non-treated cells (NT) served as control values for HC- or CY-treated cells, and those for CY-treated cells for HC+CY-treated cells.

Soluble protein quantification in cell supernatant by ELISA

The concentrations of TSP-1, vWF, and soluble PECAM-1 in cell supernatants were evaluated by ELISA. Supernatants were collected after treatment, spun for 10 min at 4,000g and then stored in several aliquots at -20°C until protein quantification. A commercial kit was used for sPECAM-1 assays according to the manufacturer's instructions (Diaclone, Besançon, France). TSP-1 was assayed as previously described.[29] vWF assays were performed according to the manufacturer's instructions (Dakocytomation, Glostrup, Denmark), and the standard curve was generated by serial dilution of purified vWF (Diagnostica Stago, Asnières, France). All ELISA results are expressed as the mean concentration in pg/ml or ng/ml for 5 independent experiments each in duplicate.

In situ quantification of extracellular matrix (ECM) proteins by ELISA

VEC were seeded (2×10^4 cells in 100 μL) onto 96-well plate in their respective medium 24 h before exposition to HC treatment with or without TNFα and IFNγ treatment for 24 and 48 h. Plates were washed with PBS 1X without $Ca2^+$ and $Mg2^+$ and confluent endothelial cell monolayers were lyzed by incubation with 100 μL of a lysis solution containing 0.1% (v/v) Triton X-100, 0.1 M NH_4OH, 1 mM PMSF (phenylmethylsulfonyl fluoride) (Fluka, Sigma-Aldrich), 1X PIC (Protease Inhibitor Cocktail, Sigma-Aldrich) for 20 min at RT.[30] ECM were washed three times with PBS 1X, and directly analyzed by *in situ* ELISA. vWF and TSP-1 quantification in ECM were carried out using Polyclonal Rabbit Anti-Human von Willebrand Factor/HRP antibody (Dako-Cytomation) and polyclonal rabbit anti-human TSP-1 (Calbiochem, Nottingham, UK), respectively.

Analysis of vWF multimer composition

Analysis of vWF multimers was carried out by SDS-agarose gel electrophoresis, using 2% HGT agarose type VI (Sigma-Aldrich). Gels were poured with running gel buffer between two glass plates (18×16 cm) pre-heated at 50°C and separated by a 0.75 mm thick spacer. Electrophoresis was carried out in a vertical gel unit SE 600 (Hoefer Inc. Holliston, MA, USA) and run in a cold room at 4°C for 21 h at 55 V with a gentle stirring of the anode buffer. The protocol was adapted from Raines *et al.*[31] and Groot *et al.*[32] After electrophoresis, proteins were transferred from the gel to a PVDF membrane (Bio-Rad, Hercules, CA, USA) by capillary blotting procedure overnight at RT in PBS 1X containing 5% methanol. After protein transfer, the Western blot analysis for vWF was carried out using Polyclonal Rabbit Anti-Human Von Willebrand Factor/HRP (1:1,000) (DakoCytomation). Multimers were detected using enhanced chemiluminescence (ECL) (GE Healthcare, Buckinghamshire, UK).

mbPECAM-1 flow cytometry analysis

Cells were labeled with a mouse anti-human PECAM-1 IgG (clone WM59, Clinisciences, Montrouge, France) and were analyzed on a FACS Calibur flow cytometer (Beckton Dinckinson, NJ, USA) which analyzes 10,000 cells for the presentation of the specific antigen. The number of copies of the antigen molecule per VEC was estimated from a calibration curve obtained with Qifikit calibration beads (DakoCytomation). For reasons of cell availability, flow cytometry analyses were carried out only with TrHBMEC and HUVEC.

Statistical analysis

Statistical analyses included the t-test in Graph Pad Prism Software (GraphPad Software, San Diego, USA). Two groups were considered to be significantly different if the *P* value was less than 0.05.

Results

Microarray analysis of adhesion-related gene expression in TrHBMEC

All data from the microarray analysis have been deposited at the Gene Expression Omnibus gene expression data repository (*http://www.ncbi.nlm.nih.gov/geo/*) (GEO accession number GSE11372). The exhaustive list of HC-target genes modulated in TrHBMEC under basal and pro-inflammatory conditions has been established previously.[24]

Adhesion events are crucial for vasoocclusion. We therefore focused on 1,275 genes related to adhesion (*Online Supplementary Table S1*). Under basal conditions, 24 h of HC treatment modulated the expression of 53 genes and 48 h of treatment modulated the expression of 49 genes (*Online Supplementary Tables S3 and S4*). Fewer genes were modulated by HC in pro-inflammatory conditions than in basal conditions: 33 genes at 24 h and 21 genes at 48 h (*Online Supplementary Tables S5 and S6*). The modulated genes included several genes encoding for collagen and ADAM (A Disintegrin And Metalloproteinase). For example, expression of *COL1A1* encoding the alpha1 chain of collagen type I was decreased by HC treatment at 24 h both in basal and pro-inflammatory conditions (5- and 1.6-fold change in basal and pro-inflammatory conditions, respectively). The metalloproteinase ADAMTS4 was increased by HC whatever the cellular context (basal or pro-inflammatory) and the treatment period (fold changes between 5 and 2). The

microarray analysis also indicated that VCAM-1, a key mediator of cell adhesion expressed in pro-inflammatory conditions, was down-regulated by HC treatment at 24 h in the presence of CY (*Online Supplementary Table S5*); this is in agreement with our previous findings.[22,24] We then individually assessed the effects of HC on the expression in TrHBMEC, HUVEC, and HPMEC of genes involved in SCD pathophysiology, namely *TSP-1*, *vWF*, *PECAM-1* and *CD36*. *CD36* is expressed in HPMEC but not in TrHBMEC or in HUVEC.[10, 25]

Effects of HC and/or CY on the two partners of adhesion: TSP-1 and CD36

HC treatment significantly decreased the amount of *TSP-1* mRNA in TrHBMEC and in HPMEC. In TrHBMEC, the decrease was observed from 10 h of HC treatment in basal conditions (58.2±9.5% of the control, *P*=0.0122) (Figure 1Ai) and 5 h of HC treatment in pro-inflammatory conditions (80.7±5.9% of the control, *P*=0.047) (Figure 1Aii). The maximal decrease was after 24 h both in basal and pro-inflammatory conditions (30.8±4.3% of the control, *P*<0.0001, and 37±6.9% of the control, *P*=0.0006, respectively) and the expression remained low after 48 h (30.80±9.6% of the control, *P*=0.0003, and 37.6±14.1% of the control, *P*=0.0002, respectively) (Figure 1Ai-ii). In HPMEC, the amount of *TSP-1* mRNA was significantly decreased by HC at 48 h in basal conditions (85.9±2.6% of the control, *P*=0.0077) and at 24 h and 48 h in pro-inflammatory conditions (79.06±6.3% of the control, *P*=0.018 and 80.4±4.9%, *P*=0.037, respectively) (Figure 1Ai-ii). In TrHBMEC treated with CY alone, the amount of this mRNA was significantly higher than in untreated cells at 5 h (145.2±14.9% of the control, *P*=0.0392) but then decreased progressively with time to 54.8±3.2% of the control (*P*=0.0002) at 48 h (Figure 1Aiii). A slight decrease was also observed in HPMEC but without reaching significance (80.2±10.2% of the control) (Figure 1Aiii). By contrast, CY alone increased *TSP-1* mRNA level in HUVEC to a maximum at 16 h (153.9±16.04% of the control, *P*=0.0153) (Figure 1Aiii). Moreover, HC did not affect *TSP-1* mRNA level in HUVEC in either basal or pro-inflammatory conditions (e.g. 144±20.9% and 108±8.4% of the control, respectively, at 48 h) (Figure 1Ai-ii).

At the protein level, TSP-1 concentration was lower in supernatants from TrHBMEC and HUVEC (669±105 ng/mL and 500.4±62 ng/mL, respectively, at 48 h for NT cells) than in HPMEC supernatants (15,350±1,960 ng/ml) (Figure 1Bi). Concerning the effect of HC, a 20-25% decrease was observed in TrHBMEC supernatants at 48 h (669.8±105.5 ng/mL for NT cells *vs.* 439±96.99 ng/mL for HC-treated cells) and in HPMEC supernatant (15,350±1,960 ng/mL for NT-cells *vs.* 12,100±2,226 ng/mLfor HC-treated cells) in basal conditions, but these differences did not reach statistical significance (Figure 1Bi-ii). Consistent with the findings for the mRNA, HC treatment did not significantly affect TSP-1 concentration in HUVEC supernatants, whatever the treatment duration, either in basal or pro-inflammatory conditions (Figure 1Bi-ii). CY did not significantly affect TSP-1 protein secretion into the supernatant by any of the three cell types: TrHBMEC (608±87.4 ng/mL), HUVEC (493±91 ng/mL) and HPMEC (14,434±2,045 ng/mL) at 48 h.

As TSP-1 is also incorporated in the ECM, *in situ* ECM ELISA was carried out to evaluate TSP-1 quantity in TrHBMEC-generated ECM following HC and/or CY treatment. Neither HC nor CY treatment seems to modify the quantity of TSP-1 in ECM whatever the treatment period (Figure 1Biii).

The *CD36* gene is not expressed in TrHBMEC or in HUVEC.[10,25] In HPMEC, its mRNA expression was not significantly affected by HC treatment. On the contrary, CY alone provoked a large decrease of *CD36* mRNA level at 24 h and 48 h (21.1±5.8% of the control, *P*<0.0001, and 18±4.8% of the control, *P*<0.0001) (Figure 1Ciii).

Effects of HC and/or CY on vWF

In basal conditions, HC significantly decreased *vWF* mRNA level in TrHBMEC after 16 h (68±3.1% of the control, *P*<0.001) and this decrease remained stable at 24 h (55.8±6.8% of the control, *P*=0.0001) and 48 h (65.8±26.1% of the control, *P*=0.042) (Figure 2Ai). The level of *vWF* mRNA was similarly decreased in TrHBMEC in pro-inflammatory conditions after 24 h of HC treatment (67±11.2% of the control, *P*=0.04), this decrease being greater at 48 h of treatment (45.4±22.7% of the control, *P*=0.005) (Figure 2Aii). In HPMEC, HC did not modulate *vWF* expression in basal conditions (Figure 2Ai), and a small decrease was observed in pro-inflammatory conditions (83.8±6.1% of the control, *P*=0.058) (Figure 2Aii). By contrast, HC significantly increased the amount of *vWF* mRNA in HUVEC at 24 h in basal conditions (155.6±19.39% of the control, *P*=0.045) (Figure 2Ai), a slight increase is also observed in pro-inflammatory conditions at 24 h (135±15%, *P*=0.086) but without reaching a statistical significance (Figure 2Aii). CY alone did not modulate *vWF* mRNA in HUVEC (Figure 2Aiii) but provoked a transient significant decrease of *vWF* mRNA level in HPMEC at 24 h (39.5±2.9% of the control, *P*<0.0001) and in TrHBMEC at 16 h and 24 h (76.6±7.7% of the control, *P*=0.0386 and 60.6±8.3% of the control, *P*=0.0091, respectively) (Figure 2Aiii).

HC and/or CY did not modulate vWF concentrations in supernatants from the three cell types, whatever the treatment period and the environment (Figure 2Bi-ii). The vWF protein concentration was higher, both in basal and in pro-inflammatory conditions, in HUVEC supernatant than in TrHBMEC and HPMEC supernatants (e.g. the vWF concentration was 342.1±120.8 ng/mL at 48 h in basal conditions in HUVEC and 132.4±77.2ng/mL in TrHBMEC and 121.8±12.8 ng/mL in HPMEC) (Figure 2Bi).

As for TSP-1, *in situ* ECM ELISA was processed to quantify vWF in TrHBMEC and HPMEC matrix. HC treatment significantly decreased vWF quantity incorporated in TrHBMEC ECM at 48 h in basal conditions (3.8±0.5 ng/cm^2 for NT and 2.1±0.2 for HC-treated cells, *P*=0.0051) (Figure 2Biii). vWF quantity incorporated in HPMEC MEC was not significantly affected whatever the treatment and the treatment period (Figure 2Biii-iv).

vWF ultralarge multimers (UL-vWF) are the most hemostatically active. Analysis of the vWF multimer pattern was conducted in the supernatants of TrHBMEC, HUVEC, and HPMEC to test for a potential HC impact on vWF multimer generation. Results in Figure 3 compare the multimer patterns in the three cell types of NT and HC-treated cells at 48 h in basal conditions. HC did not seem to modify vWF multimer patterns in the three cell types. However, interestingly, the patterns were clearly different between the three cell types. HUVEC excreted UL-vWF, but TrHBMEC and HPMEC presented profiles mostly composed of L-vWF and M-vWF multimers with only small amounts of UL-vWF. Profiles were identical in inflammatory conditions (*data not shown*).

Effects of HC and/or CY on PECAM-1

In basal conditions, *PECAM-1* mRNA was strongly down-regulated within 5 h of HC treatment in TrHBMEC (80.7±5.1% of the control, *P*=0.0049) and continued to decline at 24 h (55.8±9.5% of the control, *P*=0.0098) and 48 h (61±8.4% of the control, *P*=0.01) (Figure 4Ai). In basal conditions, HC did not modulate *PECAM-1* mRNA level in HUVEC or HPMEC (Figure 4Ai). In inflammatory conditions, the decreased expression of *PECAM-1* mRNA in TrHBMEC was delayed as compared to basal conditions: the decrease was observed from 24 h of HC treatment (80.7±5.2% of the control, *P*=0.0082), and was greater at 48 h of treatment (56.6±23.7% of the control, *P*=0.015) (Figure 4Aii). In these pro-inflammatory conditions, *PECAM-1* mRNA level in HUVEC was not affected by HC treatment but a small decrease was observed in HPMEC at 48 h (82.7±5.1% of the control, *P*=0.04) (Figure 4Aii). Treatment with pro-inflammatory CY alone did not significantly affect *PECAM-1* mRNA in HUVEC but transiently decreased its level in HPMEC at 24 h (52.4±6.5% of the control, *P*=0.0019) and in TrHBMEC from 5 h (76.8±3.5% of the control, *P*=0.0027) to 24 h (52.2±7.5% of the control, *P*=0.0032) (Figure 4Aiii).

As PECAM-1 is a transmembrane protein, we investigated the effect of HC on PECAM-1 expression on the surface of TrHBMEC and HUVEC by flow cytometry. HC and/or

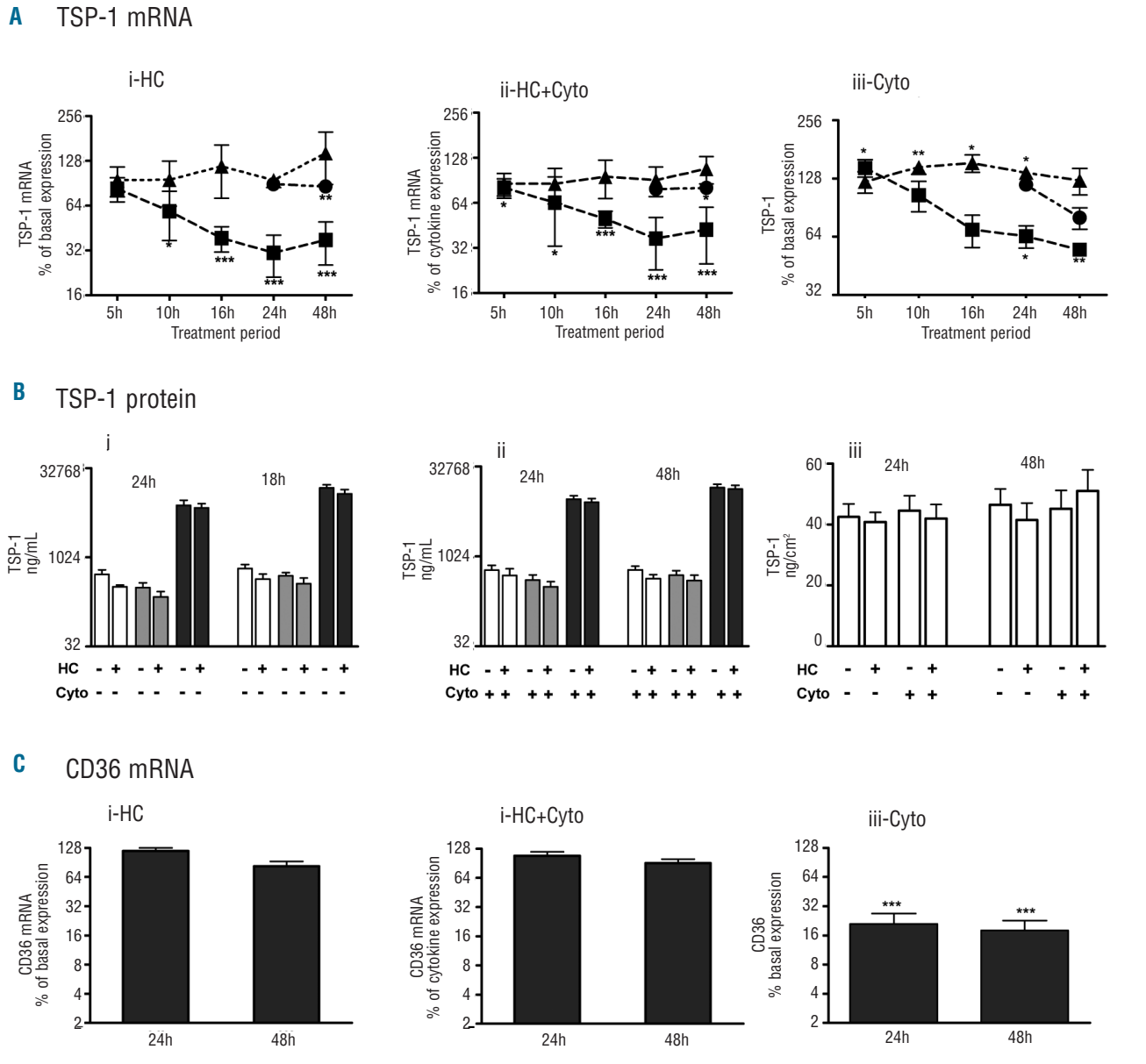

Figure 1. Effects of HC and/or Cytokines on TSP-1 and CD36 expression. (A) TSP-1 gene expression was analyzed under HC (i), HC+Cyto (ii) and Cyto (iii) treatment in TrHBMEC (■), HUVEC (▲) and HPMEC (●). Results are expressed as % expression of the control value. (B) TSP-1 protein concentrations in culture supernatants and ECM of TrHBMEC (white bars), HUVEC (gray bars), and HPMEC (dark bars). Results are expressed in ng/ml under HC (i) and HC+Cyto (ii) treatment and in ng/cm² for ECM analysis (iii). (C) Modulation of CD36 gene expression in HPMEC (dark bars) is expressed as % expression of the control value under HC (i), HC+Cyto (ii) and Cyto (iii) treatment. A $\log_2$ scale is used except for ECM analysis. **P*<0.05, ***P*<0.01, *** *P*<0.001.

CY did not affect either the numbers of positive cells or the numbers of sites per cell for whichever VEC type, irrespective of treatment duration (*data not shown*).

The soluble form of PECAM-1 (sPECAM-1) was not detected in TrHBMEC or HPMEC supernatants. But sPECAM-1 was detected in HUVEC supernatants after 24 h of culture and its concentration was significantly diminished after 48 h of HC treatment in basal conditions (704.6±6 pg/mL for NT cells and 633.4±11.7 pg/mL for HC-treated cells, *P*=0.0006) (Figure 4Bi). CY alone provoked a small decrease of the sPECAM-1 concentration in HUVEC supernatants after 24 h of treatment: 560±10.4 pg/mL for NT and 514±11.6 pg/mL for CY-treated cells, *P*=0.01 (Figure 4Bi-ii).

Discussion

This study is the first in which a global approach (microarray analysis) has been used to investigate the effect of HC and/or CY on the expression of genes related to adhesion events in VEC. In addition, the expression of genes of interest, as inferred from this approach, was then tested individually on VEC models of the microcirculation that are specifically relevant to SCD and VOC, i.e. the TrHBMEC cell line and primary cultures of HPMEC. This was carried out in comparison with HUVEC, the most widely utilized macrocirculation model.

In our microcirculation models, TrHBMEC and HPMEC, HC reduced the expression of TSP-1, vWF, and PECAM-1 encoding genes at the mRNA level. TSP-1 is synthesized and secreted by VEC and circulating cells such as platelets and monocytes. This multifunctional adhesive protein is present in soluble form in the plasma (sTSP-1) and in the VEC basement membrane. These two forms play an important role in the increased RBC adhesion to the endothelium and the subendothelial matrix. Indeed, the concentration of sTSP-1 is higher than normal in SCD patient plasma, implicating this factor in vasoocclusive events.[33] It has also been shown that sickle RBC adhere to immobilized TSP-1 under flow conditions.[34] We observed a slight but consistent decrease of sTSP-1 in TrHBMEC and HPMEC supernatants. However, it did not reach statistical significance and thus did not match the clear results observed at the mRNA level. Similarly, we did not find statistically significant differences in the TSP-1 ECM content. A protein effect is obviously

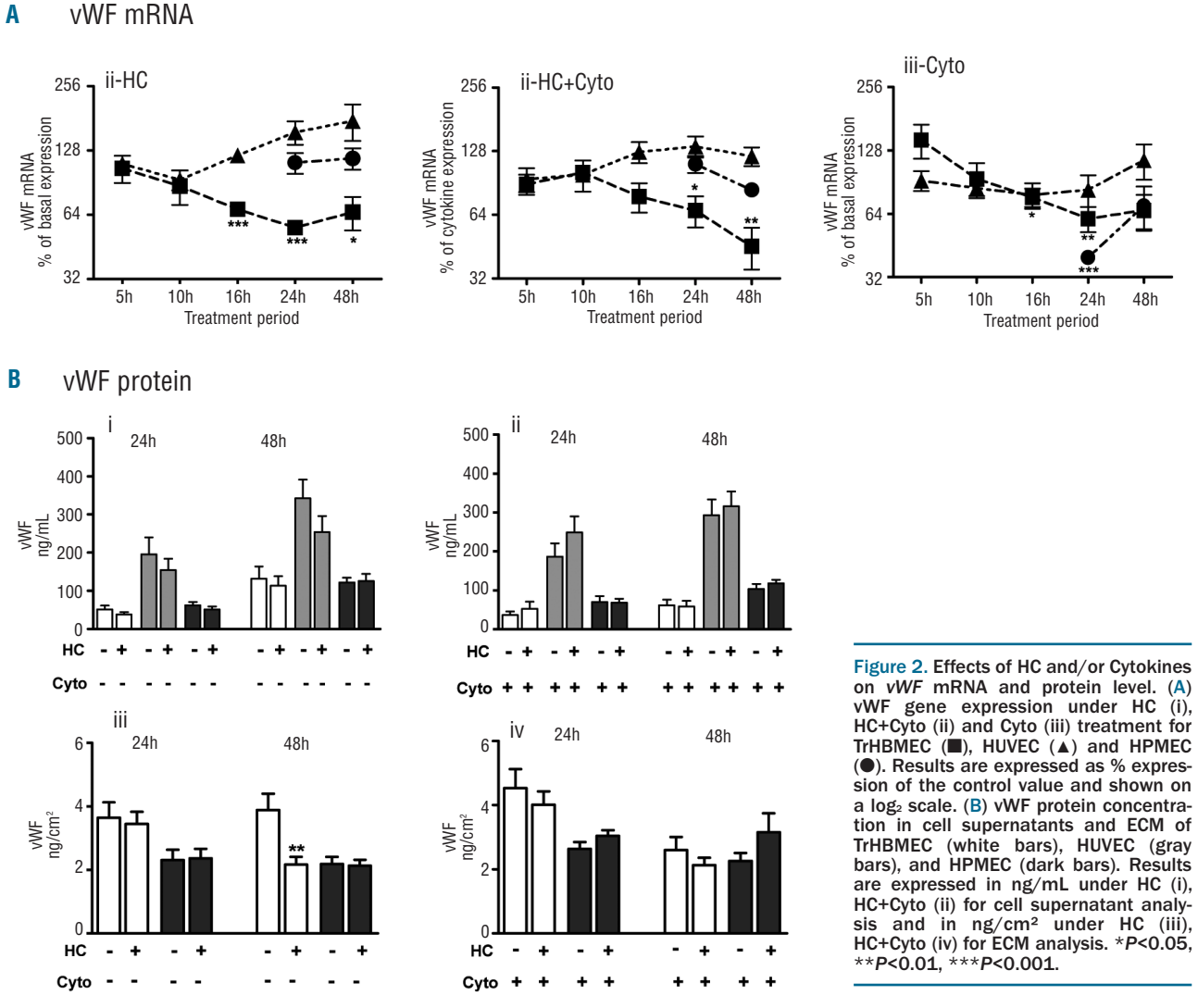

Figure 2. Effects of HC and/or Cytokines on *vWF* mRNA and protein level. (A) vWF gene expression under HC (i), HC+Cyto (ii) and Cyto (iii) treatment for TrHBMEC (■), HUVEC (▲) and HPMEC (●). Results are expressed as % expression of the control value and shown on a $\log_2$ scale. (B) vWF protein concentration in cell supernatants and ECM of TrHBMEC (white bars), HUVEC (gray bars), and HPMEC (dark bars). Results are expressed in ng/mL under HC (i), HC+Cyto (ii) for cell supernatant analysis and in ng/cm² under HC (iii), HC+Cyto (iv) for ECM analysis. **P*<0.05, ***P*<0.01, ****P*<0.001.

delayed *versus* the mRNA effect and it is possible that a 48 h treatment was insufficient to observe a significant difference at the protein level. However, longer incubations with HC are not possible because of the drug toxicity; cells are not viable beyond this period of time.

Hillery *et al.* described decreased adhesiveness to TSP-1 of RBC from sickle patients treated with HC.[18] This raises the issue of TSP-1 receptors, including the first to be described, i.e. CD36. Soluble TSP-1 connects two CD36 molecules on the sickle RBC and VEC, respectively. Several studies have shown that HC decreases CD36 expression on sickle RBC membranes.[18,19] In HPMEC, the only cell type in which the *CD36* gene was expressed in our study, its expression was not affected by HC, but strongly inhibited by CY. Actually, the contribution of CD36 in vasoocclusive events is controversial. Lee *et al.* reported that the clinical course of CD36-deficient SCD patients is similar to that of CD36-positive SCD patients, suggesting that CD36 does not play a crucial role in vasoocclusion.[35] However, Trinh-Trang-Tan *et al.*, using sickle cell SAD mice, showed recently that erythrocyte CD36 contributes to the increased SAD RBC adhesion, while endothelial CD36 is not indispensable.[36] The issue is complex because CD36 is less strongly expressed on RBC from SAD than wild-type mice. Thus a potential CY-induced decrease of CD36 at the surface of HPMEC might not directly correlate with decreased adhesive properties in inflammatory conditions. In platelets, the phosphorylation state of CD36 dictates its ligand specificity and only dephosphorylated CD36 binds TSP-1.[37] Thus, phosphorylation, another level of regulation of the TSP-1/CD36 adhesion processes in SCD, should be considered in addition to the expression level. Another TSP-1 receptor on RBC is CD47 (or IAP, integrin-associated protein).[38] Unexpectedly, Odièvre *et al.* described an increase of CD47 at the RBC surface in SCD patients treated with HC.[19] However, here again, the situation is complex because the main involvement of CD47 in adhesion events might not be through its direct interaction with subendothelial TSP-1 but as an intermediate of a signaling pathway in which plasma TSP-1 binding to CD47 initiates a G-protein-PKA-dependent cascade leading to the activating phosphorylation of the $\alpha_4\beta_1$ integrin.[39] Thus it can be expected that even a modest decrease of sTSP-1 under HC treatment, which would be coherent with our results, may lead to significant variations in the number of adhesion events between RBC and VEC.

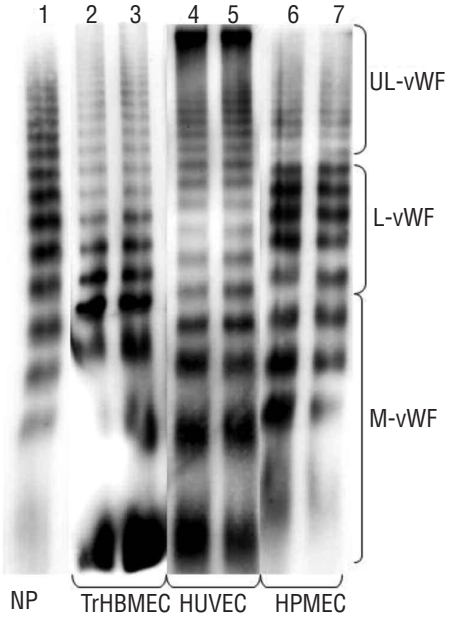

Figure 3. vWF multimeric pattern at 48 h of treatment. 1: normal plasma, 2: NT TrHBMEC, 3: HC-treated TrHBMEC, 4: NT HUVEC, 5: HC-treated HUVEC, 6: NT-HPMEC and 7: HC-treated HPMEC

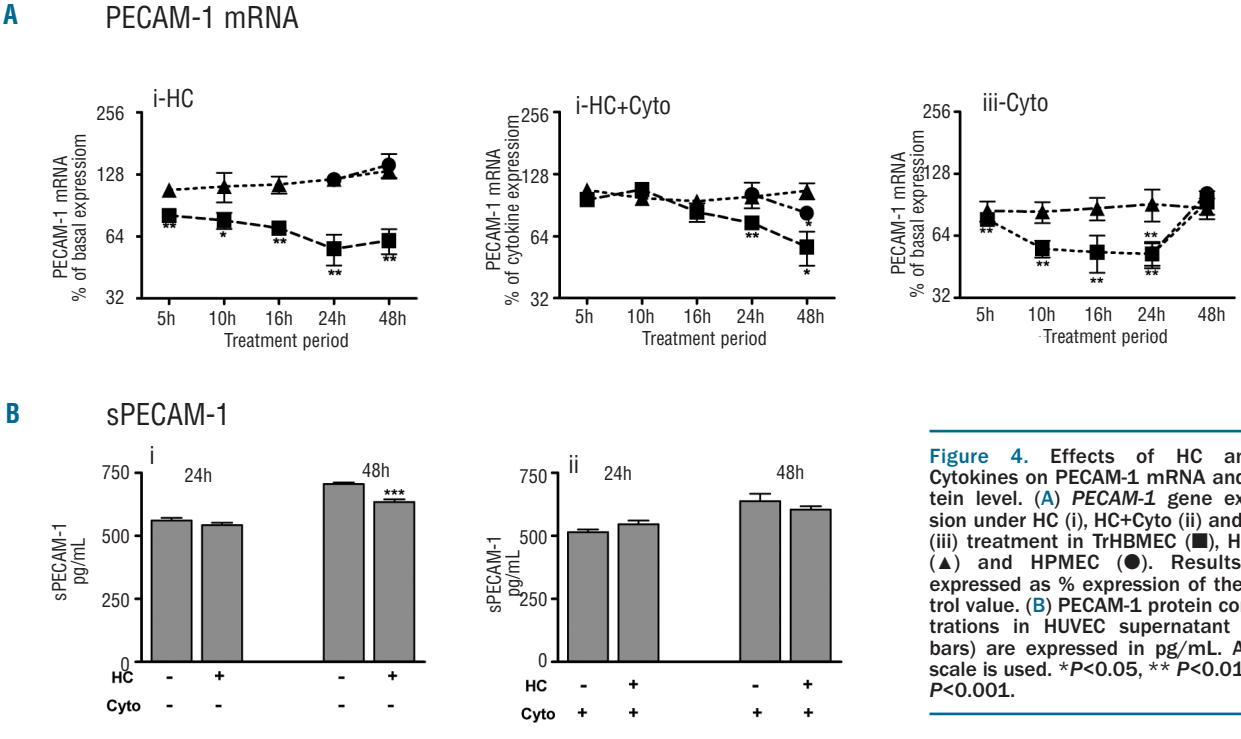

Figure 4. Effects of HC and/or Cytokines on PECAM-1 mRNA and protein level. (A) *PECAM-1* gene expression under HC (i), HC+Cyto (ii) and Cyto (iii) treatment in TrHBMEC (■), HUVEC (▲) and HPMEC (●). Results are expressed as % expression of the control value. (B) PECAM-1 protein concentrations in HUVEC supernatant (gray bars) are expressed in pg/mL. A $\log_2$ scale is used. **P*<0.05, ** *P*<0.01, *** *P*<0.001.

Concerning the effect of inflammation mediators, expression of the *TSP-1* gene was decreased by CY treatment (TNFα and IFNγ). Previous findings concerning the effects of TNFα on TSP-1 level are controversial;[40,41] IFNγ seems to increase TSP-1 expression in monocytes.[42] Thus, the increased plasmatic TSP-1 concentration observed in SCD patients is probably not a direct result of increased levels of TNFα on VEC but may be the consequence of multi-cellular and multi-molecular signals. In our study, *CD36* mRNA expression was strongly decreased by CY treatment in HPMEC. In previous studies, CY did not alter CD36 expression modulation in monocytes and CD36 expression was not induced in HUVEC after treatment by TNFα or IFNγ.[10,42]

Like TSP-1, vWF may promote RBC adhesion, and this may be particularly true for unusually large vWF multimers (ULvWF) which are the most hemostatically active.[43,44] TSP-1 and vWF show partially related functions, the most important one being the TSP-1 role in ADAMTS13 operated vWF multimer processing.[45] Like platelets, RBC adhere to VEC exposing vWF at high shear stress.[44,46] As a constituent of the subendothelial matrix, vWF can also promote adhesion between circulating cells and a damaged vessel wall. Our data show that HC down-regulates *vWF* gene expression by VEC from the microcirculation (HPMEC and TrHBMEC). This was not accompanied by a decrease in soluble vWF in the cell supernatant, or changes in the vWF multimer profile. However, we observed a marked decrease in vWF in the ECM of HC-treated TrHBMEC at 24 h in basal conditions. This downregulation of vWF by HC may contribute significantly to the reduction of adhesion events.

We found that HC decreases *PECAM-1* mRNA level in TrHBMEC, but it did not affect protein expression at the cell surface; sPECAM-1 was undetectable in the supernatant of the two microcirculation cell types tested whatever the conditions. A different effect was observed in HUVEC from the macrocirculation: although HC did not affect *PECAM-1* mRNA or its expression at the cell surface, it significantly reduced sPECAM-1 in the cell supernatant. PECAM-1 is a multifunctional molecule that plays a key role in inflammation and vascular biology.[47] It has been implicated in adhesion events and specifically in leukocyte/monocyte transmigration during inflammation processes. Incubation of sickle RBC with HUVEC induces monocyte extravasation.[48] PECAM-1 plays an important role in ischemia/reperfusion injury and increased sPECAM-1 levels have been reported in patients with acute ischemic stroke.[49] The current data suggest that PECAM-1 blockade can be protective in numerous animal models of ischemia/reperfusion injury. Thus our observation that HC reduces sPECAM-1 produced in an endothelial cell model from the macrocirculation may suggest a potential beneficial effect of HC for stroke prevention in SCD patients. This would be of interest in ongoing randomized trials addressing the role of HC in the prevention of large vessel cerebral disease.[50] However, this cannot be shown at the protein level only because the adhesion function of PECAM-1 is activated by phosphorylation mediated by the PKC pathway.[51]

Altogether, our gene expression results suggest an anti-adhesogenic effect of HC on VEC, but by different mechanisms depending upon the VEC type. For instance, at the protein level HC decreases vWF in TrHBMEC ECM and sPECAM-1 in HUVEC supernatant. However, this hypothesis still remains to be tested at the functional level. One has also to keep in mind the importance of posttranslational phosphorylation-mediated regulation of the adhesion properties of some adhesion molecules. In this context, of particular interest is the recent observation by Bartolucci *et al.*[52] that the decreased RBC adhesion to laminin in SCD patients treated with HC is associated with inhibition of erythroid Lu/BCAM protein phosphorylation. Thus, it is clear that in the future, analysis of HC action on adhesion processes should no longer be restricted to mRNA/protein levels.

Finally, our analysis of microarray data indicated that the expression of genes encoding several collagens and metalloproteases was modulated by HC and/or CY. Together with our observation of the modified expression of vWF in ECM, this is possibly suggestive of altered remodeling of the subendothelial matrix and thus be relevant in the context of the systemic vasculopathy associated with SCD.

In conclusion, our analysis shows that variations in the expression profile of genes related to adhesion events in response to HC and/or CY treatment differ between VEC of different origins. This differential response to the same stimuli is consistent with the substantial heterogeneity of VEC notably exemplified by Chi *et al.* at the transcriptomic level.[9] Similarly, it illustrates, on novel markers, the heterogeneity of VEC responsiveness to CY, an issue of important pharmacological consequences in many diseases involving inflammatory processes.[53] Consequently, the exact mode of action and efficacy of HC, as well as response to inflammatory stress, in SCD patients, may differ according to the vascular site.

Authorship and Disclosures

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References

1. Solovey A, Lin Y, Browne P, Choong S, Wayner E, Hebbel RP. Circulating activated endothelial cells in sickle cell anemia. N Engl J Med. 1997;337(22):1584-90.
2. Belcher JD, Marker PH, Weber JP, Hebbel RP, Vercellotti GM. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion. Blood. 2000;96(7):2451-9.
3. Wun T, Cordoba M, Rangaswami A, Cheung AW, Paglieroni T. Activated monocytes and platelet-monocyte aggregates in patients with sickle cell disease. Clin Lab Haematol. 2002;24(2):81-8.
4. Croizat H. Circulating cytokines in sickle cell patients during steady state. Br J Haematol. 1994;87(3):592-7.
5. Cartron JP, Elion J. Erythroid adhesion molecules in sickle cell disease: effect of hydroxyurea. Transfus Clin Biol. 2008;15(1-2):39-50.
6. Lawson ND, Scheer N, Pham VN, Kim CH, Chitnis AB, Campos-Ortega JA, et al. Notch signaling is required for arterial-venous differentiation during embryonic vascular development. Development. 2001;128(19):3675-83.
7. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. Circ Res. 2007;100(2):158-73.
8. Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ Res. 2007;100(2):174-90.
9. Chi JT, Chang HY, Haraldsen G, Jahnsen FL,

Troyanskaya OG, Chang DS, et al. Endothelial cell diversity revealed by global expression profiling. Proc Natl Acad Sci USA. 2003;100(19):10623-8.
10. Swerlick RA, Lee KH, Wick TM, Lawley TJ. Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. J Immunol. 1992;148(1):78-83.
11. Muller AM, Hermanns MI, Skrzynski C, Nesslinger M, Muller KM, Kirkpatrick CJ. Expression of the endothelial markers PECAM-1, vWf, and CD34 in vivo and in vitro. Exp Mol Pathol. 2002;72(3):221-9.
12. Brawley OW, Cornelius LJ, Edwards LR, Gamble VN, Green BL, Inturrisi CE, et al. NIH consensus development statement on hydroxyurea treatment for sickle cell disease. NIH Consens State Sci Statements. 2008;25(1):1-30.
13. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med. 1995; 332(20):1317-22.
14. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, Kutlar A, et al. Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA. 2003;289 (13):1645-51.
15. Maier-Redelsperger M, Labie D, Elion J. Long-term hydroxyurea treatment in young sickle cell patients. Curr Opin Hematol. 1999;6(2):115-20.
16. Ware RE, Eggleston B, Redding-Lallinger R, Wang WC, Smith-Whitley K, Daeschner C, et al. Predictors of fetal hemoglobin response in children with sickle cell anemia receiving hydroxyurea therapy. Blood. 2002;99(1):10-4.
17. Ware RE. How I use hydroxyurea to treat young patients with sickle cell anemia. Blood. 2010;115(26):5300-11.
18. Hillery CA, Du MC, Wang WC, Scott JP. Hydroxyurea therapy decreases the in vitro adhesion of sickle erythrocytes to thrombospondin and laminin. Br J Haematol. 2000;109(2):322-7.
19. Odievre MH, Bony V, Benkerrou M, Lapoumeroulie C, Alberti C, Ducrocq R, et al. Modulation of erythroid adhesion receptor expression by hydroxyurea in children with sickle cell disease. Haematologica. 2008;93(4):502-10.
20. Styles LA, Lubin B, Vichinsky E, Lawrence S, Hua M, Test S, et al. Decrease of very late activation antigen-4 and CD36 on reticulocytes in sickle cell patients treated with hydroxyurea. Blood. 1997;89(7):2554-9.
21. Adragna NC, Fonseca P, Lauf PK. Hydroxyurea affects cell morphology, cation transport, and red blood cell adhesion in cultured vascular endothelial cells. Blood. 1994; 83(2):553-60.
22. Brun M, Bourdoulous S, Couraud PO, Elion J, Krishnamoorthy R, Lapoumeroulie C. Hydroxyurea downregulates endothelin-1 gene expression and upregulates ICAM-1 gene expression in cultured human endothelial cells. Pharmacogenomics J. 2003;3(4): 215-26.
23. Lapoumeroulie C, Benkerrou M, Odievre MH, Ducrocq R, Brun M, Elion J. Decreased plasma endothelin-1 levels in children with sickle cell disease treated with hydroxyurea. Haematologica. 2005;90(3):401-3.
24. Laurance S, Pellay FX, Dossou-Yovo OP, Verger E, Krishnamoorthy R, Lapoumeroulie C, et al. Hydroxycarbamide stimulates the production of proinflammatory cytokines by endothelial cells: relevance to sickle cell disease. Pharmacogenet Genomics. 2010;20 (4):257-68.
25. Schweitzer KM, Vicart P, Delouis C, Paulin D, Drager AM, Langenhuijsen MM, et al. Characterization of a newly established human bone marrow endothelial cell line: distinct adhesive properties for hematopoietic progenitors compared with human umbilical vein endothelial cells. Lab Invest. 1997;76(1):25-36.
26. Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest. 1973;52(11):2745-56.
27. De Franceschi L, Bachir D, Galacteros F, Tchernia G, Cynober T, Neuberg D, et al. Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease. Br J Haematol. 2000;108(2):284-9.
28. Bieche I, Onody P, Laurendeau I, Olivi M, Vidaud D, Lidereau R, et al. Real-time reverse transcription-PCR assay for future management of ERBB2-based clinical applications. Clin Chem. 1999;45(8 Pt 1):1148-56.
29. Poon RT, Chung KK, Cheung ST, Lau CP, Tong SW, Leung KL, et al. Clinical significance of thrombospondin 1 expression in hepatocellular carcinoma. Clin Cancer Res. 2004;10(12 Pt 1):4150-7.
30. Bonnefoy A, Harsfalvi J, Pfliegler G, Fauvel-Lafeve F, Legrand C. The subendothelium of the HMEC-1 cell line supports thrombus formation in the absence of von Willebrand factor and collagen types I, III and VI. Thromb Haemost. 2001;85(3):552-9.
31. Raines G, Aumann H, Sykes S, Street A. Multimeric analysis of von Willebrand factor by molecular sieving electrophoresis in sodium dodecyl sulphate agarose gel. Thromb Res. 1990;60(3):201-12.
32. Groot E, Fijnheer R, Sebastian SA, de Groot PG, Lenting PJ. The active conformation of von Willebrand factor in patients with thrombotic thrombocytopenic purpura in remission. J Thromb Haemost. 2009;7(6): 962-9.
33. Browne PV, Mosher DF, Steinberg MH, Hebbel RP. Disturbance of plasma and platelet thrombospondin levels in sickle cell disease. Am J Hematol. 1996;51(4):296-301.
34. Joneckis CC, Shock DD, Cunningham ML, Orringer EP, Parise LV. Glycoprotein IV-independent adhesion of sickle red blood cells to immobilized thrombospondin under flow conditions. Blood. 1996;87(11):4862-70.
35. Lee K, Gane P, Roudot-Thoraval F, Godeau B, Bachir D, Bernaudin F, et al. The nonexpression of CD36 on reticulocytes and mature red blood cells does not modify the clinical course of patients with sickle cell anemia. Blood. 2001;98(4):966-71.
36. Trinh-Trang-Tan MM, Vilela-Lamego C, Picot J, Wautier MP, Cartron JP. Intercellular adhesion molecule-4 and CD36 are implicated in the abnormal adhesiveness of sickle cell SAD mouse erythrocytes to endothelium. Haematologica. 2009;95(5):730-7.
37. Ash AS. Outcomes analysis and the practice of medicine. Hosp Pract (Off Ed). 1993;28 (10):10-1.
38. Brittain JE, Mlinar KJ, Anderson CS, Orringer EP, Parise LV. Integrin-associated protein is an adhesion receptor on sickle red blood cells for immobilized thrombospondin. Blood. 2001;97(7):2159-64.
39. Brittain JE, Han J, Ataga KI, Orringer EP, Parise LV. Mechanism of CD47-induced alpha4beta1 integrin activation and adhesion in sickle reticulocytes. J Biol Chem. 2004;279(41):42393-402.
40. Morandi V, Cherradi SE, Lambert S, Fauvel-Lafeve F, Legrand YJ, Legrand C. Proinflammatory cytokines (interleukin-1 beta and tumor necrosis factor-alpha) down regulate synthesis and secretion of thrombospondin by human endothelial cells. J Cell Physiol. 1994;160(2):367-77.
41. Narizhneva NV, Razorenova OV, Podrez EA, Chen J, Chandrasekharan UM, DiCorleto PE, et al. Thrombospondin-1 up-regulates expression of cell adhesion molecules and promotes monocyte binding to endothelium. Faseb J. 2005;19(9):1158-60.
42. Yesner LM, Huh HY, Pearce SF, Silverstein RL. Regulation of monocyte CD36 and thrombospondin-1 expression by soluble mediators. Arterioscler Thromb Vasc Biol. 1996;16(8):1019-25.
43. Kaul DK, Nagel RL, Chen D, Tsai HM. Sickle erythrocyte-endothelial interactions in microcirculation: the role of von Willebrand factor and implications for vasoocclusion. Blood. 1993;81(9):2429-38.
44. Wick TM, Moake JL, Udden MM, Eskin SG, Sears DA, McIntire LV. Unusually large von Willebrand factor multimers increase adhesion of sickle erythrocytes to human endothelial cells under controlled flow. J Clin Invest. 1987;80(3):905-10.
45. Bonnefoy A, Hoylaerts MF. Thrombospondin-1 in von Willebrand factor function. Curr Drug Targets. 2008;9(10):822-32.
46. Andre P, Denis CV, Ware J, Saffaripour S, Hynes RO, Ruggeri ZM, et al. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. Blood. 2000;96(10):3322-8.
47. Woodfin A, Voisin MB, Nourshargh S. PECAM-1: a multi-functional molecule in inflammation and vascular biology. Arterioscler Thromb Vasc Biol. 2007;27(12): 2514-23.
48. Sultana C, Shen Y, Rattan V, Johnson C, Kalra VK. Interaction of sickle erythrocytes with endothelial cells in the presence of endothelial cell conditioned medium induces oxidant stress leading to transendothelial migration of monocytes. Blood. 1998;92(10):3924-35.
49. Zaremba J, Losy J. sPECAM-1 in serum and CSF of acute ischaemic stroke patients. Acta Neurol Scand. 2002;106(5):292-8.
50. Gulbis B, Haberman D, Dufour D, Christophe C, Vermylen C, Kagambega F, et al. Hydroxyurea for sickle cell disease in children and for prevention of cerebrovascular events: the Belgian experience. Blood. 2005;105(7):2685-90.
51. Rattan V, Sultana C, Shen Y, Kalra VK. Oxidant stress-induced transendothelial migration of monocytes is linked to phosphorylation of PECAM-1. Am J Physiol. 1997;273(3 Pt 1):E453-61.
52. Bartolucci P, Chaar V, Picot J, Bachir D, Habibi A, Fauroux C, et al. Decreased sickle red blood cell adhesion to laminin by hydroxyurea is associated with inhibition of Lu/BCAM protein phosphorylation. Blood. 2010;116(12):2152-9.
53. Molema G. Heterogeneity in endothelial responsiveness to cytokines, molecular causes, and pharmacological consequences. Semin Thromb Hemost. 2010;36(3):246-64.