Case Report

Treatment of Catastrophic Antiphospholipid Syndrome with Defibrotide, a Proposed Vascular Endothelial Cell Modulator

ARSINUR BURCOGLU-O'RAL, DORUK ERKAN, and RONALD ASHERSON

ABSTRACT. ***Objective.*** To define at the molecular level the vascular endothelial cell (VEC) injury characteristics of catastrophic antiphospholipid syndrome (CAPS) and to report successful therapeutic use of a VEC modulator, defibrotide.

Methods. We describe a 55-year-old man with primary APS with an intractable prothrombotic state (CAPS) resistant to combined therapy with heparin, warfarin, aspirin, and dipyridamole. Treatment with defibrotide was conducted in the context of an investigational phase II protocol where the dose was regulated and individualized by disease/patient-specific molecular and clinical markers.

Results. The patient entered complete remission with defibrotide treatment. During treatment, dose dependent pharmacological actions of defibrotide and key stress markers for VEC injury were identified. Evidence of defibrotide's polypharmacology included downregulation of cytokines, notably tumor necrosis factor-α, as the earliest effect, cellular differentiation of VEC, possibly with direct regulatory effect over cellular genes, and the reversal of platelet consumption and prothrombotic state. Von Willebrand antigen levels were used as the sole marker to guide therapy.

Conclusion. This case demonstrates effective remission of CAPS with defibrotide treatment. In contrast to theories that CAPS is triggered by ischemic and thrombotic tissue damage, these data present VEC injury as the primary and representative lesion of CAPS. The pathogenesis may involve concurrent impairment of different VEC functions. Achieving remission may require a polypharmacologic approach, represented here by use of defibrotide. (J Rheumatol 2002;29:2006–11)

Key Indexing Terms:

CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME
MULTIPLE ORGAN DYSFUNCTION SYNDROME DEFIBROTIDE

In contrast to the "classic" antiphospholipid syndrome (APS), venous or arterial large blood vessel occlusions do not dominate the clinical picture in catastrophic APS (CAPS). Rather, severe multiorgan dysfunction syndrome, characterized by diffuse small vessel ischemia and thrombotic occlusion of the microvasculature, predominantly affects the parenchymal organs[1-6]. The extensive tissue damage caused by this process results in the liberation of excessive amounts of cytokines, which lead as the afferent arm of the injury to further perturbations of vascular endothelial cell (VEC) functions[7,8], ultimately expressing themselves as the efferent arm in thrombotic lesions[9-11]. The main therapies for CAPS, either alone or in combination, include antithrombotic, fibrinolytic and antiplatelet agents, corticosteroids, and immunosuppressives[3]. Plasmapheresis is often combined with these pharmacologic agents[9]. No single modality has been shown thus far to provide survival value[2].

We propose VEC as the primary target of therapy, not addressed by current treatments. VEC injury not only involves the impairment of VEC regulatory functions within the circulatory, thrombotic, fibrinolytic, neuroendocrine vasoactive, immune, renal, and respiratory systems, but also compromises VEC coordinating functions over the renin-angiotensin system, endothelins, natriuretic peptides, prostaglandins, leukotrienes, and dopaminergic and purinergic systems, which leads to a state of functional failure ultimately characterized as multiorgan dysfunction syndrome[5,12]. Based on the potential impairment of such a multitude of functions that precede the final event of thrombus formation, it would be prudent to address this spectrum of pathology with a polypharmacologic approach. We propose using a single agent, defibrotide, for the reconstitution of these multiple VEC functions[12-17].

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The primary objective is to define at the molecular level the VEC injury characteristic of CAPS, and secondarily we describe successful therapeutic use of defibrotide as a polypharmacological agent in CAPS.

MATERIALS AND METHODS

Case report. The patient was a 55-year-old white man with history of hypertension, primary APS (postphlebitic syndrome of the left calf, thrombotic occlusion of left deep femoral vein 10 years ago, positive lupus anticoagulant (LAC) test, maintained taking warfarin), and asymptomatic proteinuria of 7000 mg/day for the past 6 years. His antihypertensive therapy had recently been changed to an angiotensin converting enzyme (ACE) inhibitor with subsequent onset of an intractable prothrombotic state (CAPS) with new deep vein thrombosis (DVT) of the right deep femoral vein, and progressive soft tissue necrosis involving all digits of his hands. On presentation, he had demarcation lines outlining the necrotic tissue that extended to the proximal metacarpophalangeal (MCP) joints. A venogram of the bilateral lower extremities showed possible new thrombus formation superior to the organized, old, deep left femoral and left popliteal thrombi, and a new, deep, right femoral thrombus. Arteriography of the hands showed severe narrowing, sclerosis, and intimal proliferation of the digital arterial beds bilaterally. Serologically, titers for IgG and IgM anticardiolipin antibodies (aCL) were 9 and 74 U/ml, respectively. The quantitative 24 h urine collection showed 7.9 g protein. Serum creatinine was 1.8 mg/dl.

Initially, he received intravenous (IV) heparin, aspirin, and dipyridamole in addition to warfarin. However, on Day 10 of admission, the clinical course deteriorated with the development of adult respiratory distress syndrome (ARDS) and a subendocardial myocardial infarction, which was confirmed by echocardiography, and elevations in the creatinine phosphokinase (CPK-MB) and troponin enzymes. He was intubated and all antithrombotic, antiplatelet, and antihypertensive medications were stopped. Defibrotide was initiated as the sole therapeutic agent at a dose of 80 mg/kg/day administered as a 24 h continuous infusion.

Defibrotide. Defibrotide is an alkali metal salt of single stranded DNA (Crinos Farmacobiologica SpA, Milan, Italy). It is an adenosine receptor agonist with affinity to adenosine receptors A1 and A2, an effect that appears to have antithrombotic function. Three of the aptamers isolated from the precursor molecule have highly conserved base sequences and act as potent inhibitors of thrombin induced platelet aggregation, as well as thromboxane biosynthesis[15]. The literature described a multitude of functions of defibrotide including: (1) upregulation of endogeneous prostaglandins (PG) E_2 and PGI_2; (2) downregulation of prothrombotic leukotriene B4[16-20]; (3) reconstitution of platelet deaggregatory actions via stimulation of vascular prostacyclin[20]; (4) upregulation of cyclic adenosine monophosphate[21]; (5) inhibition of cathepsin G secretion from activated polymorphonuclear leukocytes[22]; (6) inhibition of monocyte superoxide anion generation[23] and calcium influx into polymorphonuclear cells[24]; (7) stimulation of fibrinolysis primarily by the downregulation of plasminogen activator inhibitor-1 (PAI-1), and secondarily by increasing endogeneous tissue plasminogen activator (TPA) activity[13]; (8) upregulation of adenosine triphosphate, adenosine diphosphate; and (9) downregulation of endothelin-1 (ET-1)[19]. This multitude of defibrotide actions has been loosely defined as the VEC modulatory actions of defibrotide[14,16,17].

Defibrotide has a relatively short half-life of 10–30 min after IV administration. This half-life, however, refers to its sugar moiety, and not to the biological pharmacological half-life of the drug. Defibrotide may also be given orally using double the IV dose. Adverse effects may include flushing, transient mild systolic hypotension, nausea, and abdominal discomfort in less than 5% of patients.

Defibrotide use in this case was monitored by the regulatory structure for compassionate use of drugs. An investigational new drug (IND) protocol, an investigator's IND, and an individual patient IND were assigned. Eligibility, therapy, laboratory investigations, and patient followup were discussed with the US Food and Drug Administration (FDA), with the formation of an individual patient file for FDA records.

Treatment protocol. The patient described here is one of 17 others with various diseases who made up the population of an investigational phase II pilot trial. The eligibility criteria were the clinical diagnosis of multiorgan dysfunction syndrome accompanied at the molecular level by VEC injury. The objective of the protocol was to develop a dose system that would optimize the polypharmacologic actions of defibrotide by regulating the levels of the cellular markers that define the type and degree of ongoing cellular events.

A master panel of assays was constructed for a multisystem evaluation (including cytokines, thrombosis, fibrinolysis, neuroendocrine systems, and platelets) of the events around the VEC injury and included: coagulation factors I–XII, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time, reptilase time and the respective mixes; fibrin split products, fibrin monomers, euglobulin lysis time, and TPA; PAI-1; protein S, protein C, plasmin, antiplasmin, antithrombin III, platelet aggregation studies with arachidonic acid, adenosine diphosphate, epinephrine, collagen, interleukin 1 (IL-1), IL-2, IL-6, tumor necrosis factor-α (TNF-α), endothelin-1 (ET-1), and von Willebrand antigen (vWag).

Von Willebrand antigen was utilized as a surrogate marker for defibrotide induced VEC modulation and differentiation. The 46 assay panel was performed at baseline and every 3 days thereafter or at each dose escalation, for a total 37 times within a period of 275 days. After analysis of the total pilot trial from 17 patients with multiorgan dysfunction syndrome, a panel of the following molecular stress markers was constructed and reproducibly identified as being representative of VEC injury in multiorgan dysfunction syndrome: PAI-1, platelet count, arachidonic acid induced platelet aggregation (AA-agg), ET-1, TNF-α, IL-1, IL-2, IL-6, and vWag. The stratified dose ranges were individually analyzed for each of the VEC injury molecules for their minimum and maximum modulatory activities.

Upon analyzing the mean and median of the minimum and maximum doses at which each marker in each patient was modulated, a wide variation between patients was observed (data not shown). This variation was undoubtedly due to the type of injury and the molecular and cellular factors of patients. Thus after analyzing the phase II pilot data, the recommended dose for VEC injury in CAPS and multiorgan dysfunction syndrome has been amended to 100–275 mg/kg/day to be administered for a minimum of 3 weeks. The objective was not to lose any slow or partial responders by initiating therapy at lower doses.

RESULTS

Figure 1 shows the intensities of the molecular stress markers of TNF-α as the afferent arm of the injury and PAI-1 as the efferent arm, plotted as a function of dose and number of days of treatment. On Day 10, before initiation of defibrotide, both TNF-α and PAI-1 levels were high, consistent with the universal molecular spectrum of VEC injury. On Day 13, after 3 days of therapy, slight improvement in the PAI-1 level and normalization of TNF-α were seen, accompanied by improvement in oxygenation. The patient was extubated after 3 days, an unexpectedly fast recovery for ARDS.

Figure 2 shows the intensities of the molecular stress markers of soluble IL-2 receptor (sIL-2R) as the afferent arm of the injury and ET-1 as the efferent arm. In Figure 3, the platelet count and AA-agg, both efferent arms, are plotted as a function of dose and number of days of treatment. Before initiation of defibrotide, both sIL-2R and ET-1 were elevated, whereas platelet count and AA-agg were impaired. On Day 13, after 3 days of therapy, while sIL-2R and ET-1 decreased to normal levels, platelet consumption persisted, again showing a dose

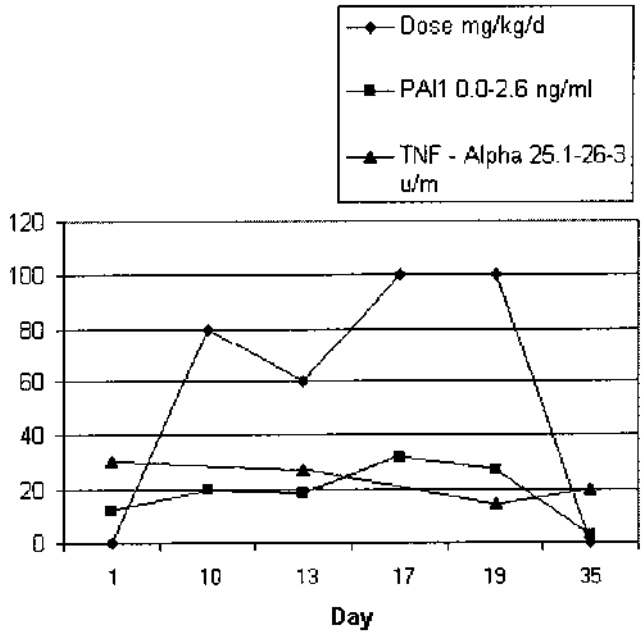

Figure 1. Modulation of plasminogen activator inhibitor-1 (PAI-1) and TNF-α as a function of dose and duration of therapy.

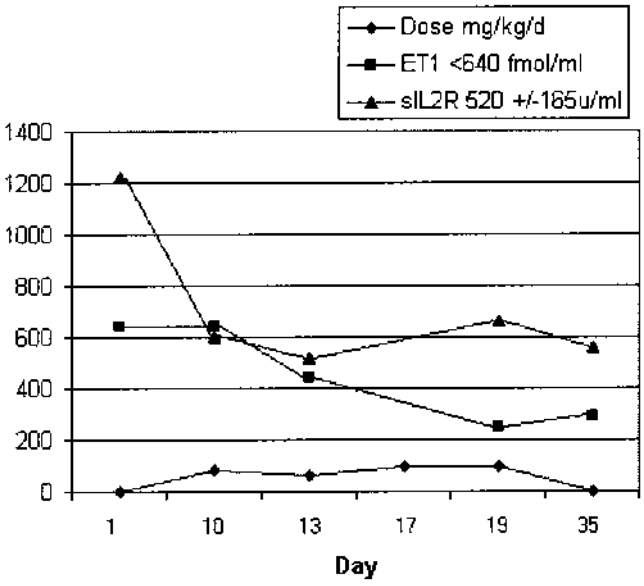

Figure 2. Correlation of daily dose per kg body weight with modulations of endothelin-1 (ET-1) and soluble IL-2 receptor levels (sIL-2R).

stratified polypharmacology. Extubation of the patient was temporally and exactly related to normalization in cytokines and ET-1, the latter being highly associated with ARDS and myocardial infarction.

According to the principles of the marker directed dose system (MDDS) used in the trial, persistence of abnormality in the PAI-1, the platelet count, and AA-agg would have led to escalation of the daily dose. However, the reverse was done (the patient was enrolled in the early phase of the trial before

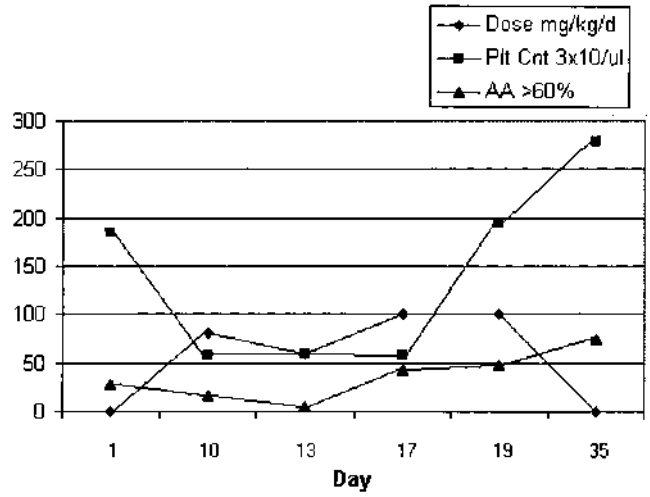

Figure 3. Remission in platelet counts and arachidonic acid (AA) induced platelet aggregation.

the principles were set), with the dose decreasing to 60 mg/kg/day for 10 days, resulting in further upregulation in the PAI-1 level (Figure 1), persistent consumptive thrombocytopenia (Figure 3), and worsening proteinuria of 13 g/day (Figure 4). Further, expansion of the soft tissue necrosis involving all digits was observed. These events supported the dose dependency of both the clinical and molecular markers, with both deteriorating concurrently as the defibrotide dose decreased.

On Day 17, the daily dose was escalated as per MDDS requirements to 100 mg/kg/day. After 2 days of therapy, the platelet count normalized (Figure 3), accompanied by complete remission in all the inflammatory signs of the postphlebitic syndrome and bilateral DVT. PAI-1 levels and AA-agg improved slightly yet remained abnormal, again representing a state of partial remission. Following the MDDS requirements, which dictate either prolongation of therapy or escalation of the daily dose in the face of partial remission, the former option was selected and therapy was continued. On Day 35, after 18 more days of therapy, the complete spectrum of VEC injury markers normalized (Figures 1, 2, 3). Temporally correlating with the remission induction at the molecular level, changes in the clinical events included complete normalization of the chest radiograph, pulmonary function tests, and blood pressure, as well as reduced proteinuria to 895 mg/day and complete remission of the soft tissue necrosis. This improvement further confirmed the profound dose dependency of remission induction and stratified manifestations of the polypharmacology of defibrotide.

On Day 35, LAC was undetectable, and the levels of IgG and IgM aCL had decreased to 4 and 9 U/ml, respectively. Repeat venography showed persistence of the left deep femoral and popliteal thrombi, but lysis of the right deep femoral thrombus. Digital arteriography, however, showed deterioration of the arterial circulation in both hands, in direct

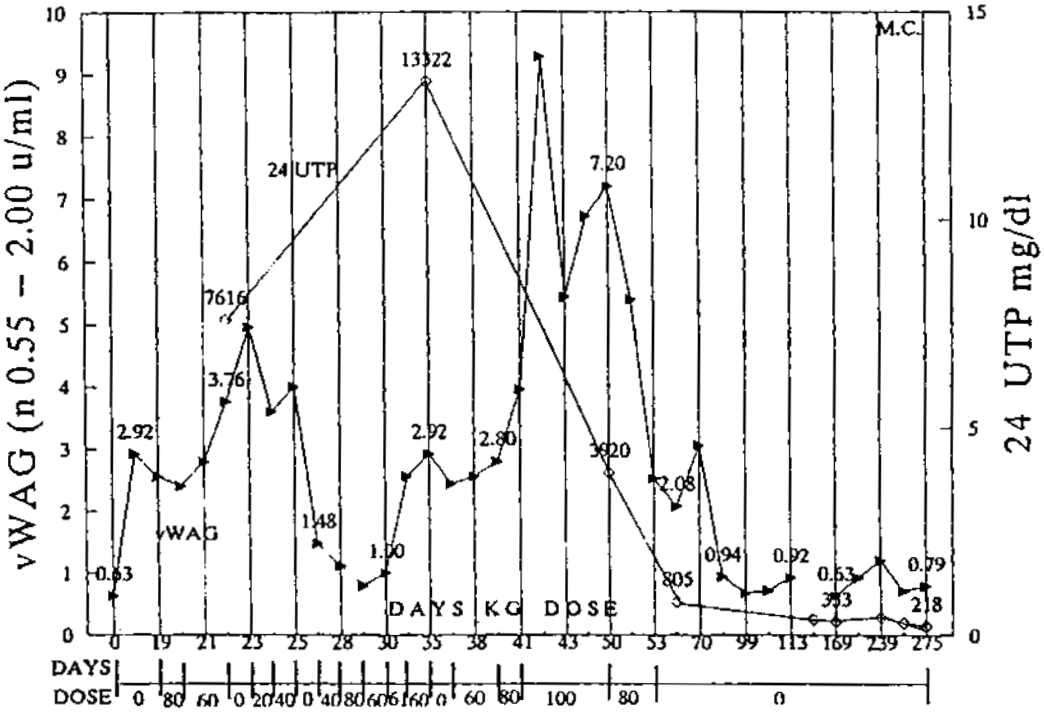

Figure 4. Escalation of proteinuria to 13,320 mg/day with decreasing defibrotide 60/mg/day on Day 30 of therapy. Direct dose dependency of remission in proteinuria with escalation of defibrotide dose to 100 mg/kg/day is seen by Day 35. Improvement in proteinuria continues after cessation of defibrotide therapy up to Day 275. ▲: vWag concentration, ○: protein secretion, mg/day.

contrast with the remission of tissue necrosis, suggesting a mechanism other than resumption of capillary circulation to account for the cytotropic actions of defibrotide. With remission induction in all the molecular and clinical measures, defibrotide therapy was terminated on Day 35.

On Day 235, the patient was no longer taking antihypertensives and had complete healing of the digits, and proteinuria had decreased to 299 mg/day. There was also a sustained remission in the postphlebitic syndrome symptomatology for the first time in 10 years. On Day 400, the patient was still off defibrotide. Titers of aCL had increased to 11 and 76 U/ml for IgG and IgM, respectively, with no relapse in thrombotic complications, despite withholding warfarin.

Figure 5 shows direct correlation between vWag levels and the defibrotide dose. This was representative of all other responders.

DISCUSSION

This case represents the first published report of effective use of defibrotide as a polypharmacologic agent in CAPS. In contrast to the more widely accepted ischemic and thrombotic tissue damage, these data present the VEC injury as the primary and representative lesion of CAPS. Although only one patient is described here, the molecular description of the state of injury was accomplished by extensive molecular analysis of the events around VEC.

In this patient, the modulation of stress molecules at different defibrotide doses was examined to identify the pathologic molecular markers. To assure their validity, each marker was examined for its biological relation to a clinical finding and the dose related variation in intensity. A panel of potential surrogate markers was constructed (after analyzing 17 patients with multiorgan dysfunction syndrome together in a phase II pilot trial) that included TNF-α, IL-1, IL-2, IL-6, PAI-1, ET-1, sIL-2R, platelet count, and AA-agg. We propose that this panel will reflect a spectrum pointing to the desirability of a polypharmacologic therapy.

Our second objective was to evaluate defibrotide as a polypharmacologic agent whose actions would include concurrent anticytokine, antithrombotic, platelet deaggregatory, and vasodilatory effects. This is the first report of application of a dose system that is regulated by ongoing cellular events. Further, this report illustrates the dose dependency of the state of remission and the stratification of various pharmacological actions that manifest themselves at different dose levels. There was clear expression of dose dependency in our patient, with stress markers first improving, then deteriorating, and then again improving to a state of complete remission at dose levels of 80 mg/kg/day for 3 days, 60 mg/kg/day for 10 days, and 100 mg/kg/day for 12 days, respectively.

The data also show that stratification of dose becomes a defining factor for the stratification of the polypharmacologic

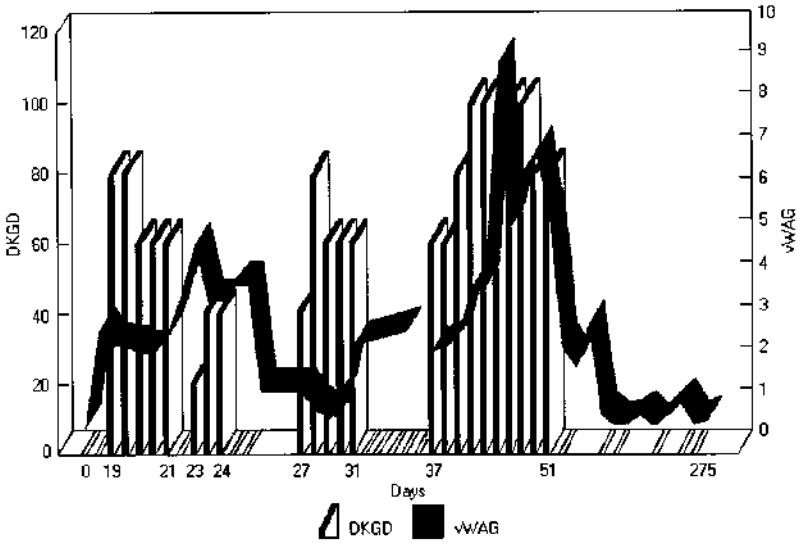

Figure 5. Direct correlation between von Willebrand antigen (vWag) levels and daily dose of defibrotide; highest level of vWag reaching a plateau represented the most effective daily dose. Upregulation of vWag levels served as a quantitative marker for defibrotide induced VEC differentiation. DKGD: dose/kg body weight/day.

actions. Eighty mg/kg/day for 3 days in this patient, for example, led to stable remission in the molecules of ET-1, sIL-2R, and TNF-α, over which VEC have a known coordinating role, but not PAI-1, platelet count, or AA-agg, over which VEC have a direct regulatory role. For the duration that pathologic markers are present, dose escalation is needed for complete treatment of a specific state of injury. As shown in our phase II pilot data, patients may require up to 275 mg/kg/day.

The impairments seen in the VEC functions may actually reflect perturbations in the expression of cellular genes as a result of injury by TNF-α, IL-1, IL-2, IL-6, and prostacyclins[25-28]. Defibrotide's regulatory role over cytokines is reported for the first time here and its unique characteristics are described. Downregulation by defibrotide continues only until normal levels are reached (never extending below normal even if the administration of defibrotide is continued), unlike the effects of anti-TNF antibodies, where continuous administration leads to subnormal levels. This may in turn explain why anti-TNF therapies are associated with increased mortality in multiorgan dysfunction syndrome. A second characteristic of defibrotide's modulatory effects over cytokines is that multiple cytokines are downregulated simultaneously, the only prerequisite being abnormally high levels in any one of the cytokines.

VEC regulate the cellular genes of the renin-angiotensin system, endothelins, natriuretic peptides, prostaglandins, and leukotrienes. Reports show that precisely the same systems are also regulated by defibrotide[19]. ET-1 is the most potent vasoconstrictor molecule in humans, with receptor distribution in the lung, heart, kidney, and vascular smooth muscle. ET-1 is the primary factor in the pathogenesis of ARDS, myocardial infarction, and hypertension, specifically applicable to this patient. The remission induced by defibrotide was indirect by virtue of the downregulation of ET-1. Thus, we believe that defibrotide mimics the coordinating role of VEC over these interactive systems.

Discussion of transcriptional regulation of cellular genes by defibrotide is beyond the scope of this report. However, the dose related downregulations of the abnormally upregulated molecules PAI-1 and ET-1 and cytokines may imply a defibrotide induced re-regulation in the perturbed expressions of these cellular genes. This potential regulation would be consistent with defibrotide's known cellular differentiating actions in endothelial cell cultures[29].

Von Willebrand antigen is an accepted marker for the quantitative measurement of cellular differentiation and multiplication in endothelial cell cultures. Defibrotide upregulates vWag more strongly than endothelial cell growth factor in endothelial cell cultures, whereas endothelial cell growth factor induces cell multiplication at a higher level than defibrotide. These actions demonstrate that defibrotide has mild growth factor-like effects, but a notable cellular differentiation effect[29]. This patient had direct dose dependent variations in vWag levels *in vivo* (Figure 5), a finding that was reproducible in all the other responders (data not shown). vWag levels became a direct indication of the adequacy of the daily dose because it is a direct quantitative measurement of VEC differentiation. vWag plateau levels reflected the maximum efficacious daily dose. In nonresponders and in healthy controls vWag levels were not affected by defibrotide, which translated to the absence of any pharmacologic action (due to absence of state of injury in controls). These observations supported the validity of our hypothesis regarding defibrotide's ability to reconstitute VEC functions.

In Europe, defibrotide has been commercially recognized as an antithrombotic agent with monopharmacologic action and has been used with empiric doses ranging from 10 to 30 mg/kg/day[30]. Richardson, *et al* used empiric doses of defibrotide in 19 patients with hepatic venoocclusive disease following stem cell transplantation (all with evidence of multiorgan dysfunction), and 42% of their patients had resolution of the venoocclusive disease[31]. Our case suggests that defibrotide should be studied further as a polypharmacologic agent for diseases characterized by multiple cellular, tissue, and organ failures.

In summary, catastrophic antiphospholipid syndrome is a multiorgan disease state and VEC represent the primary target of injury. Cellular injury is characterized by tissue and organ dysfunction that originates from perturbation in the expression of cellular genes. The primary means of VEC injury seem to be related to direct damage by cytokines. We propose that the ideal therapy is a polypharmacologic approach aimed at reconstituting the normal cellular and coordinating functions of VEC. Defibrotide's dose dependent induction of cellular differentiation, shown previously *in vitro*, and now *in vivo*, also supports this model. MDDS may be a necessary dose system for the maximal expression of defibrotide's polypharmacologic and cellular differentiation effects. The escalation of the dose and the duration of therapy should be directed by the levels of the surrogate markers of VEC injury.

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