

Q₁₀ Quest for New Therapies to Prevent Antiphospholipid Antibody–Mediated Thrombosis

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Although the antiphospholipid syndrome (APS) has been clearly defined for over 3 decades, it remains a clinical challenge. Treatment of this syndrome, the association of certain autoantibodies with thrombosis and recurrent pregnancy loss, is on one hand straightforward. Patients who have had a thrombotic event and persistently positive antiphospholipid antibodies (aPL) are at high risk for recurrent thrombosis and, in most cases, can be successfully managed with oral anticoagulation and reduction or elimination of modifiable thrombotic risk factors. However, this therapeutic approach is far from ideal given the significant risk of bleeding associated with long-term anticoagulation. Importantly, this strategy is not supported for primary prevention of an initial thrombotic event despite advances in identification of high-risk aPL profiles. Other medications may help reduce risk, but have limitations. Daily low-dose aspirin has been recommended for many years, and retrospective studies show some protective effect.¹ However, a randomized, double-blind, placebo-controlled trial (albeit underpowered) did not demonstrate clear benefit.² Hydroxychloroquine, an antimalarial agent with mild antiplatelet activity commonly used in the management of systemic lupus erythematosus (SLE), may be helpful in preventing thrombosis in SLE patients with aPL; however, most data are retrospective, and the applicability to patients without SLE is unknown. In light of these limitations, the study by Perez-Sanchez et al³ in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology* offers a novel approach.

See accompanying article on page 1923

A 2-hit hypothesis of aPL-mediated thrombosis is widely held.⁴ Briefly, aPL interact with plasma, circulating blood cells, and vascular endothelium and produce a systemic thrombotic diathesis that constitutes the first hit. The second hit is presumed to be a local perturbation that triggers thrombosis at a particular vascular site and time. Traditional anticoagulation probably acts primarily by preventing the second hit and is associated with significant risks of bleeding. In theory, therapeutic approaches aimed at the first hit could counteract the prothrombotic effects of aPL with minimal hemostatic consequences.

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In the accompanying article Perez-Sanchez et al³ demonstrate that treatment of APS patients with ubiquinol, the reduced equivalent of coenzyme Q₁₀ (CoQ₁₀), significantly reduced cellular markers of oxidative stress in a double-blind, placebo-controlled, crossover trial (Figure). CoQ₁₀, an endogenously synthesized lipid-soluble antioxidant, constitutes a critical component of the mitochondrial electron transport chain facilitating transfer of electrons from complexes I and II to complex III. In addition to its role in mitochondrial respiration, CoQ₁₀, in its reduced form, protects against lipid peroxidation and is of potential interest as an antiatherogenic agent.⁵ The 200 mg dose of ubiquinol administered to patients with APS in this trial is comparable to that used in other clinical studies and a dose taken as a dietary supplement.

A central finding of the study is the capacity of ubiquinol treatment to reduce monocyte oxidative stress and procoagulant activity in patients with APS. Isolated primary human monocytes from patients with APS demonstrated increased levels of reduced glutathione and lower reactive oxygen species generation after ubiquinol treatment. Lipid peroxidation has previously been shown to enhance tissue factor (TF) activity through enhanced exposure of phosphatidylserine on monocytes.⁶ Consistent with the role of oxidative stress in regulating TF activity, ubiquinol treatment significantly reduced TF gene expression, cell surface exposure, and activity. Furthermore, preclinical studies suggest that myeloid cell–derived TF plays an important role in venous thrombus formation.⁷ It is interesting to consider whether this mechanism of action could provide ubiquinol with indirect anticoagulant properties in the setting of APS.

Neutrophils may serve as important drivers of venous thrombosis, in part, through the capacity of these cells to form neutrophil extracellular traps (NETs). Recent evidence supports oxidative stress as an important initiator of NET formation in SLE.⁸ Perez-Sanchez et al³ report that ubiquinol treatment reduces neutrophil reactive oxygen species production and inhibits the formation of NETs. Consistent with reduced NET formation, expression of myeloperoxidase and neutrophil elastase, proteins required for efficient NET generation, were also downregulated. Future studies should examine whether increased NET formation underlies thrombosis associated with aPL.

The authors further explore the beneficial effects of ubiquinol treatment through assessment of endothelial cell function. Ubiquinol treatment significantly improved microvascular perfusion and was accompanied by a modest but significant reduction in plasma levels of soluble VCAM1 (vascular cell adhesion molecule 1), a marker of endothelial dysfunction. In vitro treatment of primary human endothelial cells with serum from ubiquinol-treated patients reduced cell surface expression of both VCAM1 and TF. Somewhat paradoxically the authors also observed reduced expression of endothelial nitric oxide

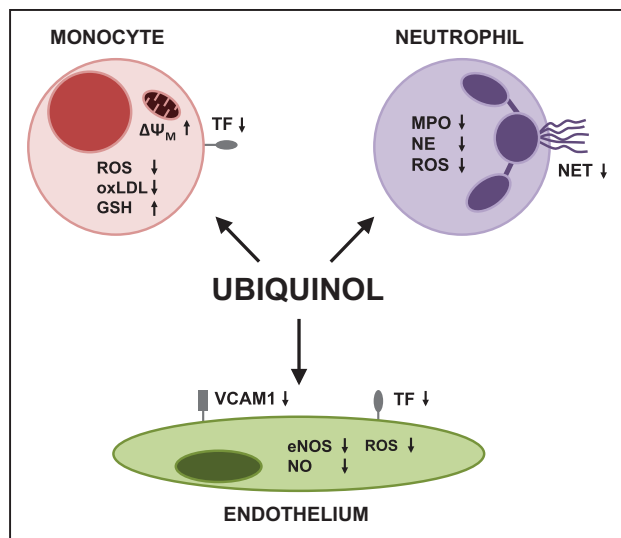


Figure. Effect of ubiquinol treatment on markers of oxidative stress and activation of procoagulant pathways. Treatment of antiphospholipid syndrome (APS) patients with ubiquinol protects monocytes, neutrophils, and endothelium from oxidative stress and is accompanied by reduced activation of procoagulant pathways. $\Delta\Psi_M$ indicates mitochondrial membrane potential; eNOS, endothelial nitric oxide synthase; GSH, reduced glutathione; MPO, myeloperoxidase; NE, neutrophil elastase; NET, neutrophil extracellular trap; NO, nitric oxide; oxLDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; TF, tissue factor; and VCAM1, vascular cell adhesion molecule.

synthase and nitric oxide generation, important regulators of endothelial function. It is possible, however, that downregulation of endothelial nitric oxide synthase expression provides protection from further nitrosative and oxidative stress resulting from endothelial nitric oxide synthase uncoupling.⁹

The predominate target of autoantibodies present in patients with APS is β_2 -GP I (β_2 -glycoprotein I).⁴ The structure of β_2 -GP I is redox sensitive because of the presence of several free thiols that can become disulfide linked under oxidative conditions. The oxidized conformation of β_2 -GP I has been found to be elevated in patients with APS and may be more antigenic. It remains to be determined whether treatment of APS patients with ubiquinol preserves β_2 -GP I in a nonoxidized state and to what extent this could limit the antigenic load.

Perez-Sanchez et al¹⁰ have previously reported that patients with APS demonstrate enhanced monocyte oxidative stress and mitochondrial dysfunction. Although it seems likely that oxidative stress contributes to the pathogenesis of APS, some caution in the interpretation of these data may be warranted because of the inclusion of patients with SLE. Oxidative stress is a hallmark of SLE, resulting from abnormal activation of the adaptive immune system and dysregulation of intracellular pro- and antioxidant pathways.¹¹

Although the current study presents a highly novel approach, it is relatively small and limited to determination of markers of oxidative stress and coagulation. Moving forward, a trial of CoQ₁₀ with well-defined clinical end points, such as decreased rate of thrombosis, may be problematic because of inherent challenges in APS study design. Although thrombosis is a significant risk over a lifetime for individuals with medium- to high-titer aPL, the annual incidence of thrombotic

events is relatively low, at $\approx 2\%$ to 3% . Thus, a study with an end point of, for example, a 50% reduction in thrombotic events would have to be quite large and long in duration to achieve adequate statistical power.

Another important issue in APS studies involves the high risk of recurrent thrombosis in patients with persistent high-titer aPL and the efficacy of oral anticoagulation in mitigating that risk. Ethically, anticoagulation cannot be withheld in a secondary prevention study. Thus, in a trial involving APS patients with a known history of thrombosis, the therapeutic benefit of novel agents would have to be observable in patients on anticoagulation. In secondary prevention studies, it may, therefore, be particularly challenging to demonstrate reduced incidence of thrombosis in patients treated with CoQ₁₀.

Disclosures

None.

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