ampio Pharmaceuticals Inc. (Greenwood Village, CO) is developing Optina (danazol) as an oral treatment for diabetic macular edema (DME). When administered to patients with DME, a reduction in central subfield retinal thickness as measured by optical coherence tomography was observed. In addition, preclinical in vitro studies in which human endothelial cells were treated with danazol have demonstrated an enhancement of endothelial barrier function with a corresponding decrease in vascular permeability. Unlike intraocular injections of drugs targeting VEGF, Optina is administered orally and has a strong proven safety profile.

**F-ACTIN CORTICAL RING AND STRESS FIBER FORMATION**

Filamentous actin (f-actin) is a major component of the cytoskeletal network and plays a critical role in the dynamic regulation of cell shape while helping to generate the force needed for migration and contraction. Polymerization of f-actin can exert control over vascular permeability through 2 divergent pathways. When organized into a submembranous cortical “ring,” f-actin increases the barrier function of endothelial cells. In this orientation, it serves as an anchor, tethering adhesion molecule complexes to the cytoskeleton, strengthening cell-to-cell adhesions.

Conversely, f-actin can also mobilize into parallel bundles called stress fibers that interact with myosin motors, driving contraction of cells and creating paracellular gaps. A hallmark of endothelial cell stimulation with edematous agents such as tumor necrosis factor-alpha (TNF-α), thrombin, and VEGF is a pronounced development of stress fibers in the cytosol. In addition, 1 end of the contractile bundle typically binds to focal contacts, weakening interaction with the extracellular matrix, compounding the effect. The possibility also exists that stress fibers can cause the removal of adhesion molecules from the surface of the cell. Treatment of endothelial cells with VEGF leads to the rapid disappearance of vascular endothelial cadherin by endocytosis.

**OPTINA**

Optina is a repurposed drug based on a low dose of the weak androgen, low-molecular-weight, very lipophilic steroid danazol. Danazol is currently approved for the treatment of endometriosis, hereditary angioedema, fibrocystic disease of the breast, and idiopathic thrombocytopenic purpura, with effective and approved dosages for these...
conditions ranging from 200 to 800 mg/day, much higher than the Optina formulation.

Preliminary studies performed in our lab sought to determine if danazol altered endothelial cell function in vitro. Danazol proved effective, in a dose-dependent manner, at attenuating both proliferation and tubulogenesis. Recent work in the lab suggests that danazol also prevented endothelial cell migration in wound-scratch assays (unpublished data). These models represent 3 of the primary phases of angiogenesis, and a danazol intervention at any step could be part of its clinical potency.

The effect of danazol on vascular permeability was then investigated using human endothelial cells of retinal, umbilical, brain, and renal microvascular origins. Our findings suggest that a biphasic dose-response exists for danazol on vascular permeability (Figure 1). Tracking the migration of horseradish peroxidase (HRP) through monolayers of human endothelial cells in a transwell system showed that at 100 to 500-nanomolar concentrations, danazol reduced passage across the cells. Increasing the concentration, however, reversed the beneficial effects of danazol and led to an increase in paracellular permeability. The beneficial effect of danazol also appeared to be very rapid. Within minutes of exposure to barrier-enhancing concentrations of danazol, endothelial cells exhibited f-actin cortical ring formation and increases in endothelial barrier function, as demonstrated by phalloidin staining and a transelectrical endothelial resistance (TEER) model (Figures 2 and 3). Furthermore, danazol at these concentrations counteracted the formation of stress fibers upon stimulation with proinflammatory molecules such as TNF-α or thrombin (Figure 3).

**PHASE 2A CLINICAL TRIAL**

A 12-week randomized placebo-controlled double-masked study to evaluate the safety and efficacy of danazol for DME was conducted at St. Michael’s Hospital in Canada. Included were patients with DME and a central subfield retinal thickness of 300 μm or greater. A total of 34 patients constituted the safety set population. The efficacy evaluable population (n = 23) was composed of patients from the safety set who completed 80% or greater of study medications at 4 weeks of treatment. The primary endpoint was change in central subfield retinal thickness from baseline to 12 weeks of treatment, and the secondary endpoints were change from baseline in retinal volume and ETDRS best corrected visual acuity (BCVA) at week 12 of treatment. The 3 danazol doses studied were 5 mg, 15 mg, and 45 mg. All treatments were administered orally twice a day.

The first significant finding was that the effect of
Danazol appears to reduce DME in a BMI dosage-adjusted manner and appears to trend toward improved visual acuity, although the trial described here was too small to make this definitive conclusion. Our in vitro data suggest that danazol has a biphasic effect on endothelial cells: At low doses, danazol decreases vascular leakage, while at higher concentrations an increase in vascular permeability is observed. This biphasic effect was supported by the effectiveness of danazol in vivo at different BMIs. A US Food and Drug Administration phase 2b trial is in progress to further the understanding and approval of this promising drug for a highly prevalent and debilitating condition.

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