Oral Danazol for DME

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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.[1] 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.[2,3]

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.[4] 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.[1] In addition, 1 end of the contractile bundle typically binds to focal contacts, weakening interaction with the extracellular matrix, compounding the effect.[1] 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.[5]

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

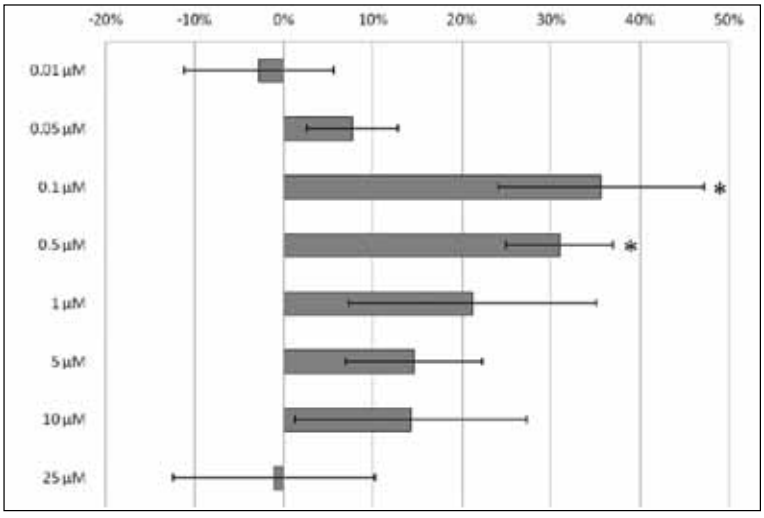

Figure 1. Percentage of change in decrease in HRP permeability across endothelial cell monolayers. Dose response of danazol effect on HRP permeability of cells treated for 24 hours. Data presented as mean + SEM calculated for 3 separate experiments, each performed in triplicate. * = *P* value < .05 vs vehicle.

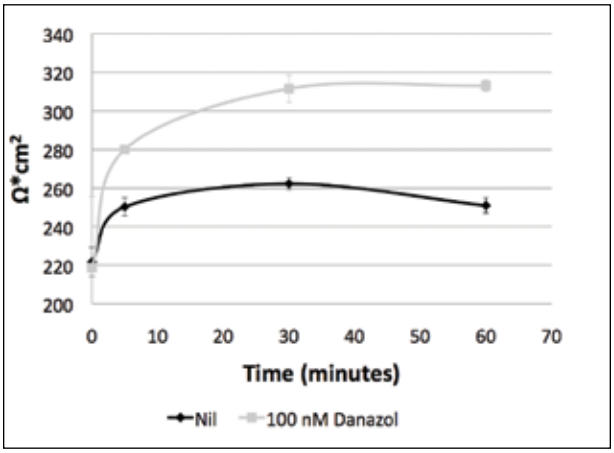

Figure 2. TEER response of danazol: Temporal effect of 0.1 µm vs vehicle. TEER measured across monolayers of endothelial cells grown on transwell inserts. Higher resistance equals greater barrier integrity.

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.[6] 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.[7] 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).

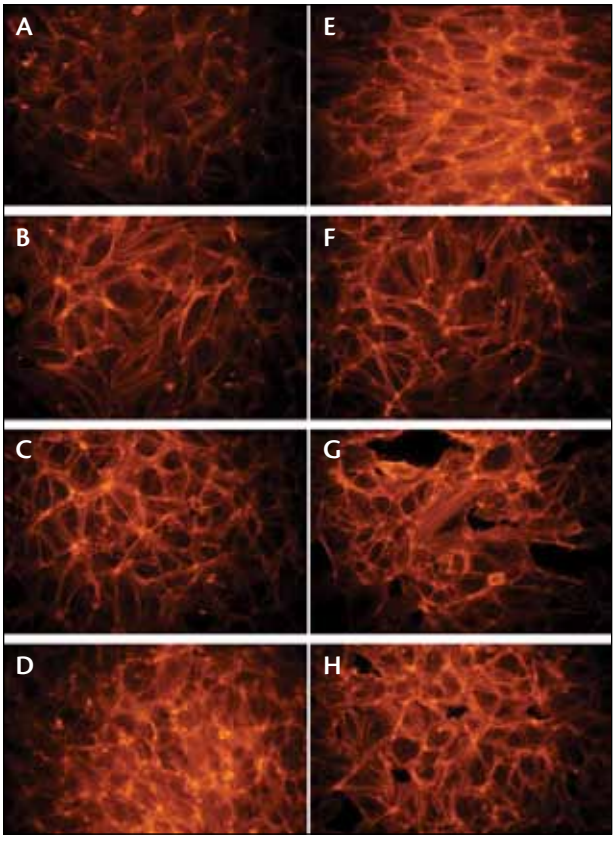

Figure 3. F-actin cortical rearrangements and danazol. Retinal endothelial cells stained with rhodamine conjugated phalloidin. Cells fixed 3 hours after treatment (thrombin exposure only 15 minutes). Treatment groups: vehicle control (A), 0.1 µm danazol (B), 1 µm danazol (C), 10 µm danazol (D), 100 ng/mL TNF-α (E), TNF-α + 0.1 µm danazol (F), 0.1 U/mL thrombin (G), and thrombin + 0.1 µm danazol (H).

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

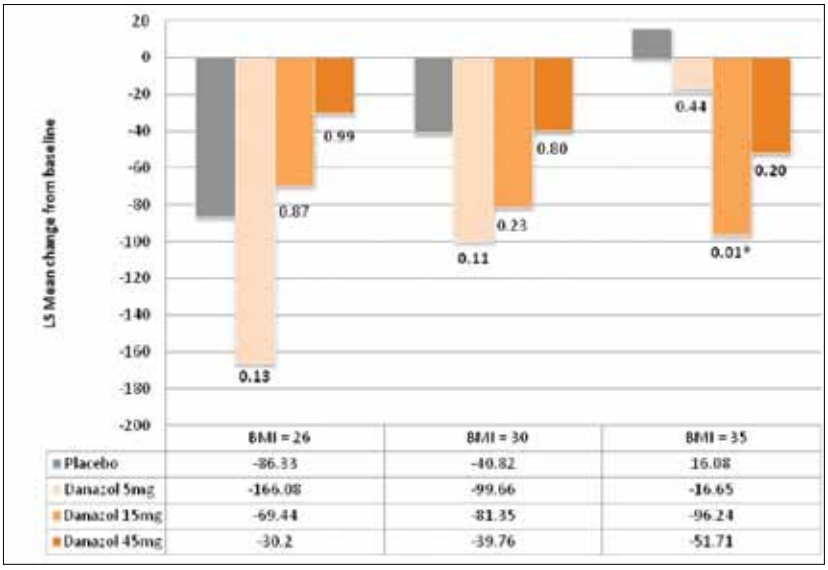

Figure 4. Least square mean change in retinal thickness from baseline by baseline BMI. Because of the significant interaction between treatment and baseline BMI, least square means and *P* values are estimated for 25%, 50%, and 75% BMI quartiles. The final ANCOVA model contains treatment effect, days from baseline, baseline retinal thickness, and BMI. The *P* value compares each active group with the placebo group; the Dunnett-Hsu method is applied for multiple comparison adjustment.

danazol on retinal thickness was dependent on baseline body mass index (BMI; *P* = .01). At lower BMI values, the lower danazol dose effectively decreased retinal thickness, whereas at higher BMIs, higher doses were more effective (Figure 4). Defined a priori, a decrease in retinal thickness of more than 11% was considered clinically significant. Nearly all subjects (86%, 6 of 7 patients) receiving the 15-mg dose had a significant decrease in retinal thickness at 12 weeks, compared with 29% (2 of 7 patients) in the placebo group. Retinal volume also decreased in the 15-mg group compared with placebo (*P* = .05).

For BCVA change in ICD-9-CM vision loss, 36% of subjects improved at least 1 category with treatment. The placebo group had the lowest proportion of subjects with improvement (14%), whereas 47% of all patients treated with danazol improved by at least 1 category.

No ocular hypertension was detected in any of the groups at baseline or week 12, and no investigational medical product-related serious adverse events were reported. Three treatment-related adverse events occurred (peripheral edema, psoriasis, and worsening depression), all of which were considered possibly related to the IMP.

SUMMARY

Oral administration of danazol to patients with DME is safe with no evidence of serious adverse events. 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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