

Abstract

Background: In addition to regulating oncogenic client proteins, the Hsp90 molecular chaperone also controls the folding of key signaling molecules required to maintain normal cell function in many organs, including the retina. In human clinical trials a number of Hsp90 inhibitors have been associated with visual disorders including blurred vision, flashes, delayed light/dark accommodation, and photophobia. These adverse effects involving injury to the retina may be attributable to photoreceptor degeneration and cell death, as previously reported in dogs following repeated doses of Hsp90 inhibitor PF-04929113^(1,2). In contrast, ganetespib, a potent Hsp90 inhibitor currently in phase III/III trials, has demonstrated promising clinical activity without manifesting ocular toxicity.

Results: In this study, we examined the relationship between retinal drug distribution profiles and photoreceptor degeneration in male SD rats treated with 17-DMAG, 17-AAG, and STA-9056 (an Hsp90 inhibitor with comparable *in vitro* activity to 17-DMAG). All compounds were tested in short-term studies administered i.v. at 1-3 dose levels. At necropsy, eyes were dissected and processed for histopathological examination. In subsets of animals, the retinal tissues, along with plasma and cerebrospinal fluid samples, were collected for analysis. Our results indicate that all compounds evaluated showed greater exposure in the retinal tissue compared to plasma and cerebrospinal fluid. 17-DMAG, for which visual changes have been reported in clinical subjects, produced marked photoreceptor cell death and was associated with a slow elimination rate (at 6 h post-dose, 50% of the drug present at 30 min remained in the retina) and a high retina/plasma (R/P) ratio. In contrast, and consistent with the absence of clinically-reported visual changes, 17-AAG at the maximum tolerated dose (MTD) did not produce detectable photoreceptor injury. At 6 h post dose, 94% of 17-AAG had been eliminated from the retina resulting in a low R/P ratio. Finally, STA-9056 showed 79% drug elimination at 6 h and an R/P ratio that was moderately low. Photoreceptor degeneration was not observed at doses of STA-9056 that are active in animal tumor models, and only minimal degeneration was seen at a higher dose.

Conclusions: Our findings suggest that the retina/plasma exposure ratio and elimination rate profiles play crucial roles in ocular toxicity and can be used as indicators of potential Hsp90 inhibitor-induced damage in rats. In summary, Hsp90 plays an important role in the retina and prolonged Hsp90 inhibition can lead to vision disorders. However, ocular toxicity may be successfully minimized by administration of Hsp90 inhibitors with favorable drug properties that include, although not necessarily limited to, lower retina/plasma exposure ratios and faster retinal elimination.

Methods

Animals:

Male Sprague Dawley (SD) or Long-Evans rats.

Treatment groups:

Vehicle: D5W or DRD (10% DMSO/14%Cre-RH40/ 75%D5W)

17-DMAG: 10, 20, or 25 mg/kg.

17-AAG: 8, 20, 60, 80/100 mg/kg

STA-9056: 10, 20, 30, 50, 100 mg/kg

Route of administration and dosing regimen:

i.v., Daily for 1-3 days, or 3x/week for 8 doses

Tissue collection and processing:

Eyes were harvested 24 h after the last dose, fixed in Davidson's solution for 24-48 h, and then washed with 70-90 % ethanol before routine processing for paraffin embedding and sectioning. Eye sections were stained with H&E and TUNEL. Millipore's ApopTag[®] peroxidase *in situ* apoptosis detection kit was used.

The histological slides were reviewed in a blind fashion by a board certified pathologist. TUNEL stained sections were scored semi-quantitatively based on the areas and density of positive stains: (-), ≤ 20 cells/section; (\pm), minimum; (+), mild; (++) moderate; (+++), severe.

HER2 degradation assay:

BT474 cells were treated with the indicated compounds over a 10-1000 nM concentration range for 24 h prior to collection and staining with a FITC-conjugated anti-HER2 antibody (BD Biosciences) for 30 min at room temperature. Cells were analyzed by flow cytometry using CellQuest software. Half maximal inhibitory concentrations (IC₅₀ values) were determined based on 100% HER2 expression levels of untreated control samples.

Determination of plasma and retinal tissue drug concentrations:

17-DMAG (20 mg/kg), 17-AAG (80 mg/kg), and STA-11-9056 (50 mg/kg) were administered intravenously to male SD rats (n = 3). These doses were selected to be levels lower than MTD or those that induced mortality. Blood plasma samples were collected at 0.5 and 6 h post-dose for bioanalysis. Retina samples were also collected at 0.5 and 6 h post dose. Retinal samples from each time point were pooled and processed with the Covaris Cryoprep system followed by homogenization in phosphate buffered saline with an IKA homogenizer. Plasma and homogenized retinal samples were extracted by protein precipitation and analyzed by LC-MS/MS. A Phenomenex Kinetex 2.6 μ C18 (30 x 2.1 mm) column was used with a run time of 3.5 min per sample.

17-DMAG Induced Ocular Toxicity Involves the Photoreceptor Outer Nuclear Layer (ONL) in Sprague Dawley Rats

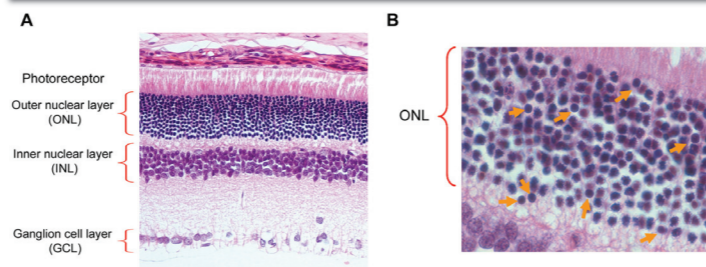


Figure 1. (A) H&E staining of normal retina in vehicle-treated Sprague Dawley (SD) rats shows orderly arrangement of photoreceptors, including the outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL). Magnification, 200X. (B) H&E stain showing degenerative changes (nuclear condensation and pyknosis, arrows) within the ONL of SD rats treated with 17-DMAG. Magnification, 1000X.

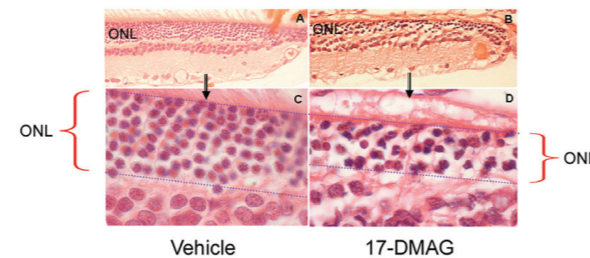


Figure 2. 17-DMAG markedly reduces the thickness of the ONL. SD rats were treated with repeated doses of vehicle or 17-DMAG (total of 8 doses on a 3x/week schedule) and retinal changes examined by H&E staining. Vehicle-treated animals (A, C) exhibited no discernible changes within the peripheral region of the retina. In contrast, the thickness of the ONL layer was markedly reduced in 17-DMAG-treated animals, with extensive evidence of cellular degeneration (B, D). Magnification: A & B, 100X; C & D, 1000X.

17-DMAG Induces Apoptotic Cell Death Within the ONL in Both Pigmented and Albino Rats

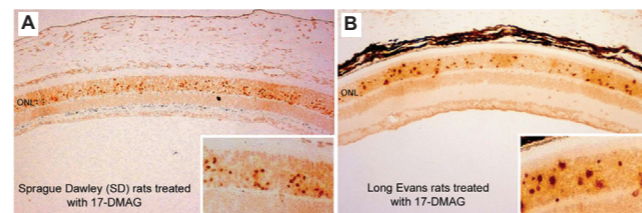


Figure 3. TUNEL staining confirms that the degenerative cellular changes within the ONL of SD rats (A) are due to apoptotic loss of photoreceptors. In addition to the SD (albino) rat, 17-DMAG also induces apoptotic photoreceptor cell death within the ONL of Long Evans (pigmented) rats (B). Insets show apoptotic effects in more detail. Original magnification, 100X.

Comparison of Ocular Toxicity in Rats Treated with 17-DMAG, 17-AAG and STA-9056

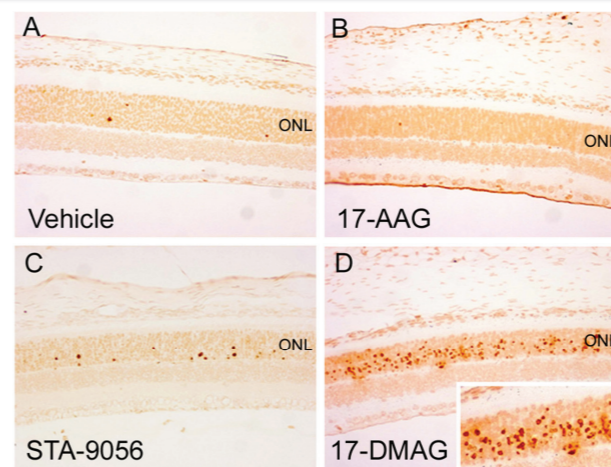


Figure 4. Apoptotic cell death within the retinal ONL following exposure to multiple Hsp90 inhibitors. SD rats were treated with vehicle (A), 17-AAG (B), STA-9056 (C) and 17-DMAG (D) and retinas subject to TUNEL staining to detect extent of apoptosis. Inset in panel D provided for greater detail. Magnification, 200X.

- These data show that 17-DMAG, for which visual changes have been reported in clinical subjects, produced marked photoreceptor cell death.
- In contrast, and consistent with an absence of clinically reported visual changes, 17-AAG at the maximum tolerated dose did not produce detectable photoreceptor injury.
- STA-9056 treatment resulted in a minimal increase in apoptotic cells compared to control or 17-AAG animals.

Compound	HER2 degradation IC ₅₀ (nM)	Treatment Doses (mg/kg, i.v.)	Retina/plasma conc.	Total # of rats	Photoreceptor degeneration / apoptosis	Systemic Toxicity
Vehicle	-	10 ml/kg	-	>10	(-) (\pm) occasionally	-
17-AAG	100-150	8, 20, 60	-	9	(-)	Mortality: 100 mg/kg
		80 (100)	Examined	6	(-)	
STA-9056	60	10, 20, 30	-	9	(-)	MTD: <100 mg/kg
		50	Examined	3	(\pm)	
17-DMAG	60	10	-	6	(\pm) - (++)	Mortality: 25 mg/kg/dx3days
		20 (25)	Examined	9	(+) - (+++)	

*histological score: (-) ≤ 20 cells/section, \pm : minimum, +: mild, ++: moderate, +++: severe
Retina/plasma concentrations were determined at the dose that did not illustrate mortality or MTD after 1-3 days treatment.

- HER2 degradation is included as a measure of *in vitro* Hsp90 inhibitory activity of each of the compounds.
- Unlike 17-DMAG, 17-AAG did not induce ocular toxicity in rats – even up to doses that resulted in mortality.
- Photoreceptor degeneration was not observed in rats treated with STA-9056 at doses 10-30 mg/kg, and only minimal/mild changes were seen at higher doses.

Retina/Plasma Exposure Ratio and Retinal Elimination Rate are Linked to Ocular Toxicity Induced by Hsp90 Inhibitors

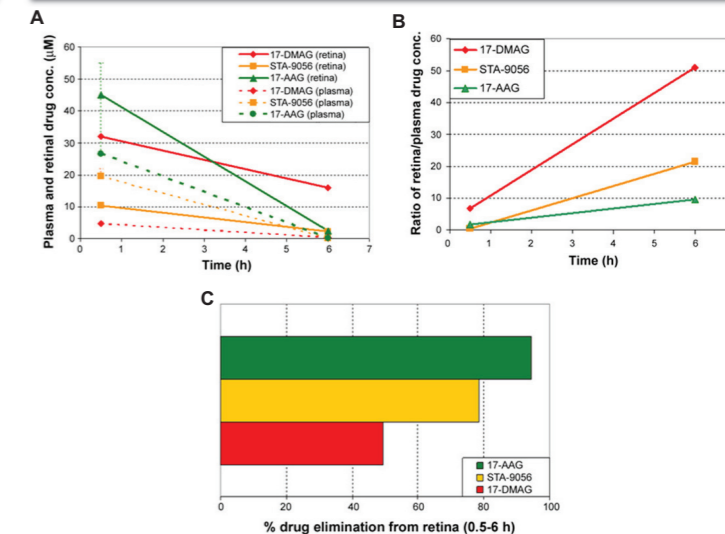


Figure 5. (A) Plasma drug concentrations do not predict for exposure in the retina. (B) Comparison of retina/plasma (R/P) drug concentration ratios for the three Hsp90 inhibitors over time. 17-DMAG, the compound which displayed the highest R/P ratio at 6 h post-dose, produced the most severe ocular effects whereas the lower R/P ratios of STA-9056 and 17-AAG were associated with less risk of retinal damage. (C) Retinal elimination profiles correlate with the degree of observed ocular toxicity. 17-AAG and STA-9056, which showed either an absence of or only minimal ocular effects, were more rapidly eliminated from the retina compared to 17-DMAG. The longer retention time of 17-DMAG within the retina also likely contributes to its adverse effects within this tissue.

- Together these data suggest that both degree (R/P ratio) and duration (elimination rate) of Hsp90 inhibitor drug exposure in the retina are linked to ocular toxicity potential.

Conclusions

- Hsp90 plays an important role in retinal function; prolonged Hsp90 inhibition can lead to vision disorders.
- Our data provide biological insights into the retinal effects induced by some Hsp90 inhibitors to account for the ocular effects observed in human subjects.
- The retina/plasma exposure ratio and elimination rate profiles play crucial roles in ocular toxicity and may be used as indicators of potential Hsp90 inhibitor-induced retinal damage in rats.
- Ocular toxicity may be successfully minimized by administration of Hsp90 inhibitors with favorable drug properties that include, although not necessarily limited to, lower retina/plasma exposure ratios and faster retinal elimination.

References

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