Toxicology and Applied Pharmacology 273 (2013) 401–409

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

A rat retinal damage model predicts for potential clinical visual disturbances induced by Hsp90 inhibitors

Dan Zhou [a,*], Yuan Liu [a], Josephine Ye [a], Weiwen Ying [a], Luisa Shin Ogawa [a], Takayo Inoue [a], Noriaki Tatsuta [a], Yumiko Wada [a], Keizo Koya [a], Qin Huang [b], Richard C. Bates [a], Andrew J. Sonderfan [a]

[a] Synta Pharmaceuticals Corp., 45 Hartwell Avenue, Lexington, MA 02421, USA
[b] Department of Pathology and Laboratory Medicine, Veterans Affairs Boston Healthcare System, 1400 VFW Parkway, West Roxbury, MA 02132, USA

ARTICLE INFO

Article history:
Received 11 June 2013
Revised 20 September 2013
Accepted 21 September 2013
Available online 1 October 2013

Keywords:
Hsp90 inhibitors
Visual impairment
Retinal degeneration
Drug safety assessment
Predictive toxicology
Rodent model

ABSTRACT

In human trials certain heat shock protein 90 (Hsp90) inhibitors, including 17-DMAG and NVP-AUY922, have caused visual disorders indicative of retinal dysfunction; others such as 17-AAG and ganetespib have not. To understand these safety profile differences we evaluated histopathological changes and exposure profiles of four Hsp90 inhibitors, with or without clinical reports of adverse ocular effects, using a rat retinal model. Retinal morphology, Hsp70 expression (a surrogate marker of Hsp90 inhibition), apoptotic induction and pharmacokinetic drug exposure analysis were examined in rats treated with the ansamycins 17-DMAG and 17-AAG, or with the second-generation compounds NVP-AUY922 and ganetespib. Both 17-DMAG and NVP-AUY922 induced strong yet restricted retinal Hsp70 up-regulation and promoted marked photoreceptor cell death 24 h after the final dose. In contrast, neither 17-AAG nor ganetespib elicited photoreceptor injury. When the relationship between drug distribution and photoreceptor degeneration was examined, 17-DMAG and NVP-AUY922 showed substantial retinal accumulation, with high retina/plasma (R/P) ratios and slow elimination rates, such that 51% of 17-DMAG and 65% of NVP-AUY922 present at 30 min post-injection were retained in the retina 6 h post-dose. For 17-AAG and ganetespib, retinal elimination was rapid (90% and 70% of drugs eliminated from the retina at 6 h, respectively) which correlated with lower R/P ratios. These findings indicate that prolonged inhibition of Hsp90 activity in the eye results in photoreceptor cell death. Moreover, the results suggest that the retina/plasma exposure ratio and retinal elimination rate profiles of Hsp90 inhibitors, irrespective of their chemical class, may predict for ocular toxicity potential.

Introduction

Heat shock protein 90 (Hsp90) is a ubiquitously expressed molecular chaperone required for the post-translational stability of its target substrates, known as client proteins, many of which are critical for cell growth, differentiation and survival (Taipale et al., 2010). In addition to playing an indispensable role in the normal homeostatic maintenance of organs and tissues, it is now recognized that the chaperone functions of Hsp90 can become subverted during tumorigenesis (Whitesell and Lindquist, 2005). In this setting Hsp90 can serve as a biochemical buffer to promote the structural and functional stability of a number of oncogenic signaling proteins causally implicated in human cancers (Trepel et al., 2010; Whitesell and Lindquist, 2005). Of note, pharmacologic inhibition of Hsp90 results in destabilization and targeted proteasomal destruction of these clients and a unique feature of Hsp90 blockade is that it provides a means to simultaneously inhibit multiple oncogenic pathways. For these reasons, Hsp90 has emerged as an attractive molecular target for the development of novel cancer therapeutics (Banerji, 2009; Kim et al., 2009).

Ocular toxicities are a common side-effect of systemic chemotherapeutic drugs (al-Tweigeri et al., 1996) and have also emerged as an important clinical concern for newer molecularly-targeted agents entering standard oncology practice (Renouf et al., 2012). The first class of targeted Hsp90 compounds characterized was the benzoquinone ansamycins, which include the natural product geldanamycin and its derivatives 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin) and 17-AAG (17-allylamino-17-demethoxygeldanamycin) (Taldone et al., 2008). In recent years, an increasing number of synthetic small molecule inhibitors of Hsp90 have also been developed that are based on a diverse variety of chemical scaffolds, including resorcinol, purine and benzamide structures (Biamonte et al., 2010; Taldone et al., 2008). In human clinical trials some Hsp90 inhibitors, including 17-DMAG, have been associated with visual disorders including blurred vision, flashes, delayed light/dark accommodation, night blindness and photophobia (Kummar et al., 2010; Pacey et al., 2011; Samuel et al., 2010; Shapiro et al., 2010). These ocular adverse effects are often reversible though dose-limiting; moreover, the clinical evaluation

* Corresponding author. Fax: +1 781 274 8228.
E-mail address: DZhou@syntapharma.com (D. Zhou).

0041-008X
http://dx.doi.org/10.1016/j.taap.2013.09.018

of the second-generation aminobenzamide-based inhibitor, PF-04929113 (SNX-5422), was recently discontinued by Pfizer based on significant drug-related ocular toxicity in both preclinical and clinical studies (Rajan et al., 2011). The molecular mechanisms underlying such visual disturbances remain undefined, although recent animal toxicology studies have suggested that retinal dysfunction linked to photoreceptor degeneration and cell death may be a contributing factor (Rajan et al., 2011).

Interestingly, a number of other Hsp90 inhibitors that have undergone clinical evaluation, including 17-AAG and ganetespib (a second generation resorcinolic compound), have not manifested the same degree of visual abnormalities seen for these other compounds (Banerji et al., 2005; Cho et al., 2011; Demetri et al., 2011; Goldman et al., 2010; Ramalingam et al., 2008; Ramanathan et al., 2005, 2007; Solit et al., 2007; Wong et al., 2011). The reasons for the superior ocular safety profile exhibited by 17-AAG and ganetespib are presently unknown. Here, histopathological changes and retinal drug distribution profiles of four Hsp90 inhibitors, with or without reported clinical visual disturbances, were evaluated in a rodent model in order to understand the observed differences in ocular toxicity profile among agents in this class. A characteristic feature of targeted Hsp90 inhibition is the induction of heat shock protein 70 (Hsp70), a related molecular chaperone that plays a key role in the chaperone complex machinery (Mayer and Bukau, 2005). In this regard, Hsp70 upregulation is a commonly used surrogate biomarker for Hsp90 blockade (Whitesell et al., 2003) and was used as a biological readout for Hsp90 inhibition in the rat retina. Results presented in this report suggest that Hsp90 plays a critical role in normal retinal function and suggest that aberrant inhibition of Hsp90 activity in the eye results in structural damage of the retina and photoreceptor cell death. In addition, the data suggest that the retina/plasma exposure ratio and retinal elimination rate profiles of individual Hsp90 inhibitor compounds, irrespective of their chemical class, represent predictive factors for assessing compounds' potential for ocular damage.

Materials and methods

Hsp90 inhibitors. 17-DMAG and 17-AAG were purchased from LC Laboratories (Woburn, MA) and NVP-AUY922 from Selleck Chemicals (Houston, TX). Ganetespib was synthesized by Synta Pharmaceuticals Corp.

Animals, tissue processing and immunohistochemistry. All in vivo procedures were approved by the Synta Pharmaceuticals Corp. Institutional Animal Care and Use Committee and carried out in strict accordance with the Guide for Care and Use of Laboratory Animals of the National Institutes of Health. Male Sprague Dawley (SD) and Long Evans rats (220 ± 60 g; Charles River Laboratories, Wilmington, MA) were maintained on a 12 h light–dark cycle. Rats were dosed with either 17-DMAG (20 mg/kg), 17-AAG (80 mg/kg), ganetespib (20 mg/kg), NVP-AUY922 (10 mg/kg) or vehicle (17-DMAG was formulated in D5W [5% dextrose in water] and the other three compounds in DRD [10% DMSO/14% Cremophor RH40/76%D5W]). All four inhibitors were dosed intravenously as this represents the clinical route of administration for each. Animals were treated on a 2 consecutive day dosing schedule. Eyes were harvested 24 h after the last dose, fixed in Modified Davidson's solution for 24–48 h, and then washed with 70–90% ethanol. Paraffin-embedded eye sections (5 μm) were subject to routine H&E staining, TUNEL staining using the ApopTag Peroxidase ISOL Apoptosis Detection kit (Millipore, Billerica, MA), or immunohistochemistry with a rabbit polyclonal antibody directed against Hsp70 (1:100 dilution; #4872, Cell Signaling Technology, Danvers, MA), according to manufacturers' protocols. Negative control staining was performed in the absence of primary antibody.

Histological assessment. Histology slides were reviewed in a blind fashion by two independent investigators followed by a board certified pathologist. TUNEL stained sections were scored semi-quantitatively as follows: negative (−), ≤20 cells/overall section; minimal (±), 21–50 positive cells/overall section; mild (+), >50 positive cells in less than 30% of overall retinal section; moderate (++), >50 positive cells in 30–60% of the overall retinal section; severe (+++), >50 positive cells in greater than 60% of the overall retinal section. Quantitative image analysis was performed on the Hsp70 immunostained sections. Three prominently stained retinal regions per rat were randomly selected from the peripheral area, proximal to the optical nerve, and within the intervening region. Images were captured under identical electronic exposure profiles and analyzed using Image-Pro Plus software (Media Cybernetics Inc., Rockville, MD) at 20× magnification, and measured as the percentage of Hsp70 positive and negative areas from the inner segment of the photoreceptor layer to the ganglion cell layer. The average % Hsp70-positive area/rat values were calculated and expressed as a fold change (median ± SD) compared to the vehicle group. Statistical significance was set at $P < 0.05$ using one-way ANOVA.

Retinal morphological evaluation. As an index of photoreceptor cell loss, a quantitative measurement of mean outer nuclear layer (ONL) thickness was performed according to previously published procedures (LaVail and Lawson, 1986; Williams and Howell, 1983). Rats were treated with vehicle or 17-DMAG at 20 mg/kg/day, every other day (q.o.d.) for 2 weeks. This dose schedule provided repeated exposure to the inhibitor, and was identified in pilot studies as the maximally tolerated dose. Twenty-four hours after the final dose, eyes were enucleated, fixed in Modified Davidson's solution and embedded in paraffin. Retinal tissue was sectioned (4-μm thickness) along the vertical meridian through the optic nerve and stained with H&E. In each of the superior and inferior hemispheres, the ONL thickness was measured quantitatively using Image-Pro Plus software. The ONL thickness of each of the superior and inferior hemispheres was measured in nine sets of three measurements each (total of 27 measurements per hemisphere). Each set was centered on adjacent 500 ± 50 μm segments of the retina, with the first point of measurement taken approximately 170 ± 30 μm from the optic nerve head and subsequent sets located toward the periphery. Comparisons of ONL thickness were made between vehicle and 17-DMAG-treated animals using 6 rats per group.

Western blotting. Retinal tissue (including the retinal pigment epithelium layer) from both eyes was dissected using previously described methodology (Barres et al., 1988) and homogenized in a lysing matrix tube (MP Biosciences, Santa Ana, CA) containing lysis buffer (Cell Signaling Technology). Lysates were clarified by centrifugation and equal amounts of protein resolved by SDS-PAGE before transfer to nitrocellulose membranes (Invitrogen, Carlsbad, CA). Membranes were blocked and then immunoblotted with antibodies directed against Hsp70/72 (C92F3A-5; Enzo Life Sciences, Farmingdale, NY) or GAPDH (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The antibody–antigen complex was visualized and quantitated using the Odyssey system (LI-COR, Lincoln, NE).

Pharmacokinetic analysis. 17-DMAG (20 mg/kg), 17-AAG (80 mg/kg), ganetespib (20 mg/kg) and NVP-AUY922 (10 mg/kg) were i.v. administered to male SD rats (n = 3). For consistency with the experimental protocol, animals were given two consecutive daily doses of inhibitor prior to PK assessment. Following retro-orbital blood collection, plasma samples were protein precipitated and analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Noncompartmental pharmacokinetic analysis was performed using Phoenix WinNonlin ver. 6.3 (Pharsight, Mountain View, CA) for individual animals and mean data reported for the group.

For the determination of plasma and retinal tissue drug concentrations, inhibitors were i.v. administered to rats (n = 3) as two consecutive daily doses and plasma and retina samples were collected at 0.5, 6 and 18 h after the final dose for bioanalysis. To do this, animals were euthanized and retinal samples from each time point were pooled and