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Application of electroretinography (ERG) in early drug development for assessing retinal toxicity in rats

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ABSTRACT

Retinal ocular toxicity is among the leading causes of drug development attrition in the pharmaceutical industry. Electroretinography (ERG) is a non-invasive functional assay used to assess neuro-retinal physiological integrity by measuring the electrical responses. To directly assess the utility of ERG, a series of studies was conducted following intravitreal and/or iv administration of pan-cyclin-dependent kinase inhibitors: AG-012,986 and AG-024,322 in rats. Both compounds have previously shown to induce retinal toxicity. Retinal injury was evaluated by ERG, histopathology and TUNEL staining. Intravitreal injection of AG-012,986 at ≥10 μg/eye resulted in decreases (60%) in ERG b-wave and microscopic changes of mild to moderate retinal degeneration, and at 30 μg/eye led to additional ophthalmic findings. Intravenous administration of AG-012,986 daily at ≥5 mg/kg resulted in dose-related decreases (25 to 40%) in b-wave and sporadic to intense positive TUNEL staining. Intravitreal injection of AG-024,322 at 30 μg/eye also resulted in decreases (50 to 60%) in b-wave, mild to marked retinal degeneration and mild vitreous debris. These experiments demonstrate that ERG can be used as a sensitive and reliable functional tool to evaluate retinal toxicity induced by test compounds in rats complementing other classical ocular safety measurements.

1. Introduction

Data summarized from Pfizer's internal drug development database revealed that ocular toxicity accounts for approximately 7% of therapeutic candidate attrition, the 4th most common cause of attrition after cardiovascular, liver, and renal toxicity. Among the aforementioned ocular toxicity, 99% were attributed to retinal toxicity. Sensory retina is the most complex of all ocular tissues, composed of multiple light-sensitive neuronal layers (nerve fiber, ganglion, bipolar, photoreceptor layers, plus retinal pigment epithelium lining the inner surface of the eye (Yang and Huang, 2012). Retinal exposure to xenobiotics/chemical compounds has the potential to lead to interruption of visual signal transmission or changes in structure morphology, resulting in retinal toxicity (Huang et al., 2009).

Current practices to assess ocular toxicity consist of in vitro and in vivo approaches. In vitro retinal toxicity screening assays, using retinal cell lines, mixed cells, or organotypic/tissue cultures, are relatively less costly and can be performed in high throughput. However, the predictivity of these assays remains debatable. By contrast, in vivo animal model screening is more translatable and definitive for retinal toxicity evaluation. Therefore in vivo animal testing is considered a standard step in preclinical safety evaluation in drug development, and is well accepted by regulatory agencies. The most common available in vivo ocular safety assessment tools are ophthalmic examinations, including ophthalmoscopy, slit-lamp, fluorescein angiogram, and histopathology, all of which are focused on the evaluation of structural/morphologic changes in the retina. Electroretinography (ERG) on the other hand, is a non-invasive test used to assess the integrity of neuro-retinal physiology by measuring the electrical responses of various retinal cell types. The ERG waveform represents the electrical response that is generated by the entire retina when stimulated by a brief stimulus of light. The light stimulus elicits a biphasic waveform recordable at the cornea. The two components of the ERG which are most commonly measured and reported are the a- and b-waves. The a-wave is the first large negative component produced by the photoreceptors (cones and rods) within the retina. The b-wave is a large positive component generated by the bipolar cells within the inner nuclear layer of the retina. Together the two waves comprise the main portion of the ERG waveform and represent a

Abbreviations: ERG, electroretinography; pan-CDK inhibitor, pan-cyclin dependent kinase inhibitor; CDK, cyclin-dependent kinase; APAP, N-acetyl-para-aminophenol/acetaminophen; Hz, hertz; H&E, hematoxylin and eosin; TUNEL, terminal deoxynucleotide transferase (TdT)-mediated dUTP nick end labeling; ANOVA, analysis of variance analysis; PRL, photoreceptor layer; OPL, outer plexiform layer; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; RPE, retinal pigment epithelium; NFL, nerve fiber layer; OLM, outer limiting membrane; NSF, no significant findings; SID, once daily; BID, twice daily.

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majority of the function within the retina. When applied at the pre-clinical stage, ERG may be utilized to evaluate potential ocular toxicity of drug candidates.

A class of small molecule compounds with reported ocular toxicities in pre-clinical species is the pan-cyclin dependent kinase (pan-CDK) inhibitors. Cyclin-dependent kinases (CDKs) are a family of serine-threonine kinases which when activated by the cyclin regulatory subunit, control the progression of normal mammalian cells through the cell cycle (Lee and Nurse, 1987).

Since inappropriate cell proliferation in cancer was identified to be closely related with overactivity of cell cycle CDKs, de-regulating the specific protein kinases has become widely pursued drug targets in pharmaceutical research. Efforts have been mainly focused on developing potent and safer CDK inhibitors for a variety of cancer indications in the past decade. (Shapiro, 2006; Krystof and Uldrijan, 2010). However, several pan-CDK inhibitors have been reported to cause unexpected retinal and peripheral nerve toxicity in mice and specific photoreceptor layer damage in Cynomolgus monkeys (Illanes et al., 2006; Saturno et al., 2007). One of these pan-CDK inhibitors is AG-012,986 which upon iv administration resulted in retinal degeneration or atrophy in CD-1 mice after 21-days of drug administration (Illanes et al., 2006). Interestingly, compound-related microscopic findings were not detected by routine histology exam in mice. Instead, a more specific staining assay such as the TUNEL assay was further applied to visualize the apoptotic retinal cells (Illanes et al., 2006). Thus sensitive or early in vivo toxicological screening is very important for the development of new drugs, and rats are widely used as one of the standard rodent species and accepted by regulatory agencies as a test species in toxicity studies. To save money and time, developing more robust, sensitive, and non-invasive assays to detect rat retinal toxicity earlier on is therefore beneficial. A retinal functional assay such as ERG is a superlative tool to evaluate ocular toxicity in drug development (Rosolen et al., 2005) and pan-CDK inhibitors would be ideal bench marker compounds to validate such assays.

In the present article, we conducted a comprehensive evaluation of retinal toxicity, using a functional test (ERG), in vivo ophthalmic examinations, and histopathology, in rats treated with pan-CDK inhibitors, AG-012,986 and AG-024,322. The sensitivity and specificity of a full-field scotopic ERG in detecting retinal toxicity were confirmed and compared to the morphological or structural changes and their relationship to in vivo ophthalmology exams. The effects of intravitreal versus iv administration of test compounds on retinal toxicity in the rat model were also studied. Additionally, the progression of the retinal lesions and possible reversibility were also investigated.

2. Materials and methods

Studies were designed to assess the sensitivity and specificity of ERG to detect ocular toxicity in Wistar Han rats administered pan-cyclin dependent kinase inhibitors by intravitreal or iv routes.

2.1. Animals and husbandry

In all studies, standard procedures and conditions were applied in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures involving laboratory animals were reviewed and approved by Pfizer's Institutional Animal Care and Use Committee. All studies were conducted in male Wistar Han IGS (CRL:WI [Han]) rats that were supplied by Charles River Laboratories, Inc., (Portage, MI, USA). The rats were 6 to 8 weeks old and weighed 200–250 g at study initiation. Environmental controls for the animal room were set to maintain a temperature range of 20 to 26 °C, relative humidity of 30% to 70%, a routine 12 h light/dark cycle and a minimum of 12 air changes per hour.

2.2. Test articles

AG-012,986 (4-((4-amino-5-(2,6-difluorobenzoyl)-1,3-thiazol-2-yl)amino)-N-((1R)-2-(dimethylamino)-1-methylethyl)benzamide) and AG-024,322 (N-((5-(3-(4,6-difluoro-1 H-benzo[d]imidazol-2-yl)-1 H-indazol-4-yl)-4-methylpyridin-3-yl)methyl)ethanamine) are chemically distinct, early generation small molecule pan-CDK inhibitors developed at Pfizer. The pan-CDK inhibitors were formulated as a nano-suspension in 2.5% povidone (PVP) K30, 0.2% Tween80 in water at 0.2, 0.6, 2.0 and 6.0 mg/ml or 0.1 M NaCl, 0.1 M acetic acid, pH 4.5 at 0.5, 1.0 and 2.0 mg/ml, for intravitreal (5 μl) or iv administration (dose volume 10 ml/kg), respectively. N-acetyl-para-aminophenol (APAP)/acetaminophen has no reported ocular effects (FDA) and was utilized as a negative control. APAP was supplied by Sigma-Aldrich (Milwaukee, WI) and formulated in 2% polyvinyl alcohol (PVA) as a nano-suspension at 12 mg/ml.

2.3. Ocular toxicity studies

Rat study designs are summarized in Table 1. Briefly, AG-012,986 was given either by iv or intravitreal injections, while AG-024,322 and APAP were administered by intravitreal injection. Groups of male Wistar Han rats were administered AG-012,986 (5 or 10 mg/kg) iv once a day, (10 mg/kg) iv twice a day to achieve better tolerability, or a single intravitreal injection of vehicle, APAP (30 and 60 μg/eye) or AG-012,986 (1, 3, 10 and 30 μg/eye) to both eyes.

In preparation for intravitreal dosing, animals were anesthetized using 2.5% isofluorane. Two drops of 2.5% phenylephrine (hydrochloride ophthalmic solution, USP, Akorn Inc., Lake Forest, IL) and 1% tropicamide (Akorn Inc., Lake Forest, IL) were administered, topically, to each eye to facilitate pupil dilation. Immediately prior to the injection, one drop of 0.5% proparacaine hydrochloride (Akorn Inc., Lake Forest, IL) was administered to numb the eyes. The animals were each placed under a surgical microscope and a contact lens was placed on the cornea using a small drop of 0.3% hypromellose (GenTeal, Novartis Pharmaceutial Corp, East Hanover, NJ). Rats were given a single 5 μl intravitreal injection, using a 30 gauge needle attached to a 10-μl Hamilton syringe, approximately 1 mm from the corneal limbus at the lateral canthus of the globe of each eye. The test article was injected slowly and the needle remained in the eye for a few seconds to allow for the dosing solution to equilibrate with the vitreous humor. The needle was then slowly removed so as to minimize the chance of dosing solution/vitreous reflux through the injection site. Topical ophthalmic antibiotic ointment was placed on the ocular surface following the procedure. Ocular signs of toxicity were assessed predose and on days 4, 8, and/or 14 of the study. Ocular irritation was scored in accordance with the Organization for Economic Cooperation and Development 1987 guidelines (OECD, 2012). Ophthalmic examination was conducted with a slit lamp and an indirect ophthalmoscope. Each eye was grossly examined and graded on a scale for the severity of changes using a modified MacDonald–Shadduck Scoring System (Aguirre et al., 2009). Fundus examinations were performed using an indirect ophthalmoscope in eyes with pupils dilated with 2.5% phenylephrine and 1% tropicamide.

Intravenous doses were administered daily via the lateral tail vein at a dose volume of 10 ml/kg. The dose volume was based on the most recent individual animal body weight (predose Day 1). The animals were assessed daily for mortality, abnormalities, and signs of pain or distress. Clinical signs of toxicity were assessed daily and body weights were recorded weekly. Ophthalmic examinations were conducted post ERG measurements predose and 4, 8 and/or 14 days post dose. Body weights were recorded during the pretest period and weekly thereafter (on days 8 and/or 15 postdose).