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Original article

Tafenoquine is not neurotoxic following supertherapeutic dosing in rats

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ABSTRACT

Background: Tafenoquine is a new drug for malaria prevention. The goal of the present work was to conduct a specific neurobehavioral study in rats with histopathological assessment of the brain.
Methods: The clinical, hematological, behavioral, motor activity, and neurohistopathologic changes induced by different dose levels of tafenoquine were evaluated following single super-therapeutic dose administration. Toxicokinetic data were generated to allow extrapolation to clinical exposures.
Results: At the highest dose (500 mg/kg), two animals (of 12) died. Surviving animals showed clinical signs of toxicity and had reduced body weight 7–8 days after dosing. Decreases in motor activity were observed on more than one occasion at doses > 9-fold higher than the clinical exposure. No statistically significant changes were observed for other behavioral endpoints. No neurohistopathological changes were noted. Changes in hematological and clinical pathology endpoints were observed at the lowest dose level (125 mg/kg). For context, the human dosing regimen is a 10 mg/kg load followed by 3.3 mg/kg weekly (in a 60 kg person).
Conclusions: As in humans, adverse events other than neurotoxicity were dose-limiting for tafenoquine in rats. This raises the prospect that a new weekly prophylactic, without neurologic liability, may become available in the near future.

1. Introduction

Tafenoquine is an 8-aminoquinoline analog of primaquine in late stage development for various malaria indications by GlaxoSmithKline, Medicines for Malaria Venture, the U.S. Army and 60 Degrees Pharmaceuticals (60P). The conferment of breakthrough therapy designation by the U.S. Food and Drug Administration [1] suggests that substantial public health benefits may accrue if tafenoquine is approved by regulators.

The long half-life of tafenoquine allows for more convenient dosing regimens. The anticipated clinical dose of tafenoquine will be 200 mg/day for three days (total of 10 mg/kg over three days in a 60 kg person) followed by 200 mg maintenance doses thereafter (3.3 mg/kg weekly in a 60 kg person [2]). In the context of travel medicine, tafenoquine would become the only available once weekly regimen useful for malaria prevention in areas of the world with chloroquine or mefloquine-resistant malaria. It would also provide travel medicine practitioners the option of being able to prescribe a chemoprophylactic agent with a weekly dosing regimen, but without the neuropsychiatric adverse event profile associated with mefloquine [3]. In some jurisdictions, concerns regarding the neuropsychiatric effects of mefloquine have resulted in very restrictive prescribing rules and there is concern that in the future this drug may not be available for special populations [4].

Dow et al. [5] reported that mefloquine, the weekly prophylactic antimalarial for which tafenoquine could be an alternative, induced degeneration of brain stem nuclei and neurobehavioral changes at threshold doses with exposure levels relevant to human dosing in female rats. The general methodology employed in that study is

List of abbreviations: 60P, 60 Degrees Pharmaceuticals; Alb, Albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Chol, cholesterol; CNS, Clinical Network Services; EDTA, ethylenediaminetetra acetic acid; F, female; FOB, Functional observation battery; G6PD, Glucose-6-phosphate dehydrogenase; H&E, Haematoxylin and Eosin stain; HCT, haematocrit; HGB, haemoglobin; LUC, large unstained cell; Lymph, lymphocyte; M, male; Mono, monocyte; Neut, neutrophil; No., number; PLT, platelet count; RBC, red blood cell; Retic, reticulocyte; SD, Sprague Dawley; TP, total protein; USAMMDA, US Army Medical Materiel Development Activity.

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required by regulators as a component of the core non-clinical safety battery included in regulatory filings [6]. Recently, 60P, as one of the commercial sponsors of tafenoquine, updated its non-clinical dossier by conducting a specific neurobehavioral study in rats with histopathological assessment of the brain. The results of this work is reported herein.

2. Materials and methods

2.1. MTD rat study

With the goal of identifying the maximum tolerated dose, groups of 5 male and 5 female Sprague Dawley (SD) rats were administered a single oral dose of 0 (vehicle), 125, 250, 400 or 700 mg/kg tafenoquine succinate (dose expressed as free base) in 1%/0.4% methylcellulose/Tween 80 in water, at a dose volume of 10 mL/kg [Note the 400 mg/kg dose group was administered an actual dose of 506 mg/kg due to a higher concentration dose formulation being prepared whereas all other groups were within 12% of nominal dose]. The day of dosing was designated Day 1. Animals were observed for 7 days following dosing.

Animal viability checks and physical observations were made daily, body weights were recorded pre-dose and twice during the study, and clinical pathology parameters were assessed on Day 7. Following Day 7 assessments animals were euthanized without further examination, although any animals dying earlier than the scheduled end of study were grossly examined at necropsy. The dose formulation for each group was analyzed to confirm the absence (control) or actual concentration of tafenoquine.

2.2. Neurobehavioral, histopathologic and toxicokinetic study

Based on the rat maximum tolerated dose study results, three groups of 12 male and 12 female SD rats were dosed once orally with 125, 250 or 500 mg/kg tafenoquine succinate (dose expressed as free base). The highest dose was anticipated to be the maximum tolerated dose. The lower doses were selected because they exceed therapeutic doses, were well tolerated in the maximum tolerated dose study, and allowed dose response to be explored. A group of 9 male and 9 female SD rats were dosed concurrently with vehicle i.e. 1%/0.4% methylcellulose/Tween 80 in distilled water.

Six rats of each sex in the control and tafenoquine-treated groups were used to assess neurobehavioral effects following dosing while the remaining 3/sex in the control group and 6/sex in the tafenoquine-treated groups were included in the toxicokinetic investigations. Blood samples (~0.5 mL) were collected from tafenoquine-treated toxicokinetic group animals (3/sex/group/time point) at 1, 3, 5, 8, 24, 48, 72 and 168 h after dosing. Blood was collected 8 h post dosing in control animals. Blood was placed in to K_2EDTA anticoagulant tubes and stored on wet ice prior to plasma separation by centrifugation. Plasma was stored frozen at approximately −80 °C (±10 °C) within 2 h of collection until analysis. Plasma was analyzed by high performance liquid chromatography with mass spectrometric detection.

All dose formulations were analysed to confirm absence (control) or concentration of tafenoquine and the homogeneity of mixtures. Daily viability checks were performed morning and evening along with general clinical observations prior to dosing and at least twice following dosing on all animals along with body weights pre-dose and on the day of necropsy. A functional observation battery (FOB) [7] was performed on neurobehavioral group animals by trained observers with no prior knowledge of treatment, pre-dosing (Day −1) and at 0.5, 3, 6, 24 and 48 h after dosing. After the FOB, pretest and at 6, 24 and 48 h post dosing, horizontal and vertical motor activity was monitored for 60 min (divided in to 12, 5 min intervals) using an automated motor monitor system.

Animals from the neurobehavioral groups were necropsied on Day 4 and 8 (3/sex/time point), i.e. 72 h and 168 h after dosing, respectively. Animals were deeply anesthetized with sodium pentobarbital before whole body perfusion via the ascending aorta with ~100 mL of saline followed by ~500 mL of 0.1 M phosphate buffer (pH 7.4 ± 0.1) containing 4% paraformaldehyde. The brain remained in situ and the carcass was refrigerated for 3–6 h, then the heads removed and post-fixed for 24–48 h with neutral buffered formalin before removal of the brain from the skull and storage in the same fixative as needed until processing.

All fixed brain tissues were processed to paraffin blocks. Rat brains were gross-trimmed according to the guide provided in Bolon *et al* for the 'best practice' approach to neuropathologic assessment in developmental neurotoxicity [8]. Since the gracile and cuneate (and potentially other brainstem) nuclei were targets for mefloquine [5], depending on the amount of tissue after the 8th slice as depicted in the Bolon *et al* recommendation, a 9th slice was taken caudal to the 8th slice and placed face down in the block. Two sections were taken from the blocks at each of levels 1 to 7 and stained with Haematoxylin and Eosin (H&E) and Bielschowsky's silver stain. The 8th and 9th (if present) blocks were step sectioned, first taking 4 serial sections on separate slides (2 stained with H&E and Bielschowsky's stain, the remaining 2 as spares) and then microtoming 50 μm deeper to take another 2 sections for H&E and Bielschowsky's staining.

H&E and silver-stained sections from control and high-dose rats were evaluated by a board certified pathologist with knowledge of dose groups. The study protocol called for blinded examination of all tissues in all dose groups if differences were noted between the high and vehicle-dosed groups.

3. Results

3.1. Maximum tolerated dose study

The dose formulation analysis confirmed the actual dose concentrations were within 12% of target concentration except for the 400 mg/kg dose group, where the formulation was 126% higher than the nominal solution concentration of 40 mg/mL thus achieving a dose of 506 mg/kg as opposed to 400 mg/kg (Note the results for this group are referred to by the nominal dose of 400 mg/kg). This was not considered to have adversely affected the aim of the study.

Clinical and physiological changes following single dose administration of tafenoquine are summarized in Table 1. One male died on Day 6 following the single oral administration of 700 mg/kg on Day 1. Clinical signs following the single administration of tafenoquine were whole body pallor (all animals at ≥400 mg/kg), dark or dull bilateral eyes at ≥400 mg/kg and thin appearance (2 females), staining on head (abnormal red color, in 1 female) and rales (1 female) at 700 mg/kg. No clinical signs were noted at 125 or 250 mg/kg. Dose related statistically significant decreases in body weight was seen at all doses in males, and at 700 mg/kg in females. Food consumption was decreased at 400 and 700 mg/kg in males and females compared to pretest baseline values. One male rat dosed with 700 mg/kg was found dead on Day 6 and had gross pathology of enlarged liver, small right testis and thymus with dark areas.

The main clinical pathology changes included decreases in red blood cell (RBC) parameters, increase in neutrophils as well as increases in liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The decreases in red blood cell mass (hemoglobin, hematocrit, and RBC count) at all doses in females were associated with a regenerative response (increased