**Introduction**

Retigabine (RTG) (other names: Potiga, ezogabine)1
- Potassium channel opener - KV7.2-7.5 (KCNQ2/3) in brain neurons
- Adjacent treatment of refractory partial-onset seizures in adults

FDA Safety Communication (April 2013)
- RTG could cause changes in retinal pigment epithelium, pigmentation and discoloration of skin resulting in a blue appearance
  (Figure 1)
- Associated with long RTG treatment intervals of over four years on average
- 38 of 605 patients had developed skin discoloration (6.3%)
- 11 of 36 patients given eye exams were found to have retinal pigment abnormalities – unknown if these changes could result in vision loss

**WARNING: RETINAL ABNORMALITIES AND POTENTIAL VISION LOSS**

GSK initiated numerous studies to gain mechanistic understanding of the origins of retinal pigment changes. One of these efforts was a long term ocular toxicity risk assessment in rats. MALDI IMS was used to determine the presence and distribution of RTG and its metabolites in the rat eye following 13 and 29 weeks of dosing.

**Methods**

All animal procedures were conducted in an American Association for the Accreditation of Laboratory Animal Care (AALAC)-accredited facility at GlaxoSmithKline (GSK) in accordance with GSK policies on the care, welfare and treatment of laboratory animals and they were reviewed and approved by GSK’s Institutional Animal Care and Use Committee (IACUC) as appropriate. RTG was administered to pigmented male Long Evans (HsdBlLE) rats for 91 days (Group 2) or 271 days (Group 3) by oral diet administration at a dose of 0 (vehicle; Group 1) or nominal dose of 100 mg/kg/day (Groups 263). Rats were necropsied on days 91 or 272 and eyes were collected on days 91 or 272 for evaluation by MALDI IMS.

Eye tissues were embedded in pHPMA (150 mg/mL, pHPMA in H2O). Thin sections (6 μm) of the embedded eye tissues were collected in a cryostat and mounted onto ITO-coated glass microscope slides. An optical image of each tissue section was generated using an ApoScropScope (Leica, Buffalo Grove, IL) digital slide scanner (20x magnification) prior to MALDI application. Serial sections (6 μm) were collected for H&E staining.

DHB matrix was applied to the tissues using a custom-built sublimation apparatus operated under vacuum (~200 mTorr) heated to ~140 °C. Approximately 50 mg of DHB was sublimed to completion ~15 min. Matrix Coated slides were incubated for approximately 20 min in a chamber saturated with methanol at room temperature. MALDI IMS was performed on a 7T Solarix FT-ICR MS (Bruker Daltonics, Billerica, MA). Positive ion mass spectra were acquired in full scan mode (m/z 200-1000) with pixel dimensions ranging from 5.25 μm.

**Results**

MALDI IMS of eye tissue sections from pigmented male Long Evans rats administered RTG revealed the presence of RTG (Figure 2A), an N-acetyl metabolite of RTG (NAMR) (Figure 2B), and several species corresponding to the dimerization of RTG and NAMR. These included: i) three phenazine dimers (Figure 2C) corresponding to RTG-RTG, NAMR-NAMR, and RTG-NAMR, and the corresponding phenazine species, RTG-RTG+, NAMR-NAMR+, and RTG-NAMR+ (Figure 2D) which are formed by the loss of the fluorobenzyl group through quaternary amine hydrolysis. The dimer species appear purple in solution (RTG-RTG+ (UV/vis = 551 nm), NAMR-NAMR+ (UV/vis = 549nm))

**Conclusions**

The detection of the three phenazine dimers (RTG-RTG, NAMR- NAMR, RTG-NAMR) in the melanin containing ocular tissue, their absence in plasma or other non-melanin containing ocular tissues, and the high melanin association for both RTG and NAMR tissues suggests that the formation of these dimers occurs from melanin bound RTG and NAMR. In this hypothesis, the melanin binding of RTG and NAMR effectively concentrates the two compounds to enable mixed condensation reactions to occur when the binding provides the proper geometry in the oxidative environment of the ocular tract. Based upon their purple appearance and UV spectra, we propose that these dimers could also be responsible for the dyspigmentation (purple-grey appearance) in melanin containing skin tissues of patients with long term RTG treatment.

Melanin binding within the retina, the region of the eye containing the photoreceptors, could have a greater potential risk for adverse events. Thus, the high resolution IMS experiments (Figure 7 and B) demonstrate that no detectable levels of RTG related material including the dimers were able to penetrate the blood-retinal barrier associated with the RPE. This is consistent with the results of the histopathological analysis of rat eyes in this study. Conversely, no ocular toxicity findings were reported. Though convincing in the rat, it should be noted that it is not clear how our findings in the rat model relate to the FDA warning of changes in retinal pigmentation associated with long term use of RTG in patients.

**References**


**Figure 1.** RTG blue discoloration of skin in a rat in a controlled environment. MALDI Imaging Safety Communication.

**Figure 2.** Distribution of several of RTG-related species detected by MALDI IMS (25 μm pixel dimensions) from two separate rats are displayed in Figures 3 and 4. The same spatial distribution was observed for these species and they appeared to be localized to the melanin containing layers of the uveal tract including the choroid, ciliary body and iris (see eye schematic).

**Figure 3.** Group of rats (n=4) were administered RTG (vehicle (Veh) or RTG at oral doses of 100 mg/kg/day) by diet for 91 days. All animals were necropsied and the following organs were collected for MALDI IMS analysis: retina, choroid, ciliary body, iris, and uvea. A) Representative MALDI-MS images overlaid with Ion Intensity and MALDI-MS images of representative ions of RTG-NAMR* (m/z 531), NAMR (m/z 501), and RTG (m/z 321) at 50X magnification. B) Representative MALDI-MS images overlaid with Ion Intensity and MALDI-MS images of representative ions of RTG-NAMR* (m/z 531), NAMR (m/z 501), and RTG (m/z 321) at 100X magnification. C) D) MALDI-MS images of ion intensity from 200X magnification overlaid with ion intensity from MALDI-MS images of representative ions of RTG-NAMR* (m/z 531), NAMR (m/z 501), and RTG (m/z 321). MALDI-MS images were generated from tissue sections collected on day 91 from rats administered RTG (Veh or RTG).