Mechanism of Voriconazole-Induced Transient Visual Disturbance: Reversible Dysfunction of Retinal ON-Bipolar Cells in Monkeys

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PURPOSE. To investigate the mechanism of voriconazole-induced transient visual disturbance in humans.

METHODS. Standard full-field electroretinograms (ERGs) were recorded from monkeys treated intravenously with voriconazole. In addition, photopic ERGs elicited by long-duration stimuli (ON-OFF response) were also recorded from monkeys receiving intravenous voriconazole or intravitreal 2-amino-4-phosphonobutyric acid (APB).

RESULTS. Characteristic changes were observed in the waveform of the standard full-field ERGs obtained immediately after dosing of voriconazole as follows: electronegative combined rod-cone response (markedly attenuated b-wave and oscillatory potentials), undetectable rod response (eliminated b-wave); slightly abnormal single-flash cone response (flattened appearance in the bottom of the a-wave, mildly attenuated b-wave); and slightly abnormal 30 Hz flicker (mildly attenuated b-wave). The above changes fully recovered to baseline 24 hours after each dosing, along with a decrease in plasma voriconazole concentration. In addition, the change in the waveform of the ON-OFF response recorded in voriconazole-treated monkeys was quite similar to that recorded in APB-treated monkeys as follows: the b-wave was eliminated or prominently attenuated; and the a- and d-waves were not apparently attenuated.

CONCLUSIONS. The results strongly suggest that voriconazole induces selective and reversible dysfunction of the retinal ON-bipolar cells in both the rod and cone pathways in monkeys. From the results obtained in monkeys in this study, it is suggested that the function of the retinal ON-bipolar cells was selectively and reversibly affected in voriconazole-treated humans who complained of transient visual disturbances.

Investigative Ophthalmology & Visual Science, July 2011, Vol. 52, No. 8

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limbus. An equal volume of physiologic saline (0.05 mL) was injected into the left vitreous of each animal as vehicle control.

**Animal Preparation for ERG Recording**

The animals were anesthetized with intramuscular injection of ketamine hydrochloride (Ketalar Intramuscular, 500 mg; Daiichi-Sankyo Co., Ltd., Tokyo, Japan) (10 mg/kg initial dose, 5 to 10 mg/kg per hour maintenance dose) and 0.6 mg/kg xylazine hydrochloride (Celactal; Bayer Medical Ltd., Osaka, Japan). The pupils were dilated with topical 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P ophthalmic solution; Santen Pharmaceutical Co., Ltd., Osaka, Japan); the corneas were anesthetized with topical 0.4% oxybuprocacline hydrochloride (Benoxil ophthalmic solution 0.4%; Santen Pharmaceutical Co., Ltd.) and protected with topical hydroxyethylcellulose (Scopisol 15; Takeda Chemical Industries, Ltd., Osaka, Japan).

**Visual Stimulation**

Two systems for visual stimulation were used in this study. One was for obtaining the standard full-field ERGs, and the other was for obtaining the ON-OFF response. The standard full-field ERGs were elicited by white xenon photostrobe flashes with a nominal flash duration of approximately 30 μsec that were generated in a dome (Ganzfeld Stimulator Model 2503S; LKC Technologies Inc., Gaithersburg, MD). The dome also had a rod-desensitizing white light as the background. The maximum flash intensity measured in the dome was 6.5 cd/s/m². The stimulus intensity was attenuated with neutral-density gelatin filters (Wratten; Eastman Kodak Company, Rochester, NY). The ON-OFF response was elicited by a long flash of white light that was generated with a light-emitting diode (LED). The LEDs were built into contact lens recording electrodes (Contact Lens Electrode with Built-in LED; Mayo Corporation, Aichi, Japan). The luminance and duration of the flashes and the background luminance were controlled by a LED stimulator (LS-C; Mayo Corporation).

**ERG Recording and Analysis**

The standard full-field ERGs were recorded according to the guideline of the International Society for Clinical Electrophysiology of Vision (ISCEV).<sup>9</sup> A bipolar contact lens electrode (H6515NFC; Mayo Corporation) was placed on the corneal surface of the left eye. A ground electrode (TN208–016; Unique Medical Co., Ltd., Tokyo, Japan) was attached to the parietal region of the scalp. After 40 minutes or more of dark adaptation, the rod response and the combined rod-cone response were elicited by light flashes at an intensity of 0.007 and 2.7 cd/s/m², respectively. Subsequently, after 10 minutes of light adaptation at 40 cd/m², the single-flash cone response and the 30 Hz flicker were elicited by light flashes at an intensity of 2.7 cd/s/m² under a background light at 40 cd/m². Each ERG mentioned above was obtained both immediately after and 24 hours after intravenous dosing.

To obtain the ON-OFF response, a contact lens electrode with built-in LED was placed on the corneal surface of the left eye, and needle electrodes (TN208–016; Unique Medical Co., Ltd.) were attached to the parietal region of the scalp and the temporal region as indifferent and ground electrodes, respectively. In bilateral recordings (before and after intravitreal injection of APB), a contact lens electrode with built-in LED was placed on each eye. The ON-OFF response was elicited by a 200 msec flash at the stimulus luminance of 63 cd/m² under a background light at 32 cd/m². The ON-OFF responses were recorded immediately before and after the intravenous injection of voriconazole, or immediately before and 60 to 90 minutes after the intravitreal injection of APB.

All responses mentioned above were amplified with a band pass from 0.5 to 1000 Hz and stored in the evoked potential test equipment (MEB-9104; Nihon Kohden Corporation, Tokyo, Japan). A limited number (three to six) of waveforms for each response was averaged to reduce variability and background noise. For the waveform analysis of the standard full-field ERGs, the a-wave amplitude was measured from baseline to the a-wave trough for the combined rod-cone response and the single-flash cone response, the b-wave amplitude was measured from the a-wave trough to the b-wave peak for all the responses, and the b/a wave ratio was calculated as the ratio of the b-wave amplitude to the a-wave amplitude for the combined rod-cone response. For the waveform analysis of the ON-OFF response, the amplitude of the b- and d-waves were measured from the a-wave trough to the b-wave peak and from the baseline at the time point of stimulus offset to the d-wave peak, respectively.

**Figure 1.** Typical waveforms of the standard full-field ERGs in a voriconazole-treated monkey. Voriconazole was administered intravenously with increasing doses of 0 (vehicle), 3, 6, and 12 mg/kg at intervals of one week or more, and the standard full-field ERGs were obtained as described in the text. Arrowheads indicate onset of the light flashes. The responses recorded immediately after each dosing (black trace) are superimposed on those recorded 24 hours after each dosing (gray trace). Each trace represents an average of three responses.
**Measurement of the Plasma Voriconazole Concentration**

A volume of approximately 0.5 mL of blood was collected immediately after the recording of the standard full-field ERGs or the ON-OFF response from the femoral vein to measure the plasma voriconazole concentration. The blood was collected immediately after recording the standard full-field ERGs and the ON-OFF responses, corresponding to 37 to 55 and 16 to 22 minutes after the start of the 10-minute infusion of voriconazole, respectively. Several months after recording the ON-OFF response from monkeys receiving 6 mg/kg of voriconazole, 6 mg/kg of voriconazole was again administered to the same animals in the same manner. Then, blood was collected in the same manner before dosing, 10 and 30 minutes, and 1, 2, 4, 7, and 24 hours after the start of dosing. The plasma was prepared from the blood samples by centrifugation at 10,000 rpm for 5 minutes at 4°C. The plasma was then stored at −80°C until measurement. The plasma concentration of voriconazole was determined by liquid chromatography mass spectrometry/mass spectrometry (LC/MS/MS) (HPLC; Waters Alliance 2795 Separations Module, Waters Corp., Milford, MA; MS/MS; Quattro Premier XE, Waters Corp.).

**Statistics**

For the statistical analysis of the measured parameters in the standard full-field ERGs, the paired t-test was used to assess the significance between vehicle-treated and voriconazole-treated values. For the statistical analysis of the measured parameters in the ON-OFF response, the paired t-test and the Student’s t-test were used to assess the significance between the values before and after dosing and between vehicle-treated and voriconazole-treated groups, respectively. The differences were considered to be significant when \( P < 0.05 \).

**RESULTS**

**Standard Full-Field ERGs in Voriconazole-Treated Monkeys**

Typical waveforms of the standard full-field ERGs in a voriconazole-treated monkey are shown in Figure 1. Measured parameters of each response are summarized in Table 1.
The combined rod-cone response was electronegative (the amplitude of the b-wave was smaller than that of the a-wave); marked attenuation in the b-wave and oscillatory potentials were observed. The decreases in the b-wave amplitude and the b/a wave ratio immediately after the dosing of voriconazole were statistically significant \((P < 0.01\) at 12 mg/kg) in comparison with vehicle-control. No significant change was noted in the a-wave amplitude. The rod response was undetectable; the b-wave was strongly attenuated or eliminated. The decrease in the b-wave amplitude immediately after voriconazole was statistically significant \((P < 0.05 \text{ at }3\text{ mg/kg}, P < 0.01 \text{ at } 6\text{ and }12\text{ mg/kg})\) compared with vehicle-control. The single-flash cone response was slightly abnormal: flattened appearance at the bottom of the a-wave and mild attenuation in the b-wave were seen. The decrease in the b-wave amplitude after voriconazole dosing was statistically significant \((P < 0.01 \text{ at } 6\text{ and }12\text{ mg/kg})\) in comparison with vehicle-control. No significant decrease was detected in the a-wave amplitude of the single-flash cone response. The 30 Hz flicker was also slightly abnormal: mild attenuation in the b-wave was observed. The decrease in the b-wave amplitude immediately after voriconazole was significant \((P < 0.05 \text{ at } 6\text{ mg/kg}, P < 0.01 \text{ at } 12\text{ mg/kg})\) compared with vehicle-control. All changes that were observed immediately after dosing fully recovered to baseline 24 hours after each dosing.

ON-OFF Responses in Voriconazole- or APB-Treated Monkeys

Typical waveforms of the ON-OFF response in monkeys. The ON-OFF responses were recorded immediately before and after intravenous dosing of vehicle (white column) or 6 mg/kg voriconazole (black column). Data are expressed as mean ± SD of three animals. Significant differences in the b-wave amplitude were detected by the paired \(t\) test \((P < 0.01)\) and the Student’s \(t\) test \((P < 0.01)\).

The plasma concentrations of voriconazole at the time of recording the standard full-field ERGs immediately after 3, 6, and 12 mg/kg dosing were 1.33 ± 0.106, 3.35 ± 0.155 and 7.13 ± 0.415 \(\mu g/mL\), respectively, and those at the time of ERG recording 24 hours after 3, 6, and 12 mg/kg dosing were below the lower limit of quantitation \((< 0.01 \mu g/mL), 0.07 ± 0.064 \mu g/mL, and 0.61 ± 0.427 \mu g/mL\), respectively (Table 2). The plasma concentration of voriconazole at the time of recording the ON-OFF responses immediately after the 6 mg/kg dosing was 4.24 ± 2.610 \(\mu g/mL\) (Table 2). The plasma concentration of voriconazole in the same animals as those used for obtaining the ON-OFF response mentioned above were 37.60 ± 6.239 \(\mu g/mL, 5.62 ± 1.271 \mu g/mL, \text{ and } 0.28 ± 0.192 \mu g/mL\) (Table 3).

**DISCUSSION**

The retinal function of voriconazole-treated monkeys was examined electrophysiologically to investigate the mechanism of voriconazole-induced transient visual disturbances in humans.

It has been reported that transient visual disturbances generally occurred early (within 30 minutes of dosing) in both healthy volunteers and patients enrolled in clinical trials with voriconazole; and the majority of those events resolved within 30 minutes. It has also been reported that the visual adverse events were related to the plasma voriconazole concentration in all the subjects who had received two 1-hour infusions of 6 mg/kg voriconazole separated by 12 hours (recommended clinical initial dose) transiently complained of visual disturbances, and the mean \(\text{AUC}_{0-24}\) and \(\text{C}_{\text{max}}\) of the nine subjects were 13.2 \(\mu g/mL\) and 4.70 \(\mu g/mL\), respectively. Therefore, the system with predosing and vehicle-control values (Fig. 4). In the d-wave, no apparent attenuation was seen in either voriconazole- or APB-treated monkeys.

**Plasma Voriconazole Concentration**

The plasma concentrations of voriconazole at the time of recording the standard full-field ERGs immediately after 3, 6, and 12 mg/kg dosing were 1.33 ± 0.106, 3.35 ± 0.155 and 7.13 ± 0.415 \(\mu g/mL\), respectively, and those at the time of ERG recording 24 hours after 3, 6, and 12 mg/kg dosing were below the lower limit of quantitation \((< 0.01 \mu g/mL), 0.07 ± 0.064 \mu g/mL, and 0.61 ± 0.427 \mu g/mL\), respectively (Table 2). The plasma concentration of voriconazole at the time of recording the ON-OFF responses immediately after the 6 mg/kg dosing was 4.24 ± 2.610 \(\mu g/mL\) (Table 2). The plasma concentration of voriconazole in the same animals as those used for obtaining the ON-OFF response mentioned above were 37.60 ± 6.239 \(\mu g/mL, 5.62 ± 1.271 \mu g/mL, \text{ and } 0.28 ± 0.192 \mu g/mL\) (Table 3).

**Table 2. Plasma Voriconazole Concentrations at the Time of ERG Recording in Monkeys**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Number of Animals</th>
<th>Time Point (After Dosing)</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Full-Field ERGs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>Immediately</td>
<td>1.33 ± 0.106</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Immediately</td>
<td>3.35 ± 0.155</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>Immediately</td>
<td>7.13 ± 0.415</td>
</tr>
<tr>
<td>ON-OFF Response</td>
<td>6</td>
<td>3</td>
<td>Immediately</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. NC, not calculated.

* Five individual values were below the lower limit of quantitation (0.01 \(\mu g/mL\)).
<table>
<thead>
<tr>
<th>Number of Animals</th>
<th>Dose (μg/kg)</th>
<th>Plasma Concentration (μg/mL)</th>
<th>Time After the Start of 10-min Infusion</th>
<th>Individual Values Below the Lower Limit of Quantitation (0.01 μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6</td>
<td>5.42 ± 1.616</td>
<td>Pre</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.92 ± 0.862</td>
<td>10 min</td>
<td>0.371 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.62 ± 0.622</td>
<td>30 min</td>
<td>0.362 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.62 ± 0.622</td>
<td>1 h</td>
<td>0.28 ± 0.192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.62 ± 0.622</td>
<td>2 h</td>
<td>0.28 ± 0.192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.62 ± 0.622</td>
<td>4 h</td>
<td>0.28 ± 0.192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.62 ± 0.622</td>
<td>7 h</td>
<td>0.28 ± 0.192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.62 ± 0.622</td>
<td>24 h</td>
<td>0.28 ± 0.192</td>
</tr>
</tbody>
</table>

Table 3. Plasma Concentrations and Toxicokinetic Parameters after Voriconazole Dosing in Monkeys

Data are expressed as mean ± SD. NC, not calculated.

In the standard full-field ERGs from voriconazole-treated monkeys in this study, apparent attenuation was detected in all responses under scotopic and photopic conditions. Furthermore, it was noteworthy that the ERGs were altered relatively more under scotopic than under photopic conditions. In terms of the effects of voriconazole on each waveform component, no apparent attenuation of the a-wave was observed in either the scotopic ERG (i.e., the combined rod-cone response) or the photopic ERG (i.e., the single-flash cone response). Meanwhile in the b-wave, a noticeable and reversible reduction was observed in the scotopic ERGs (i.e., the rod response and the combined rod-cone response) and the photopic ERGs (i.e., the single-flash cone response and the 30 Hz flicker). These findings in the scotopic and photopic ERGs suggested that the functions of both the rod and cone photoreceptors were relatively preserved, but those of the bipolar cells in both the rod and cone pathways were reversibly affected in monkeys receiving voriconazole.

Then, to make a more in-depth investigation into the functional effects of voriconazole on the retinal bipolar cells, the photopic ERG elicited by a long-duration stimulus (ON-OFF response) was evaluated in voriconazole-treated monkeys. The ON-OFF response was also recorded in monkeys treated with APB, which is known to block neurotransmission from the photoreceptors to the ON-bipolar cells.10 We found that the change of waveform in the voriconazole-treated monkeys was very similar to that in APB-treated monkeys: the descending phase of the a-wave was not affected; the b-wave was eliminated or prominently attenuated; and the d-wave appeared to be unchanged. Origins of the a-, b- and d-waves in the photopic ERG from Macaca monkeys have been shown to be as follows: the a-wave is generated from the OFF-pathway postsynaptic to the cone photoreceptors as well as from the cone photoreceptors themselves8; the activity of the retinal ON-bipolar cells is a requisite for the b-wave production6; and the d-wave originates from the activity of both the cone photoreceptors and the OFF-pathway postsynaptic to the cone photoreceptors.12 Thus, it was suggested from the changes of the ON-OFF response in monkeys in this study that the function of the ON-bipolar cells in the cone pathway was predominantly affected, but those of the cone photoreceptors and the OFF-bipolar cells in the cone pathway were preserved in monkeys treated with voriconazole. Meanwhile in the rod pathway, the rod photoreceptors in mammals are thought to synapse with a single type of bipolar cells, which depolarize after light stimulation (i.e., the ON-bipolar cells).13 Therefore, from the results of the electrophysiological evaluation of the retinal function in monkeys in this study, it was strongly suggested that voriconazole has the potential to induce selective and reversible dysfunction of the retinal ON-bipolar cells in both the rod and cone pathways.

In addition, we have also found that the ERG changes in voriconazole-treated monkeys in this study were quite similar to those reported in human patients with the complete-type of congenital stationary night blindness (cCSNB). cCSNB is a non-progressive retinal disease characterized by congenital night blindness with very characteristic changes in five types of ERG14,15: a single bright-flash ERG with a normal a-wave and...
extremely reduced b-wave (negative-shaped ERG); a rod ERG without the b-wave (nonrecordable ERG); a nearly normal cone ERG with flattened appearance in the bottom of the a-wave and mild attenuation in the b-wave; a nearly normal 30-Hz flicker ERG with mildly attenuated b-wave; and, a long-flash photopic ERG with significantly small b-wave and normal d-wave. The defect in the neural pathway of cCSNB patients is generally considered to lie in the signal transmission from the photoreceptors to the ON-bipolar cells or the ON-bipolar cells themselves in both the rod and cone pathways.\textsuperscript{16,17} Hence, these pronounced similarities in the waveform of the ERGs between voriconazole-treated monkeys and cCSNB patients strongly supports our idea that voriconazole selectively acts on the retinal ON-bipolar cells.

However, the detailed mechanisms of how voriconazole acts on the retinal ON-bipolar cells remain unclear. Fluconazole and itraconazole are triazole antifungal agents as is voriconazole. As far as we know, fluconazole- or itraconazole-induced visual disturbances have not been identified in humans, and no effects of triazole antifungal agents on retinal function has been reported either in humans or animals. Therefore, it is not likely that the voriconazole-induced dysfunction of the retinal ON-bipolar cells is related to the mechanism of antifungal action of the drug (i.e., inhibition of fungal 14α-demethylase). To date, three mutated genes have been identified in cCSNB patients: NYX gene\textsuperscript{18,19} which encodes nyctalopin, a leucine-rich proteoglycan; GRM6 gene\textsuperscript{20,21} which encodes the metabotropic glutamate receptor 6 (mGluR6); and TRPM1 gene\textsuperscript{22–25} which encodes the transient receptor potential cation channel, subfamily M, member 1 (TRPM1). mGluR6 and TRPM1 have been shown to colocalize on the dendrites of retinal ON-bipolar cells and to be required for signal transmission from the photoreceptors to the ON-bipolar cells in mice.\textsuperscript{26,27}suggesting that one of these molecules, expressed specifically on retinal ON-bipolar cells, might be the target of voriconazole. Further studies will be required to elucidate the molecular mechanism of the voriconazole-induced transient visual disturbances.

We have found that voriconazole exerted very rapid APB-like effects on retinal function after intravenous injection in monkeys. To our knowledge, this report represents the first description of quite rapid and specific drug effects on particular retinal neurons after systemic administration. In conclusion, our results strongly suggested that voriconazole induced selective and reversible dysfunction of the retinal ON-bipolar cells in both the rod and cone pathways in monkeys. From the results obtained in monkeys in this study, it is suggested that the function of the retinal ON-bipolar cells was selectively and reversibly affected in voriconazole-treated humans who complained of transient visual disturbances.

Acknowledgments

The authors thank Naozumi Samata of Daiichi-Sankyo RD Associe Co., Ltd., for measurement of plasma voriconazole concentration.

References

26. Morgans CW, Zhang J, Jeffrey BG, et al. TRPM1 is required for the dendrites of retinal ON-bipolar cells and to be required for signal transmission from the photoreceptors to the ON-bipolar cells in mice.\textsuperscript{26,27}suggesting that one of these molecules, expressed specifically on retinal ON-bipolar cells, might be the target of voriconazole. Further studies will be required to elucidate the molecular mechanism of the voriconazole-induced transient visual disturbances.

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