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. (*Invest Ophthalmol Vis Sci.* 2011;52:5058–5063) DOI:10.1167/iovs.11-7183

Voriconazole is a triazole antifungal agent with potent activity against a broad spectrum of clinically significant pathogens.[1–3] Voriconazole has been generally well tolerated in clinical trials[4] and postmarketing surveillances[5–7] with frequently reported adverse events of transient visual disturbances, which are described as enhanced/altered light perception, photopsia, photophobia, blurred vision, or color vision changes without any abnormality in the fundus oculi. Very few studies have focused on the detailed effect of voriconazole on retinal function, although the retina is generally considered to be the site of the visual disturbances because reversible decreases in the amplitude of the electroretinogram (ERG) were noted in voriconazole-treated humans.[4]

Therefore, the purpose of this study was to investigate the mechanism of the voriconazole-induced transient visual disturbances that occur in humans. For this purpose, we electrophysiologically assessed the retinal function after administration of voriconazole to monkeys, a species in which the anatomic structure of the eye is widely known to be similar to that in humans.

Methods

Animals

A total of ten cynomolgus monkeys (*Macaca fascicularis*) between three and eight years of age were used in this study. The animals were housed individually in stainless steel cages in an animal study room where the environmental condition was set as follows: room temperature, 24°C; relative humidity, 60%; illumination, 12-hour lighting (7:00 to 19:00) at 150 to 300 luces. The animals were fed 100 g per animal per day of pellet food for monkeys (PS; Oriental Yeast Co., Ltd., Tokyo, Japan). Tap water from a feed-water nozzle was supplied ad libitum to the animals. All experimental procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Institutional Animal Care Committee of Daiichi-Sankyo Co. Ltd.

Drug Administration

Voriconazole (VFEND for Intravenous Use; Pfizer Inc., New York, NY) was dissolved in physiologic saline. The dose formulation was administered intravenously at a rate of 0.2 mL/kg per minute for ten minutes to six animals with increasing doses of 0, 3, 6 and 12 mg/kg at intervals of one week or more, and the standard full-field ERGs were recorded as described below. Several months after the 12 mg/kg dosing, voriconazole was administered to three animals at 0 mg/kg and to another three animals at 6 mg/kg in the same manner, and the photopic ERG elicited by a long-duration stimulus (the ON-OFF response) was recorded as described below.

Intravitreal injection of 2-amino-4-phosphonobutyric acid (APB) (Sigma-Aldrich; St. Louis, MO) was also conducted in two animals several weeks after the last dosing of voriconazole mentioned above. The techniques for intravitreal injection have been described in detail elsewhere.[8] In brief, APB was dissolved in sterile physiologic saline at the concentration of 40 mM, and 0.05 mL of the APB solution was injected into the vitreous of the right eye with a 30-gauge needle inserted through the pars plana approximately 4 mm posterior to the

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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% oxybuprocaine 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 6.3 cd·s/m^2. 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).[9] 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/m^2, respectively. Subsequently, after 10 minutes of light adaptation at 40 cd/m^2, the single-flash cone response and the 30 Hz flicker were elicited by light flashes at an intensity of 2.7 cd·s/m^2 under a background light at 40 cd/m^2. 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 femoral 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/m^2 under a background light at 32 cd/m^2. 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.

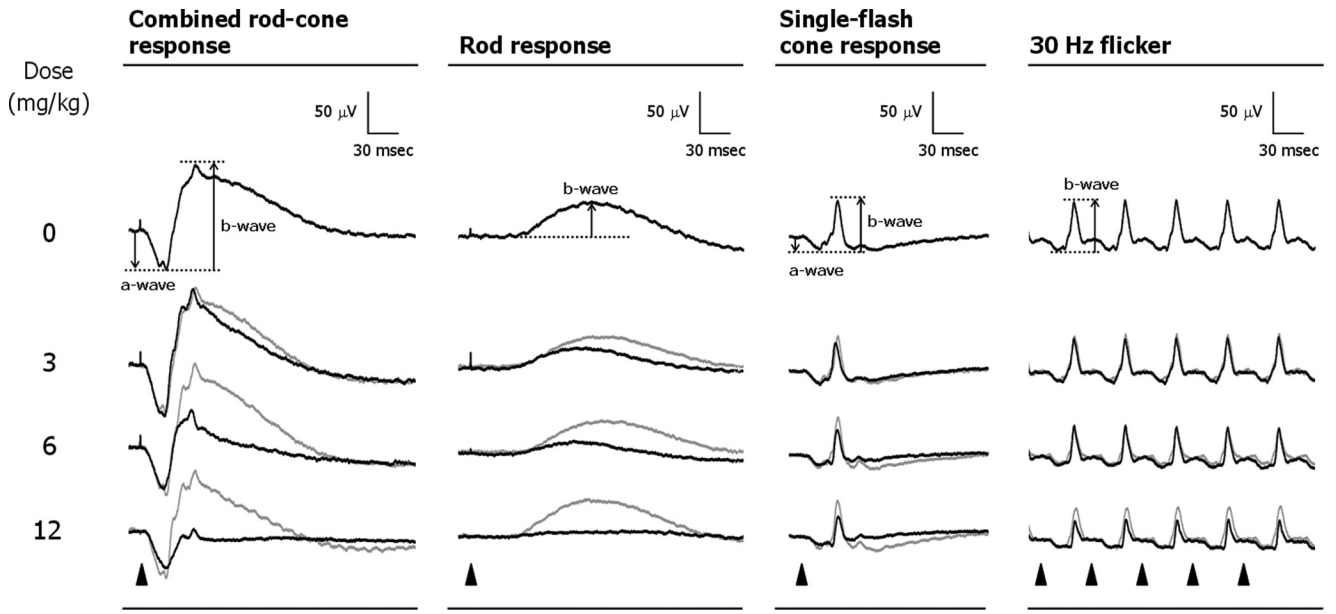

Figure 1. Typical waveforms of the standard full-field ERGs in a voriconazole-treated monkey. Voriconazole was administered intravenously with increasing doses of 0 (vehicle), 3, 6, and 12 mg/kg at intervals of one week or more, and the standard full-field ERGs were obtained as described in the text. *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.

TABLE 1. Effects of Voriconazole on the Amplitude of the Standard Full-field ERGs in Monkeys

	Dose (mg/kg)						
	0*	3		6		12	
	Immediately After	Immediately After	24 h After	Immediately After	24 h After	Immediately After	24 h After
Standard Rod-Cone Response, Amplitude (μV)							
a-Wave	52.8 ± 9.10	49.0 ± 8.97	51.0 ± 7.50	45.4 ± 8.77	54.8 ± 6.53	46.1 ± 12.40	63.7 ± 8.50
b-Wave	123.5 ± 24.93	121.9 ± 22.94	126.1 ± 21.81	89.9 ± 14.79	132.5 ± 19.17	60.5 ± 13.88†	135.7 ± 21.41
b/a Wave ratio	2.4 ± 0.34	2.5 ± 0.28	2.5 ± 0.22	2.0 ± 0.45	2.4 ± 0.31	1.3 ± 0.16†	2.1 ± 0.15
Rod Response, Amplitude (μV)							
b-Wave	43.9 ± 9.97	34.2 ± 13.80‡	44.8 ± 13.93	16.5 ± 6.47†	47.7 ± 8.23	9.7 ± 5.73†	40.8 ± 12.03
Single-Flash Cone Response, Amplitude (μV)							
a-Wave	12.7 ± 1.81	12.9 ± 2.36	12.7 ± 1.21	11.1 ± 2.34	13.5 ± 2.22	11.0 ± 2.67	14.6 ± 2.31
b-Wave	53.8 ± 6.63	47.7 ± 7.17	55.3 ± 5.42	41.3 ± 5.77†	60.3 ± 11.23	33.0 ± 5.11†	64.2 ± 12.23
30 Hz Flicker, Amplitude (μV)							
b-Wave	52.4 ± 6.62	48.1 ± 8.04	55.3 ± 3.80	45.6 ± 6.08‡	59.6 ± 12.32	35.3 ± 5.09†	58.1 ± 11.89

Data are expressed as the mean ± SD of six animals. All animals were treated with low to high doses at intervals of one week or more.

* Physiologic saline was administered as a vehicle control.

† $P < 0.01$; ‡ $P < 0.05$, significantly different in comparison with the vehicle-control value by the paired *t*-test.

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.

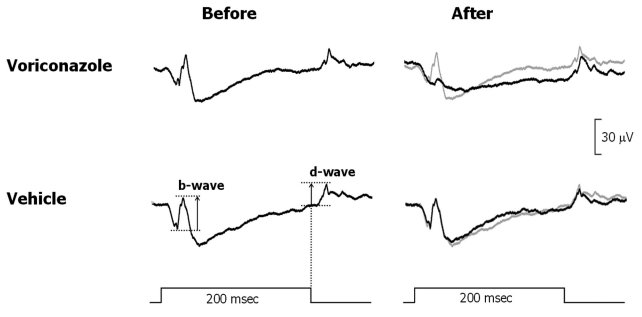

FIGURE 2. Typical waveforms of the ON-OFF response in voriconazole- and vehicle-treated monkeys. Voriconazole at a dose of 6 mg/kg or vehicle was administered intravenously, and the ON-OFF responses were recorded as described in the text. The responses obtained before dosing (*gray trace*) are superimposed on those obtained after dosing (*black trace*) in the right column. Each trace represents an average of six responses.

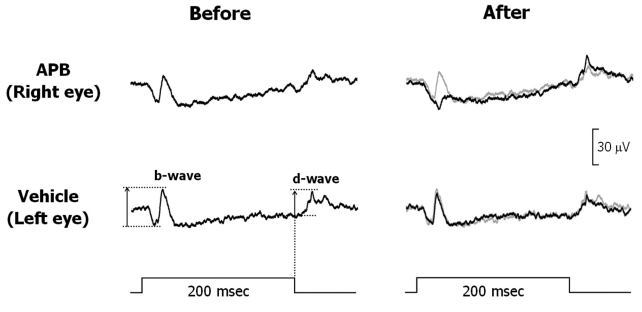

FIGURE 3. Typical waveforms of the ON-OFF response from APB- and vehicle-treated eyes in a monkey. APB and vehicle were injected into the right and left eyes, respectively, of the animal, and the ON-OFF responses were recorded as described in the text. The responses obtained before injection (*gray trace*) are superimposed on those obtained after injection (*black trace*) in the right column. Each trace represents an average of six responses.

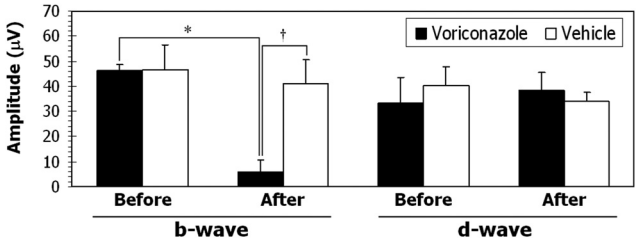

FIGURE 4. The effect of voriconazole on the b- and d-waves amplitude of the ON-OFF response in monkeys. The ON-OFF responses were recorded immediately before and after intravenous dosing of vehicle (*white column*) or 6 mg/kg voriconazole (*black column*). Data are expressed as mean + SD of three animals. 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 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 ($P < 0.05$ at 3 mg/kg, $P < 0.01$ at 6 and 12 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 ($P < 0.01$ at 6 and 12 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 ($P < 0.05$ at 6 mg/kg, $P < 0.01$ at 12 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 voriconazole- and APB-treated monkeys are shown in Figures 2 and 3, respectively.

The descending phase of the a-wave appeared not to be affected in either voriconazole- or APB-treated monkeys. In contrast, elimination or prominent attenuation of the b-wave was observed in both voriconazole- and APB-treated monkeys. The decrease in the b-wave amplitude immediately after the dosing of voriconazole was significant ($P < 0.01$) in comparison 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 μg/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 μg/mL), 0.07 ± 0.064 μg/mL, and 0.61 ± 0.427 μg/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 μg/mL (Table 2). $AUC_{0-24\ h}$, C_{max} and t_{max} after single intravenous administration of 6 mg/kg voriconazole in the same animals as those used for obtaining the ON-OFF response mentioned above were 37.60 ± 6.239 μg·h/mL, 5.62 ± 1.271 μg/mL, and 0.28 ± 0.192 hour, respectively (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.[4] It has also been reported that the visual adverse events were related to the plasma voriconazole concentration in phase 2 and 3 therapeutic trials with voriconazole.[6] During the first week of voriconazole administration, the median plasma voriconazole concentration for patients experiencing visual adverse events was 3.52 μg/mL in those trials. Therefore, the time of occurrence of the apparent alterations in the ERGs in voriconazole-treated monkeys in this study (16 to 55 minutes after the start of dosing) basically corresponded to that of the transient visual disturbances in humans receiving voriconazole. In addition, the plasma voriconazole concentration at the time of the changes of the ERGs in monkeys receiving 6 mg/kg of voriconazole in this study (3.35 ± 0.155 and 4.24 ± 2.610 μg/mL for the standard full-field ERG and the ON-OFF response, respectively) were basically comparable with those in humans experiencing voriconazole-induced transient visual disturbances. It was also reported that three out of nine human 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 AUC_τ and C_{max} of the nine subjects were 13.2 μg·h/mL and 4.70 μg/mL, respectively.[5] Therefore, the sys-

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

	Dose (mg/kg)	Number of Animals	Time Point (After Dosing)	Concentration (μg/mL)
Standard Full-Field ERGs	3	6	Immediately	1.33 ± 0.106
			24 h	NC*
	6	6	Immediately	3.35 ± 0.155
			24 h	0.07 ± 0.064
	12	6	Immediately	7.13 ± 0.415
			24 h	0.61 ± 0.427
ON-OFF Response	6	3	Immediately	4.24 ± 2.610

Data are expressed as mean ± SD. NC, not calculated.
* Five individual values were below the lower limit of quantitation (0.01 μg/mL).

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

Dose (mg/kg)	Number of Animals	Plasma Concentration (μg/mL)							$AUC_{0-24\ h}$ (μg · h/mL)	C_{max} (μg/mL)	t_{max} (h)
		Time After the Start of 10-min infusion									
		Pre	10 min	30 min	1 h	2 h	4 h	7 h	24 h		
6	3	NC*	5.42 ± 1.616	4.61 ± 0.862	3.74 ± 0.722	3.62 ± 0.442	2.89 ± 0.291	1.79 ± 0.391	0.05 ± 0.039	37.60 ± 6.239	5.62 ± 1.271	0.28 ± 0.192

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

* Individual values were below the lower limit of quantitation (0.01 μg/mL).

temic exposure to voriconazole in monkeys in this study substantially exceeded that reported in humans experiencing the voriconazole-induced transient visual disturbances. The plasma voriconazole concentration in monkeys 24 hours after dosing, when the waveforms of the standard full-field ERGs were basically equivalent to those recorded after vehicle dosing, was near the lower limit of quantitation, indicating that the ERG changes in voriconazole-treated monkeys depended on the plasma voriconazole concentration and were reversible. This reversibility of the changes in monkeys was considered to correspond to the transient manner of the voriconazole-induced visual disturbances in humans.

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 themselves[11]; the activity of the retinal ON-bipolar cells is a requisite for the b-wave production[8]; 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 nonprogressive retinal disease characterized by congenital night blindness with very characteristic changes in five types of ERG[14,15]: 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.[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[18,19] which encodes nyctalopin, a leucine-rich proteoglycan; GRM6 gene[20,21] which encodes the metabotropic glutamate receptor 6 (mGluR6); and TRPM1 gene[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,[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.

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References

1. Cuenca-Estrella M, Rodríguez-Tudela JL, Mellado E, Martínez-Suárez JV, Monzón A. Comparison of the in-vitro activity of voriconazole (UK-109,496), itraconazole and amphotericin B against clinical isolates of Aspergillus fumigatus. *J Antimicrob Chemother.* 1998; 42:531–533.
2. Espinel-Ingroff A. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. *J Clin Microbiol.* 1998;36:198–202.
3. Chandrasekar PH, Manavathu E. Voriconazole: A second-generation triazole. *Drugs Today (Barc).* 2001;37:135–148.
4. Ghannoum MA, Kuhn DM. Voriconazole - better chances for patients with invasive mycoses. *Eur J Med Res.* 2002;31:242–256.
5. Purkins L, Wood N, Greenhalgh K, Eve MD, Oliver SD, Nichols D. The pharmacokinetics and safety of intravenous voriconazole - a novel wide-spectrum antifungal agent. *Br J Clin Pharmacol.* 2003;56:2–9.
6. Tan K, Brayshaw N, Tomaszewski K, Troke P, Wood N. Investigation of the potential relationships between plasma voriconazole concentrations and visual adverse events or liver function test abnormalities. *J Clin Pharmacol.* 2006;46:235–243.
7. Eiden C, Peyrière H, Cociglio M, et al. Adverse effects of voriconazole: analysis of the French Pharmacovigilance Database. *Ann Pharmacother.* 2007;41:755–763.
8. Sieving PA, Murayama K, Naarendorp F. Push-pull model of the primate photopic electroretinogram: a role for hyperpolarizing neurons in shaping the b-wave. *Vis Neurosci.* 1994;11:519–532.
9. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M. ISCEV standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol.* 2008;118:69–77.
10. Slaughter MM, Miller RF. 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science.* 1981;211:182–185.
11. Bush RA, Sieving PA. A proximal retinal component in the primate photopic ERG a-wave. *Invest Ophthalmol Vis Sci.* 1994;35:635–645.
12. Ueno S, Kondo M, Ueno M, Miyata K, Terasaki H, Miyake Y. Contribution of retinal neurons to d-wave of primate photopic electroretinograms. *Vision Res.* 2006;46:658–664.
13. Wässle H, Boycott BB. Functional architecture of the mammalian retina. *Physiol Rev.* 1991;71:447–480.
14. Miyake Y, Yagasaki K, Horiguchi M, Kawase Y, Kanda T. Congenital stationary night blindness with negative electroretinogram. A new classification. *Arch Ophthalmol.* 1986;104:1013–1020.
15. Miyake Y, Yagasaki K, Horiguchi M, Kawase Y. On- and off-responses in photopic electroretinogram in complete and incomplete types of congenital stationary night blindness. *Jpn J Ophthalmol.* 1987;31:81–87.
16. Sieving PA. Photopic ON- and OFF-pathway abnormalities in retinal dystrophies. *Trans Am Ophthalmol Soc.* 1993;91:701–773.
17. Miyake Y. Establishment of the concept of new clinical entities–complete and incomplete form of congenital stationary night blindness [in Japanese]. *Nippon Ganka Gakkai Zasshi.* 2002;106:737–755.
18. Bech-Hansen NT, Naylor MJ, Maybaum TA, et al. Mutations in NYX, encoding the leucine-rich proteoglycan nyctalopin, cause X-linked complete congenital stationary night blindness. *Nat Genet.* 2000; 26:319–323.
19. Pusch CM, Zeitz C, Brandau O, et al. The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein. *Nat Genet.* 2000;26:324–327.
20. Dryja TP, McGee TL, Berson EL, et al. Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. *Proc Natl Acad Sci USA.* 2005;102:4884–4889.
21. Zeitz C, van Genderen M, Neidhardt J, et al. Mutations in GRM6 cause autosomal recessive congenital stationary night blindness with a distinctive scotopic 15-Hz flicker electroretinogram. *Invest Ophthalmol Vis Sci.* 2005;46:4328–4335.
22. Audo I, Kohl S, Leroy BP, et al. TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet.* 2009;85:720–729.
23. van Genderen MM, Bijveld MM, Claassen YB, et al. Mutations in TRPM1 are a common cause of complete congenital stationary night blindness. *Am J Hum Genet.* 2009;85:730–736.
24. Li Z, Sergouniotis PI, Michaelides M, et al. Recessive mutations of the gene TRPM1 abrogate ON bipolar cell function and cause complete congenital stationary night blindness in humans. *Am J Hum Genet.* 2009;85:711–719.
25. Nakamura M, Sanuki R, Yasuma TR, et al. TRPM1 mutations are associated with the complete form of congenital stationary night blindness. *Mol Vis.* 2010;16:425–437.
26. Morgans CW, Zhang J, Jeffrey BG, et al. TRPM1 is required for the depolarizing light response in retinal ON-bipolar cells. *Proc Natl Acad Sci USA.* 2009;106:19174–19178.
27. Koike C, Obara T, Uriu Y, et al. TRPM1 is a component of the retinal ON bipolar cell transduction channel in the mGluR6 cascade. *Proc Natl Acad Sci USA.* 2010;107:332–327.