Functional Abnormalities in Vincristine-Induced Night Blindness

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Various noninvasive test procedures were used to evaluate retinal function in a patient who had become night blind following vincristine chemotherapy. The results obtained were strikingly similar to those reported previously in subjects with recessively inherited stationary night blindness; the dark-adaptation curve was monophasic (ie, no evidence of a scotopic branch), rhodopsin kinetics were entirely normal, and spectral threshold data revealed the presence of residual rod-mediated vision. Also like the heritable condition, the b-wave of the ERG was depressed grossly despite normal a-wave potentials. These findings, and the fact that vincristine is known to disrupt the structural integrity of neuronal microtubules, suggest that the drug-induced defect involves the process of synaptic transmission between the photoreceptors and their second-order neurons. Invest Ophthalmol Vis Sci 25:787–794, 1984

Extensive clinical evaluation has shown that several alkaloids isolated from the periwinkle plant, Vinca rosea Linn, are therapeutically effective against a broad range of malignancies.1,2 Vincristine, one of the more potent of these agents, is a complex dimeric alkaloid whose primary therapeutic action results from inactivation of the mitotic spindle and the blockage of mitosis at the stage of metaphase.3 However, its usefulness is often limited by debilitating side-effects. Although the neuromuscular apparatus appears to be particularly susceptible,4 ocular toxicity is not uncommon, and there are numerous reports indicating that the Vinca alkaloids can induce a variety of ocular abnormalities.5

In this paper, we report a case of night blindness associated with vincristine chemotherapy. Of particular interest were the results obtained with photochemical, electrophysiologic, and subjective test procedures, which revealed striking similarities between the drug-induced visual anomalies and those seen in a recessively inherited night-blinding disorder we had studied previously.6,7

Materials and Methods

Case History

The patient (PDF), a 30-year-old male Caucasian, was referred to the Retinal Clinic at New York University in April 1981 for evaluation because of poor night vision that he became aware of 1 year earlier. He also complained of an intermittent sensation of “flickering” or “pulsating” lights that could occur at any time of day. In all other respects his vision seemed normal, and he claimed to have no ocular problems prior to the onset of the night vision defect.

The patient's medical records showed that in October 1979 a malignant melanoma was detected on his neck, and in November of that year he was placed on a 5-day regimen of chemotherapy that consisted of Da-carbazine (DTIC-Dome), 50 mg/kg on days 1 through 5; 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-urea (CCNU-Bristol), 1.6 mg/kg and bleomycin sulfate (Blenoxane-Bristol), 0.2 mg/kg on days 1 and 4; and vincristine sulfate (Oncovin-Lilly), 0.032 mg/kg on days 1 and 5. During the course of therapy the patient experienced alopecia, vertigo, nausea, vomiting, and severe abdominal pain with alternating constipation and diarrhea that persisted for several weeks after medication was withdrawn. The melanoma progressed rapidly in extent, and 6 weeks later the patient had a second course of chemotherapy. The side-effects noted above were even more severe and persisted longer than previously, but therapy was followed by a gradual reduction in the size of the tumor. However, by May 1980 the patient noted difficulty adjusting to dim room lights, and he soon became aware of an inability to move about at night. Liver function tests were normal and vitamin A therapy (125,000 IU/day) was without

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Supported by research grants EY-00283 and EY-02179, and a research center award (EY-01842) from the National Eye Institute (NIH), U.S. Public Health Service, by the National Retinitis Pigmentosa Foundation, and by an unrestricted award from Research to Prevent Blindness, Inc.

Submitted for publication: July 8, 1983.

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effect. He had no difficulty in daylight, and his visual acuity did not seem to be affected during this period. The fact that the patient had developed night blindness was confirmed by dark adaptometry (see Results).

Because the patient's symptoms and the results of our subsequent test procedures approximated so closely those seen in hereditary stationary night blindness, he and his wife were questioned intensively with regard to his night vision prior to the time that chemotherapy was initiated. Particularly noteworthy in his past history was the fact that he had graduated in 1972 from flight school at the Air Force Academy in Colorado Springs. While there, he participated in night-training exercises in wooded areas and flew and landed in planes at night, all without visual difficulties. Such evidence left little doubt that until the time he experienced his current symptoms, his night vision was normal.

Preliminary Examination

An extensive routine ophthalmological examination was uneventful. The patient was emmetropic in both eyes, the ophthalmoscopic appearance of his fundus was normal, and uncorrected visual acuity was 20/20 in each eye. Visual fields on the Goldmann perimeter were normal to a III-4 (white) test light, and to the same target size reduced in intensity by 1 log unit. Color vision was normal with both the H-R-R pseudoisochromatic plates and the Farnsworth D-15 panel.

Dark Adaptometry

Thresholds during dark adaptation were determined monocularly in the mid-peripheral retina (15 degrees temporal to the fovea) on a modified Goldmann-Weekers Adaptometer after a 7-min preadapting exposure to a large opal glass field whose luminance was $1.3 \times 10^3$ ft. lambert; ie, the retinal illumination for the patient's dilated (1% cyclopentolate hydrochloride) pupil was 5.22 log scot td. Test flashes, 1 sec in duration, were presented at intervals > 15 sec and were attenuated by a calibrated neutral density wedge interposed between the tungsten source and the uniformly illuminated opal test field; the latter subtended 1.25 degrees of visual angle. Following adaptometry, the fixation point was moved in orthogonal directions with respect to the test field in order to obtain a threshold profile of the dark-adapted retina.

Fundus Reflectometry

The instrumentation, procedures and principles of measurement for computerized rapid fundus reflectometry have been described previously.8,9 In brief, absorbance difference spectra ($\Delta D_\lambda$, for $\lambda = 410$ to 690 nm) were derived from fundus reflection measurements obtained under the following conditions of retinal adaptation: (1) after 1 hr of dark adaptation; (2) after a 30-sec photic exposure (Wratten 15; 6.24 log scot td) that bleached more than 98% of the available rhodopsin; and (3) at various times after the bleaching light was extinguished. The absorbance differences obtained between conditions (1) and (2) provide an estimate of the in situ density of rhodopsin, whereas the time-dependent increases in absorbance between conditions (2) and (3) are used in determining the rate at which the bleached rhodopsin is regenerated. On a few occasions, the experimental protocol was modified to include 10-sec exposures to weaker bleaching fields. The resultant difference spectra gave the variation in the fraction of rhodopsin bleached as a function of retinal illuminance; the data were used in deriving the relative photosensitivity of rhodopsin. The reflectivity measurements were made in the temporal retina (15 degrees from the fovea) with a circular test field that subtended a visual angle of 2 degrees; the bleaching field was concentric with the test area but had an angular subtense of 4 degrees, 30 minutes.

Electroretinography

Electroretinal potentials were elicited with 10-μsec flashes from the xenon discharge lamp of a Grass photic stimulator. The lamp was shielded from the patient's view and illuminated the interior of a diffusely reflecting hemisphere that provided a ganzfeld stimulus. The spectral composition and intensity of the flash were controlled by Wratten colored and neutral density filters. Various stimulus conditions were employed in order to alter the adaptive state of the eye and elicit responses from either the scotopic or photopic mechanism. The electroretinogram (ERG) was recorded as the potential change between a contact lens electrode (Burian-Allen) and a reference electrode located on the patient's forehead. The responses were amplified, and photographed from the screen of a cathode-ray oscilloscope. The frequency response of the system was set for 3 db attenuation at 0.6 and 300 Hz for conventional electroretinography, but the time constant of the amplifier was increased to 3 sec when we attempted to obtain a recording of the c-wave potential. Although DC recording is desirable in these circumstances to avoid distortion of the response waveform, the offset potential at the recording electrodes was too great for the available bucking voltage.

Electrooculography

Chlorided-silver disc electrodes were affixed near the nasal and temporal canthi of each eye, and the subject was asked to track visually a moving light display. The
latter consisted of 19 light-emitting diodes (LEDs), sinusoidally spaced in the horizontal plane and sequentially pulsed so as to appear to move back and forth in a pendular motion with a frequency of about 1/sec. Behind the strip of LEDs, an opal glass plate trans-illuminated by a battery of fluorescent lamps provided the high levels of retinal illuminance (~10,000 scot td) required to elicit a maximum response in the eye’s standing potential. The voltages generated across each pair of electrodes were amplified and recorded on an ink-writing polygraph. Recordings were obtained each minute during a 15-min dark period and during 15 min of exposure to the steady light-adapting background. The largest potential measured in the light (light peak) divided by the lowest potential measured in darkness (dark trough) provides a measure of the light:dark ratio; for the normal observer of the patient’s age, L/D > 1.8.

Spectral Sensitivity

Thresholds were measured with a dual beam photostimulator for seven spectral regions isolated by narrow-band interference filters (half bandwidth ~ 9 nm). A dim red fixation spot was positioned so as to center the 1 degree, 50 minute test field at 15 degrees in the temporal retina, the same retinal locus examined by dark adaptometry and fundus reflectometry. Test flashes, 0.5 sec in duration, were delivered to the fully dark adapted eye at 5-sec intervals. Thresholds were determined by a modified method of limits, with flash intensity increasing from below threshold to the level at which two consecutive presentations were visible. Data were obtained during two experimental sessions, each consisting of four threshold measurements at the various test wavelengths; the order of presentation of test wavelengths was random. The energy E transmitted by the interference filters was determined with a calibrated EG & G radiometer (Salem, MA), and threshold data were converted to relative quantum intensities. The data were plotted as log relative quantum sensitivity, where a value of zero represents the maximum sensitivity of the normal dark adapted eye at 505 nm.

Results

Dark Adaptation

The results of dark adaptometry for PDF are shown in Figure 1 where the data are compared with the normal function for equivalent test conditions, and with the findings reported previously6 for a patient (RC) with recessively inherited night blindness.

In the normal, the dark-adaptation curve is bipartite, the early and late branches representing threshold changes associated with the cone and rod mechanisms, respectively. For PDF, however, the data show only a small kink after about 3 min in darkness, but thereafter describe a monophasic curve that remains above the cone plateau of the normal function, and more than 3 log units above the absolute threshold of the normal scotopic mechanism. A very similar description characterizes the findings obtained in the hereditary night blind, although thresholds throughout the course of dark adaptation are lower due primarily to the use of a larger test field in that study.

It is also of interest to note that cone sensitivity is reduced in both the acquired and inherited forms of nyctalopia. This is not a unique finding in night-blinding disorders and indicates that photopic as well as scotopic function may be affected despite the absence of patient symptoms related to cone-mediated vision. Data obtained at other loci in the midperipheral retina of the two eyes, ie, retinal profiles (not illustrated), showed comparable elevation of thresholds.

Fundus Reflectometry

Figure 2 shows difference spectra measured at 15 degrees in the temporal retina using a 2-degree test field that fell within the area examined previously by dark adaptometry. Absorbance changes induced by exposing the fully dark-adapted retina to the intense yellow light are plotted as negative values to indicate the density losses due to bleaching. The retinal ir-radiance of the 30-sec exposure (I_B = 7.72 log scot td-sec) was sufficient to bleach more than 98% of the available rhodopsin in the test area. The peak of the difference spectrum (Δmax) at 510 nm indicates that the absorbance changes are due primarily to the bleaching of rhodopsin, whereas the magnitude of the density difference at 510 nm (ΔDmax = 0.142) lies at the high end of the normal range for this retinal locus.9
Following the bleaching exposure, spectral recordings were obtained at various times during a 40-min period of dark adaptation. The results of several representative difference spectra are graphed in Figure 2 (filled circles) where it is apparent that the rhodopsin content of the retina is almost fully restored during the first 25 min of dark-adaptation. The shift of the \( \lambda_{\text{max}} \) with time in darkness, ie, from about 485 nm at the 1-min mark to about 510 nm for \( t \geq 7 \) min, has been shown to be due to short-wavelength absorbing photoproducts that form and decay while the rhodopsin content of the retina is replenished slowly.\(^{10-12}\)

A reasonable estimate of the rate at which rhodopsin is regenerated can be derived from the time-dependent changes in density at 530 nm. These data were fit satisfactorily by an exponential with time constant (\( \tau \)) of 6 min. Thus, the time course of rhodopsin regeneration for the patient is similar to that obtained with hereditary night-blind subjects,\(^6,13\) and in all cases, the kinetics are within the normal range (\( \tau = 4 \) to 6.7 min).\(^{11,14}\) In addition, difference spectra for a range of exposure intensities provided data from which to estimate the photosensitivity of rhodopsin; ie, the retinal exposure \( E_c \) that bleaches \( 1-e^{-1} \) of the available rhodopsin. The results indicated that \( E_c = 6.92 \) log td-sec, a value not significantly different from that obtained in studies on the normal retina.\(^{14,15}\)

**Spectral Sensitivity**

We had found previously that despite the severe depression of rod-mediated vision in recessively inherited night blindness, short-wavelength stimuli gave rise to threshold responses that paralleled the scotopic spectral sensitivity function of normal observers; with longer wavelengths, visual sensitivity was subserved by photopic mechanisms.

A similar result was obtained with our patient. As shown by the spectral curves of Figure 3, visual sensitivity was maximal at 500 nm, but only the data points for \( \lambda \leq 540 \) nm are adequately fit by the CIE scotopic spectral sensitivity curve that describes the data for normal, dark-adapted observers. Moreover, sensitivity in this spectral region is reduced by more than 3 log units relative to the normal function.

For wavelengths > 540 nm, the CIE scotopic curve lies well below the experimental data, suggesting that vision is mediated by the cone mechanism; the fact that these data are fit by Wald's photopic sensitivity curve for the paracentral retina\(^{16}\) is consistent with this view. But here, too, the relative sensitivity of the photopic system is subnormal, a point stressed earlier in connection with the results of dark adaptometry.
The Electroretinogram

Figure 4A compares the patient's ERG responses with those of a normal subject; the potentials were recorded from the dark-adapted eye in response to brief flashes of white light of increasing intensity. In the normal subject even the dimmer flashes (log $I_t \leq -1.5$) are well above threshold and evoke large cornea-positive (b-wave) potentials which are preceded by small, barely detectable negative (a-wave) deflections; both components grow in amplitude as flash intensity is increased. For PDF, on the other hand, the a-wave is the major potential elicited by photic stimulation, and its amplitude increases with stimulus intensity in the normal way. However, with the brighter test stimuli (log $I_t \geq -1.0$), the a-wave is followed by small positive oscillations that closely resemble those recorded by Hill et al. in their study of hereditary nyctalopia and attributed by them to the photopic system.

Although it is reasonable to suppose that the a-wave potentials of the dark-adapted retina in both the normal and PDF derive from both rod and cone photoreceptor, the question arises as to whether the rod contribution is normal in the responses of the night-blind subject. We attempted to determine whether the rod cells of PDF generate a normal receptor potential by analyzing the ERG responses to selected regions of the visible spectrum. Since a-waves could not be elicited with the ganzfeld stimulus using narrow-band interference filters (particularly from the light-adapted retina), broad-band filters (Wratten) were selected to cover the range of the visible spectrum. The ERG recordings show that in the dark-adapted retina (Fig. 5, left column), the responses to chromatic lights are similar in form to those obtained with white-light stimuli. However, despite the limited intensity range over which the a-wave was recordable, a graph of the voltage as a function of log intensity (not shown) for each of the chromatic filters revealed that the responses are not spectrally invariant; ie, they are not subserved by a single photochemical mechanism. Without more detailed action spectra, it is not possible to identify with certainty the underlying mechanisms, but it seems likely that with the shorter wavelengths the responses are rod dominated, whereas with the longer wavelengths the cones predominate. This is borne out by the recordings of Figure 5 (right column), which demonstrate that light adaptation depressed the a-wave responses to blue and green stimuli to a greater degree than those evoked by orange and red stimuli; eg, the background illumination completely extinguished the response to blue light and produced a sevenfold reduction in the a-wave response to the green flash, but caused only a two-to-threefold decrease in the response to the longer wavelength stimuli. Also noteworthy is the emergence of the positive cone-mediated potentials in the presence of the light-adapting field, which provided a retinal illuminance sufficient to saturate the rod mechanism of the normal retina (cf, Hill et al).

Electrooculography and the ERG C-Wave

It is not yet possible to assess fully the functional integrity of the retinal pigment epithelium (RPE) with remote recording techniques. Nevertheless, the results
Figure 5. ERGs recorded from the dark- and light-adapted eye of PDF in response to spectral stimuli of various intensities. Note that in the light-adapted state the a-waves are markedly suppressed and the positive photopic potentials emerge in response to all but short-wavelength (blue) stimuli.

Discussion

There is a remarkable degree of correspondence between the results of the present study and those obtained in recessively inherited night blindness. Dark adaptometry (Fig. 1) revealed that our patient is, indeed, severely night blind, and that the recovery of sensitivity in darkness follows a nearly monophasic curve with final threshold elevated more than 3 log units above that of the normal observer. It is also apparent in these data, and in the spectral sensitivity measurements of Figure 3, that the functional abnormality affects cone-mediated as well as rod-mediated mechanisms.

In hereditary nyctalopia, the authors and Alpern et al. found that the loss of visual sensitivity could not be ascribed to a lack of rhodopsin or to a defect affecting either its light-sensitive or regenerative properties. The same applies to the drug-induced condition. With fundus reflectometry we have shown that the rhodopsin content of the retina is normal (Fig. 2), and that the pigment bleaches and regenerates with normal kinetics. Moreover, the ERG recordings (Fig. 4), indicate that a relatively normal receptor potential (a-wave) is generated in response to photic stimulation, whereas the rod-mediated b-wave could not be elicited even with intense light flashes. And lastly, the spectral sensitivity function for the patient (Fig. 3), like that of the recessively inherited night blind, shows that with appropriate experimental conditions some residual rod-mediated vision can be demonstrated.

Although our patient represents an isolated case, all of the evidence we have been able to muster indicates that the onset of night blindness is associated with the medication he received in the course of cancer chemotherapy. We believe the condition to be due most likely to the vinca alkaloid, vincristine, but we cannot exclude the possibility that another of the drugs administered was responsible for, or contributed to, the visual defects. However, neurotoxicity is rare in patients receiving dacarbazine or CCNU, both of which are alkylating agents, and known to cause serious hematopoietic depression, as well as prolonged episodes of nausea and vomiting. As regards bleomycin, a mixture of glycopeptide antibiotics that act presumably through the inhibition of DNA synthesis, skin and pulmonary toxicities are the most frequent side-effects. While it is virtually impossible to rule out idiosyncratic reactions to any of these substances, to our knowledge there have been no reports of severe visual disturbances attributable directly to their use.

Vincristine therapy, on the other hand, has been shown to induce a significant number of sensory-motor aberrations, and in several instances the drug has
been implicated as the causal factor based on histopathologic study of affected tissues.\textsuperscript{31-33} Although less common, visual disorders are not rare,\textsuperscript{5} and range in severity from ptosis and oculomotor defects\textsuperscript{34} to optic neuropathy\textsuperscript{35} and irreversible blindness.\textsuperscript{36} In addition, there is abundant evidence from studies on experimental animals that vincristine, when introduced into the vitreous body, produces profound structural changes in virtually every type of retinal cell.\textsuperscript{37,38} The proliferation of microfilaments, the formation of crystallloid inclusions, the disruption of microtubules, and the impairment of axonal transport are in accord with the results obtained with vinca alkaloids in other parts of the nervous system.\textsuperscript{39-41}

In view of the toxicity of vinca alkaloids with respect to retinal tissue, it may seem surprising that visual abnormalities are not more prevalent. However, there is good evidence that ocular penetration of vincristine is impaired by the blood–retinal barrier. In their study comparing the relative effects on rabbit retina of intracocular and intravenous administration of vincristine, Vrabec et al\textsuperscript{42} demonstrated the ineffectiveness of the latter route, a point confirmed by Haas et al,\textsuperscript{43} who found that cellular damage to retinal neurons required 6 weeks of intermittent intravenous administration. It is possible, therefore, that many of the visual disorders resulting from short-term vincristine therapy occur in patients in whom the blood–retinal barrier is compromised, either pharmacologically by one of the drugs administered in the therapeutic regimen, or by the disease process itself. In this connection, it is noteworthy that the c-wave of the ERG could not be elicited in our patient, although as already indicated, this finding is of questionable significance. However, it may not be inapposite to mention that in recent experiments on the arterially perfused cat eye, we have found that the b-wave is suppressed by vincristine infusion, but the c-wave is affected earlier and even more profoundly by the drug (Ripps, Siegel, and Mehaffey, unpublished results). We are attempting currently to determine whether this observation bears any relation to the functional properties of the RPE or the integrity of the blood–ocular barrier.

Based solely on the evidence culled from noninvasive test procedures, there is no reason to suppose that the subcellular basis of the functional defects seen with vincristine is the same as that in the genetically induced condition. Nevertheless, it appears that in both instances the severe depression of scotopic vision throughout the retina derives from an abnormality in signal transmission at a distal stage of the visual pathway. The absence of the scotopic b-wave component of the ERG, despite evidence of normal receptor activity (ie, rhodopsin kinetics, a-wave), is consistent with this view. Clearly there are a number of possible sites wherein abnormalities could produce defects of this sort. Although it has been suggested that the disorder may involve a deficiency in the supply of neurotransmitter required for synaptic transmission between photoreceptors and their second-order neurons,\textsuperscript{44} the arguments favoring this hypothesis are highly speculative and need not be reiterated here. Suffice it to say that if vincristine disturbs the integrity of the microtubular system of visual cells,\textsuperscript{45} we might expect to find impairment of axoplasmic transport,\textsuperscript{46-48} failure of the cell to maintain a functional synaptic terminal,\textsuperscript{49-51} and the development of electrophysiologic and visual abnormalities similar to those found in our patient.

**Key words:** vincristine, night blindness, rhodopsin, dark adaptation, ERG, microtubules

**Acknowledgment**

The authors are grateful to Ms. Jane Zakevicius for her assistance during the course of this study and for her help in the preparation of the manuscript.

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