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# Protection of Retinal Function by Zidovudine Following Retinal Ischemic-Reperfusion Injury

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## INTRODUCTION

Retinal ischemia-reperfusion injury is a common clinical entity and it remains a common cause of visual impairment and blindness in the industrialized world<sup>6</sup>. Ischemia/reperfusion-induced damage in retinal tissue is frequently observed in acute glaucoma, diabetic retinopathy, and hypertensive retinopathy<sup>1,5,7</sup>. Ischemia-reperfusion injury often leads to initial neuronal cell death, followed by retinal inflammation, further tissue damage, and eventual retinal dysfunction. Currently there is no effective treatment for patients with retinal ischemia-reperfusion injury.

During ischemia-reperfusion injury, microglia and blood-born macrophages are activated and mediate inflammatory processes<sup>4</sup>. The NLRP3 inflammasome, consisting of the NLRP3 scaffold, the ASC (PYCARD) adaptor, and caspase-1, is believed to function as a pathogen-associated molecular pattern recognition receptor that senses varieties of particulate stimuli as well as self-danger signals<sup>8,9</sup>. It has been demonstrated that the NLRP3 inflammasome plays an important role in ischemia/reperfusion injury in the CNS<sup>4</sup>.

NRTIs were the first drugs to receive FDA approval for the treatment of HIV/AIDS. They target reverse transcriptase, an enzyme that is critical for the replication of HIV. Recently, Fowler et al. found that NRTIs inhibit P2X7-mediated NLRP3 inflammasome activation and prevent retinal degeneration in a mouse model of age-related macular degeneration (AMD). This effect was determined to be independent of inhibiting reverse transcriptase<sup>3</sup>. **In this study, we examined the neuroprotective effects of systemic administration of the NRTI zidovudine (AZT) on retinal ischemia *in vivo* by using our established animal model<sup>2,10</sup>.** This study may provide novel insights into the development of therapeutic intervention for the treatment of patients with retinal ischemic disease.

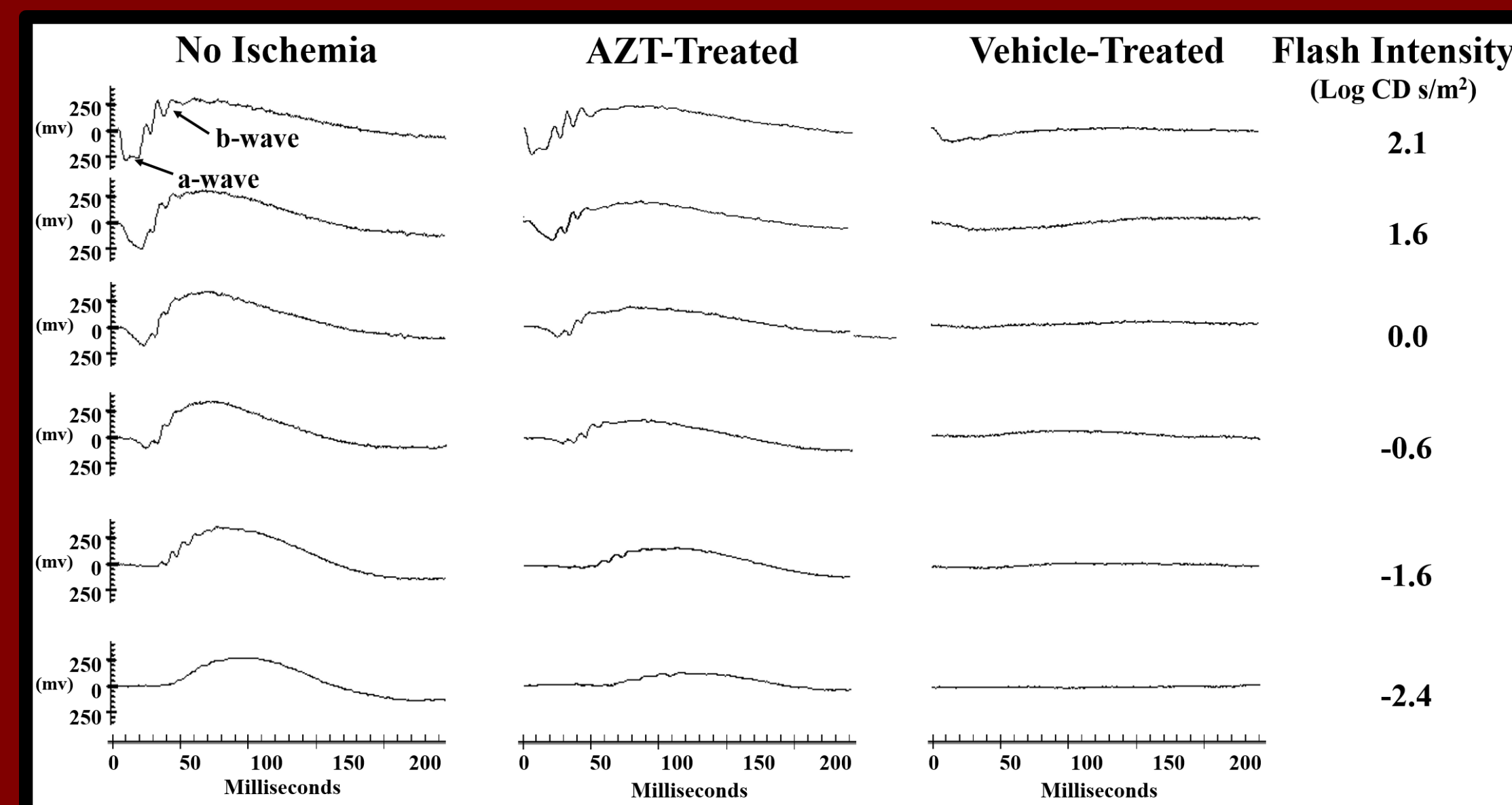
## METHODS

C57BL/6 mice (8 weeks old) were randomly assigned to one of two groups: vehicle-treated retinal ischemic injury mice (control) or AZT-treated retinal ischemic injury mice. **Subsequently, vehicle (1% DMSO in PBS) or AZT 50 mg/kg in 1% DMSO in PBS was injected intraperitoneally twice daily for 5 days.** On the second day of treatment, retinal ischemia was induced by transient elevation of intraocular pressure for 45 minutes as described below. Scotopic electroretinography (ERG) was recorded before AZT or vehicle treatment and 7 days after inducing ischemic retinal injury.

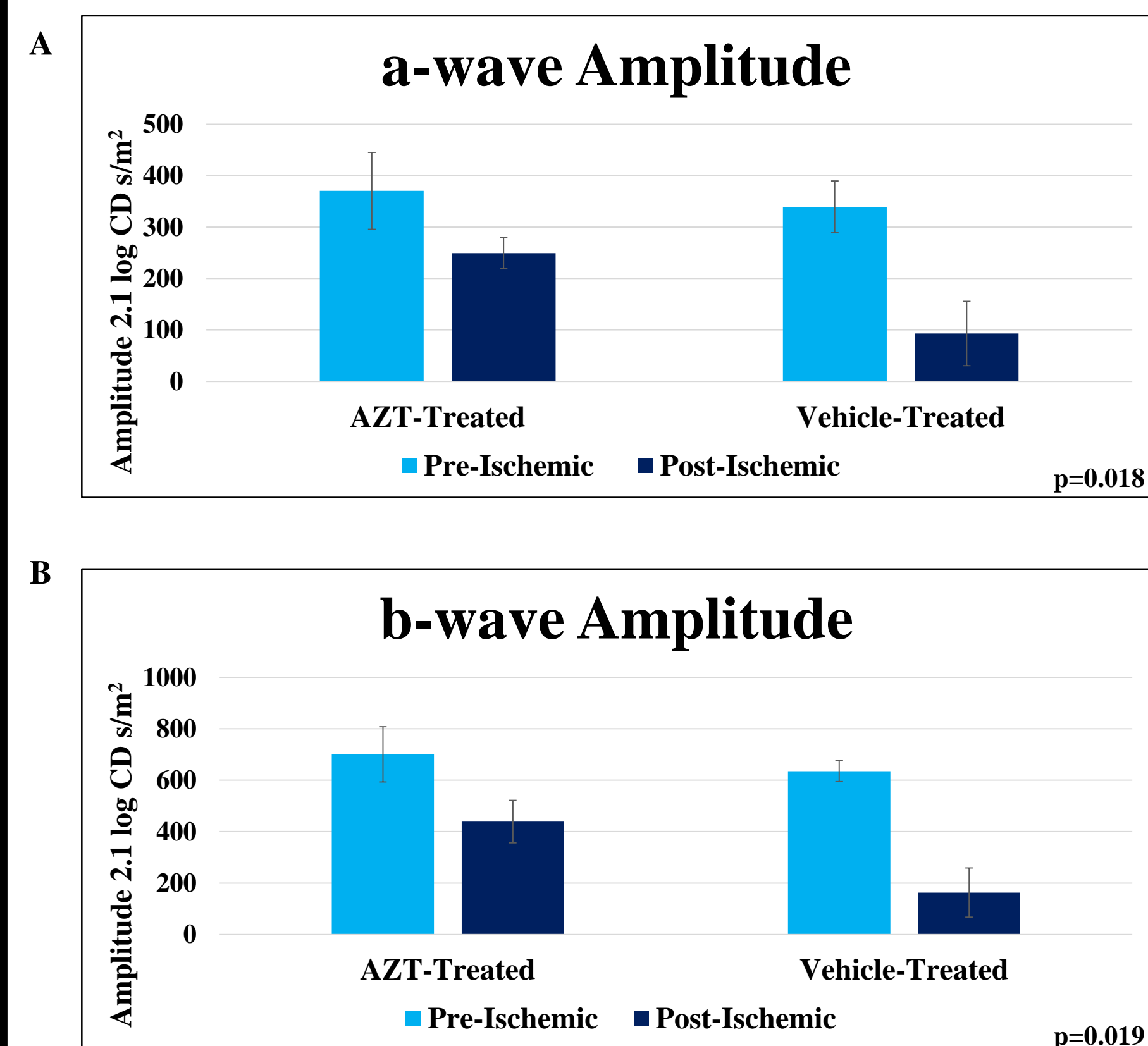
**Induction of pressure-induced ischemia:** Retinal Ischemia was induced in anesthetized mice by transient elevation of intraocular pressure (IOP) as previously described and successfully employed in our laboratory<sup>2,10</sup>. The anterior chamber of the right eye of each mouse was cannulated with a 30-gauge sterile needle connected to an elevated isotonic sterile saline bag. The IOP was raised to 110 mm Hg for 45 min, preventing blood flow to the retina. Retinal ischemia was confirmed by rapid blanching of the ocular fundus and the collapse of the retinal artery by indirect ophthalmoscope.

**Electroretinographic responses:** Retinal function was measured before inducing ischemic retinal injury and 7 days after inducing ischemic retinal injury. Mice were dark adapted overnight. Their pupils were dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride. Using a stainless steel electrode coated with 1% methylcellulose, the ERG was recorded from the corneal surface with a series of stimulus luminances<sup>2,10</sup>.

## RESULTS



**Figure 1.** Representative dark-adapted ERGs. Note the preservation of ERG a- and b-waves in the AZT-treated ischemic mice when compared with the vehicle-treated ischemic mice.



**Figure 2.** Effect of AZT treatment on retinal function following ischemic insult. Quantitative changes in ERG (A) a-wave and (B) b-wave amplitudes at 2.1 log CD s/m<sup>2</sup> flash intensity. Error bars indicate the standard deviation. (n=3 per group)

## DISCUSSION

**Figure 1** shows the morphological differences among ERG readings of non-ischemic retinas, AZT-treated post-ischemic retinas, and vehicle-treated post-ischemic retinas. Larger amplitude a- and b-waves are indicative of greater retinal function, while smaller amplitudes indicate poorer function. A flat ERG response indicates no retinal function, or blindness. Since the ERG recordings in both the AZT and vehicle-treated groups were taken 7 days post induction of retinal ischemia, we expect these ERG waves to be smaller in amplitude than those of the non-ischemic ERG. However, morphologically, the AZT-treated mice retained more retinal function compared to vehicle-treated mice.

**Figure 2** is a plot of the average a- and b-wave amplitudes both before and after ischemia in the AZT and vehicle-treatment groups. The average post-ischemic a-wave amplitude for AZT-treated mice was 249.3 ± 30.4 mv compared to 93.0 ± 62.6 mv for vehicle-treated mice (**p = 0.018, fig. 2-A**). Similarly, the average post-ischemic b-wave amplitude for the AZT-treated mice was 439 ± 82.8 mv compared to 162.8 ± 95.6 for vehicle-treated mice (**p = 0.019, fig. 2-B**).

Our data shows that AZT-treated mice demonstrated a significant preservation of ERG a-wave and b-wave amplitudes following ischemic insult as compared to controls. These preliminary findings suggest that AZT may have therapeutic value in the management of retinal ischemic diseases.

To our knowledge, this is the first study of AZT's neuroprotective effect in the context of retinal-ischemic injury. There is much left to discover in examining the use of AZT in ischemic retinal disease. As we continue this project, we aim to include a larger sample size as well as histology and morphometry to augment data analysis. Furthermore, we will study AZT's mechanism of neuroprotection in retinal ischemia.

## CONCLUSIONS

Mice treated with zidovudine (AZT) demonstrated a significant preservation of ERG a-wave and b-wave amplitudes following ischemic insult as compared to controls. Our preliminary findings suggest that AZT may have therapeutic value in the management of retinal ischemic diseases.

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