Reduced Reflectance of Retinal Nerve Fiber Layer as a Marker of Microtubule Axonal Damage in Experimental Autoimmune Encephalomyelitis

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BACKGROUND

Axonal transport, requiring intact cytoskeleton, has been suggested to precede substantial neurodegeneration in multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). OCT reflectance, a measure of signal intensity reflected from retinal tissue, is proportional to its ultrastructural directional homogeneity, which in axons relies upon microtubule network integrity. Reflectance of retinal nerve fiber layer (RNFL) has been suggested as a marker of axonal microtubule integrity. We aimed at validating the relationship between RNFL reflectance and microtubules in EAE.

METHODS

Thirty dark Agouti female rats were induced EAE through myelin oligodendrocyte glycoprotein immunization and compared to six healthy controls. Bilateral peripapillary OCT scans, flash visual-evoked potential (VEP) and optic nerve and retinal histology were performed at 14(N=6), 21(N=8), 35(N=6) and 42 dpi(N=10). RNFL reflectance Index (RRI) was calculated as signal intensity of RNFL normalized with the intensity of retinal pigment epithelium. Histology with BRN3 for retinal ganglion cell (RGC) and immunofluorescent with neurofilament and β-tubulin were quantified. EAE eyes were classified into normal VEP(nVEP) and delayed VEP(dVEP). RRI group effects tested with one-way ANOVA and correlated with histology.

RESULTS AND DISCUSSION

Already at 21 dpi, RRI was reduced in both nVEP(RRI= 0.95±0.22,p=0.007) and dVEP(RRI =0.90±0.16, p=0.003) eyes compared with healthy(RRI=1.10±0.12). Compared with healthy, dVEP eyes showed lower RGC counts(EAE:146±61, healthy:325±19, p<0.001), less percentage of neurofilaments (EAE:21.84±10.67%, healthy:40.53±12.46%, p=0.005), and β-

tubulin(EAE:33.24±14.15%, healthy:48.08±5.66%, p=0.004) within RNFL. In dVEP eyes, RRI correlated with RGC counts(r=0.651, p=0.001), percentage of β-tubulin(r=0.912, p<0.001), but not with neurofilament (p=0.067). Our results are consistent with previous in vitro studies suggesting that, at OCT wavelength, RNFL reflectance is mainly contributed by microtubules. Abnormal axonal transport may also be present without evidence of demyelination from VEP.

RNFL reflectance enables us to monitor the integrity of microtubule in vivo and may help testing therapeutic approaches targeting axonal transport in neurodegenerative diseases.