Mice transgenic for Alzheimer disease β-amyloid develop lens cataracts that are rescued by antioxidant treatment

Simon Melov, Norman Wolf, Dorothea Strozyk, Susan R. Docrow, Ashley I. Bush

Abstract

Alzheimer disease is characterized by cerebral Aβ deposition, which we have recently discovered occurs also in the lens as cataracts in Alzheimer disease patients. Here we report the presence of significantly increased cataracts in the lenses of an Aβ-transgenic mouse model for Alzheimer disease and their amelioration upon treatment with EUK-189, a synthetic SOD/catalase mimetic. These data support an oxidative etiology for AD-associated lens cataracts and their potential to be treated preventatively with antioxidants.

Keywords: Free radicals

Alzheimer disease (AD) is characterized by Aβ accumulation as an amyloid in the brain, which may be caused in humans and in Aβ-overexpressing transgenic mice (e.g., Tg2576) [1] by abnormal interaction with endogenous brain zinc and copper ions (reviewed in [2,3]). This leads to reactive oxygen species (ROS) generation and oxidative cross-linking of proteins, which are abundantly evident in the AD-affected brain (reviewed in [2,3]). Antioxidants and ROS scavengers have therefore been considered as possible therapies for AD, although a major alternative hypothesis is that Aβ is merely a self-aggregating protein that is best eradicated by clearance approaches or synthetic inhibition. Recently, we have reported that Aβ also deposits as amyloid in the human lens in AD, forming supranuclear cataracts [4], which could be a potential biomarker for AD. Here we follow up on our earlier studies of cataracts in Tg2576 mice [5], to report statistically increased and more severe cataracts compared to controls in an important animal model for AD. We further report that the development of these cataracts is inhibited by treatment of mice with EUK-189, a synthetic superoxide dismutase (SOD)/catalase mimetic efficacious against oxidative stress within the brain, similar to other compounds with demonstrable efficacy against mitochondrial oxidative stress and neurodegeneration [6,7]. EUK-189 is a slightly more lipophilic version of EUK-1891. These compounds are salen–Mn complexes whose structures have been previously reported. EUK-189 contains ethoxy, rather than methoxy, groups at the 3,3' positions of the salen rings (Fig. 1). This gives it greater intracellular [8] and neuroprotective [6] activity than EUK-
134. These structural modifications seem to allow the antioxidant to cross blood–lenticular barriers.

Experimental procedures and results

Tg2576 (APP) mice were bred as previously described [1] and maintained under barrier conditions at the Buck Institute. Mice were treated with either vehicle (water) or the synthetic catalytic antioxidant EUK-189 [6] (30 mg/kg intraperitoneally, three times weekly) from 90 days of age through to 300–400 days of age, amounting to more than a year of continuous treatment in some mice. The structure and properties of EUK-189 have been described previously [6,8,9] (Fig. 1).

The age of the animals was equally distributed between treatments. The lens of each eye was dilated with 1% tropicanimide acid and rated independently for progressive opacity in intervals of 0.5 from 0 (no opacification) to 4 (completely opaque mature cataract), using a handheld SL-14 KOWA slit lamp (Kowa, Tokyo, Japan) and read in a blinded fashion as previously described [10–12]. Eyes from vehicle-treated transgenic mice had a significantly higher (Wilcoxon Mann–Whitney \( p \) value = 0.02) cataract opacity score (median 3.0) than nontransgenic mice (median 2.0) (Table 1). This represents a significant increase in the severity of cataracts formed due to the presence of the mutant APP allele. Transgenic mice treated with EUK-189, however, had a lower median opacity score (median 2.0) than vehicle-treated transgenic mice \((p = 0.01)\). This was equivalent to that of untreated non-transgenic mice \((p = 0.99)\).

\( \chi^2 \) tests were performed to test the hypothesis that mild and severe cataracts had the same frequency in each treatment group. Based on the opacity score of all mice, cataract scores \(>2\) were assigned as “severe,” and “mild” cataracts were \(\leq 2\). Among vehicle-treated Tg2576 mice, 72.1% had severe cataracts, whereas only 33.3% of the untreated wild-type mice developed severe cataracts \((p\) value = 0.02; Fig. 2). Treatment with EUK-189 in transgenic mice led to significantly fewer severe cataracts \(42\%\), \(p\) value = 0.025) than in vehicle-treated transgenic mice, returning the opacification scores to levels comparable to those of untreated nontransgenic mice \((p = 0.6; \text{Fig. 2})\).

Although the location of cataracts can be described in the human eye with a hand-held slit lamp, in mice cataracts cannot be unequivocally described for precise location, so this specification was not noted for the individual eyes as they were examined.

Discussion

The observation of cataracts in Tg2576 mice, which recapitulates changes seen in patients with AD, encourages us to pursue the cataract phenotype in human subjects as a potential biomarker for AD [4]. Our findings also suggest that \(A\beta\) can induce sufficiently severe lens opacification, supporting the possibility that \(A\beta\) could participate in age-related cataracts, the most common form of blindness. Intriguingly, early onset cataracts and AD are common comorbid conditions in Down syndrome [13] and familial Danish dementia [14], an AD variant with cerebral \(A\beta\) amyloidosis. Our observation of the therapeutic effects of EUK-189 indicate that protein aggregation in the Tg2576 lens occurs by an oxidative mechanism rather than by seeded templating. EUK-189 is one of several salen–Mn

Fig. 1. EUK-189, a more lipophilic catalase/superoxide dismutase mimetic.

Table 1

<table>
<thead>
<tr>
<th>Cataract scores</th>
<th>Wild type ((N = 12))</th>
<th>Tg2676 ((N = 26))</th>
<th>EUK-189 ((N = 26))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median ((25\text{th–75\text{th}}\text{ percentile}))</td>
<td>2.0 (1.5–2.5)</td>
<td>3.0 (2–3)*</td>
<td>2.0 (1–3)</td>
</tr>
<tr>
<td>Mean ((\pm \text{SEM}))</td>
<td>2.0 (0.8)</td>
<td>2.6 (0.7)*</td>
<td>2.0 (1.1)</td>
</tr>
</tbody>
</table>

\* \(p\) value < 0.02 compared to wild type and EUK-189 using Wilcoxon Mann-Whitney test for comparisons of medians, t test with unequal variance for comparisons of means.

Fig. 2. Severity of cataract incidence in Tg2576 mice compared to nontransgenic, and effects of antioxidant treatment. Mild, cataract score \(\leq 2\); severe, cataract score \(>2\). The vehicle-treated Tg2576 mice have more severe cataracts than nontransgenic (wild-type) mice, whereas treatment of the Tg2576 mice with EUK-189 [6] reduces cataract severity to nontransgenic levels. The numbers of lenses in each group are wild-type \(N = 12\), Tg2576 \(N = 26\), Tg2576 + EUK-189 \(N = 26\). Opacity in each eye was scored without knowledge of genotype or treatment. The \(p\) values shown are the results of \(\chi^2\) analysis as described in the text.
complexes which, as described previously [15], have catalytic properties mimicking both SOD and catalase. As a consequence of their catalase activity, they also scavenge certain reactive nitrogen species, including peroxynitrite [16]. Salen–Mn complexes are neuroprotective and reduce tissue oxidative stress in several disease models involving ischemic [17] and excitotoxic [18] neuronal damage, as well as a mouse model for amyotrophic lateral sclerosis [19], β-amyloid peptide-induced neurotoxicity in culture [20], and dopaminergic cell death in Parkinson disease models utilizing the toxins MPTP, MPP+, or 6-hydroxydopamine [21,22]. EUK-189, an analog designed to have enhanced brain and intracellular accessibility, is the most effective [21,22]. EUK-189, an analog designed to have enhanced brain and intracellular accessibility, is the most effective [21,22].

Acknowledgments

This work was supported by grants from the NIA (RO1AG18679 to S.M., RO1AG12686 to A.I.B.), Alzheimer’s Association (to A.I.B.), NIMHRC (A.I.B.), and NEI (RO1EY11733 to N.W.) and an award from the Institute for the Study of Aging to S.M. S.M. is indebted to N. Nagulko and other members of the Melov laboratory for excellent animal care. All animal procedures were carried out under approved institutional animal protocols at the Buck Institute. Ashley I. Bush is on the scientific advisory board of Prana Biotechnology Ltd. and is a consultant and stockholder. Susan R. Doctrow is Vice President of Research at Eukarion and a co-inventor of the company’s patents relating to EUK-189.

References


