Reversals of Blood–Brain Barrier Disruption by Catalase: A Serial Magnetic Resonance Imaging Study of Experimental Optic Neuritis

John Guy,*+ Sue McGorray,† Jeffrey Fitzsimmons,§ Barbara Beck,§ Anthony Mancuso,§ Narsing A. Rao,‖ and Latif Hamed*

Purpose. To investigate serially the role of catalase detoxification of endogenous H₂O₂ in the disruption of the blood–brain barrier (BBB) and demyelination of experimental optic neuritis.

Methods. Serial contrast-enhanced magnetic resonance imaging (MRI) of the optic nerves (T₁ weighted) and T₂ weighted MRI without contrast were performed on 18 guinea pigs 3 to 14 days after sensitization with central myelin for experimental allergic encephalomyelitis. Sex and age-matched littermates were paired and sensitized with the identical antigenic emulsion. To detoxify endogenous hydrogen peroxide (H₂O₂), animals received daily intraperitoneal injections of polyethylene glycol (PEG)-catalase at a dose of 12,000 U/kg per day for 3 days, then 1,200 U/kg daily for the next week, commencing 3 days after antigenic sensitization. Littermates received an equal volume of preservative-free saline. The intensity of gadolinium-DTPA (Gd-DTPA) enhancement was quantitated by obtaining the value for a region of interest (ROI) of the right optic nerve and the left optic nerve. The effect of H₂O₂ detoxification by catalase was evaluated by differences in the intensity of Gd-DTPA enhancement and T₂ weighted signal in the ROI of the right and the left optic nerves at 7, 10, and 14 days after antigenic sensitization, from the pretreatment value obtained at day 3. The effectiveness of catalase detoxification of H₂O₂ was assessed with quantitative ultracytochemical localization of electron-dense, H₂O₂-derived cerium perhydroxide in the optic nerves.

Results. With PEG-catalase treatment, mean differences for Gd-DTPA enhancement in the ROI at 7, 10, and 14 days after antigenic sensitization were significantly reduced from the pretreatment values obtained 3 days after antigenic sensitization compared with the comparable interval values for untreated littermates. For T₂ weighted signal intensity, only the 7- and 14-day values were significantly less with PEG-catalase compared with values for littermates obtained at comparable intervals. Quantitative ultracytochemical localization of H₂O₂-derived cerium perhydroxide reaction product revealed significant reductions in the median number of cerium particle counts of the optic nerve head, sheath, and myelinated retrobulbar nerve.

Conclusions. PEG-catalase reduced H₂O₂-derived cerium perhydroxide reaction product in the optic nerve but did not eliminate it, reversed disruption of the BBB as measured by Gd-DTPA enhancement, and reduced demyelination and edema as measured by T₂ weighted signal intensity, suggesting detoxification of H₂O₂ as a new treatment strategy for disorders of primary demyelination of the central nervous system. Invest Ophthalmol Vis Sci. 1994; 35:3456–3465.

Disruption of the blood–brain barrier (BBB) plays a major role in the pathogenesis of experimental and human disorders of primary autoimmune demyelination of the central nervous system (CNS). Magnetic resonance imaging (MRI) with intravascular administration of gadopentetate dimeglumine (Gd), chelated to diethylentriamine pentaacetic acid (DTPA) to reduce biologic toxicity, reveals the foci of BBB disruption in vivo by accumulation of Gd-DTPA on T₁
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with a spinal cord emulsion (1.0 ml/kg body weight) of Gd-DTPA enhancement in the region of interest of the optic nerves is similar 3 days after antigenic sensitization but before commencement of PEG-catalase. Mean values are lower with PEG-catalase treatment at 7 days (4 days of PEG-catalase treatment), 10 days (7 days of PEG-catalase treatment), and 14 days (11 days of PEG-catalase treatment) after antigenic sensitization. Values for the untreated group were at their highest on day 14.

![Graph showing mean signal intensity (and standard error boxes) of Gd-DTPA enhancement over regions of interest (ROI) in untreated and catalase-treated guinea pigs across days 3, 7, 10, and 14 after antigenic sensitization.](image)

**FIGURE 1.** Mean signal intensity (and standard error boxes) of Gd-DTPA enhancement in the region of interest of the optic nerves is similar 3 days after antigenic sensitization but before commencement of PEG-catalase. Mean values are lower with PEG-catalase treatment at 7 days (4 days of PEG-catalase treatment), 10 days (7 days of PEG-catalase treatment), and 14 days (11 days of PEG-catalase treatment) after antigenic sensitization. Values for the untreated group were at their highest on day 14.

The overall degree of demyelination may be estimated in vivo by increases in T2 weighted signal aberrations. The MRI lesions of experimental and human optic neuritis appear similar, suggesting a similar pathogenesis.

Reactive oxygen species, such as superoxide and hydrogen peroxide (H2O2), have been implicated in CNS injury. Agents that attenuate tissue injury from reactive oxygen species have been used in the treatment of CNS disorders. Studies have shown detoxification of H2O2 with polyethylene glycol (PEG)-catalase reduced edema and demyelination of the optic nerve and nervous system and suppressed BBB permeability. Conjugation of PEG to catalase increases the half-life and reduces the antigenicity of catalase while not altering the immune response or tissue pathology.

To evaluate the role of detoxification of the reactive oxygen species H2O2 on BBB disruption and demyelination in vivo, serial MRIs and quantitative ultrastructural correlation of H2O2 localization in the optic nerves were performed in PEG-catalase-treated guinea pigs sensitized for experimental allergic encephalomyelitis (EAE), a primary disorder of CNS demyelination, and were compared to untreated littermates.

**MATERIALS AND METHODS**

Twenty-four strain-13 guinea pigs purchased from Crest Caviary (Raymond, CA) were sensitized for EAE with a spinal cord emulsion (1.0 ml/kg body weight) in complete Freunds adjuvant (Difco Laboratories, Detroit, MI) that was injected subdermally into the nuchal area. Guinea pigs were humanely cared for in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Sex- and age-matched littermates were paired, and each pair of animals received the identical antigenic emulsion. Group 1 received daily intraperitoneal injections of PEG-catalase (specific activity = 39,600 U/mg protein, molecular weight of PEG = 5,000 daltons [Sigma, St. Louis, MO]) at a dose of 12,000 U/kg per day for 3 days, then 1,200 U/kg dissolved in normal saline daily for the next week, commencing 3 days after antigenic sensitization and after the MRIs performed on days 3, 7, 10, and 14. To control for an effect of dehydration (EAE animals may imbibe less water), the paired mates received an equal volume of preservative-free saline commencing 3 days after antigenic sensitization.

Magnetic resonance imaging of 18 guinea pigs sensitized for EAE was performed 3, 7, 10, and 14 days after antigenic sensitization with a 2.0 Tesla 32-cm bore superconducting magnet (Oxford Instruments Limited, Oxford, UK) with a SUN computer-based acquisition and processing system (Spectroscopy Imaging Systems, Freemont, CA) using a 6-cm field of view, a 256 x 192 matrix with four repetitions and a section thickness of approximately 1.25 mm. A specially designed surface coil was placed over the head for an improved signal-to-noise ratio.

Suppression of orbital fat was accomplished using a frequency selective saturation pulse method with a T1 weighting (T1w) of TR = 600 msec and a TE = 20 msec and a T2 weighting (T2w) of TR = 2,000 msec and TE = 80 msec. T1w imaging was again performed immediately after intravascular administration of Gd-DTPA (Berlex Laboratories, Wayne, NJ) at a dose of 0.2 mmol/kg of body weight. Images were acquired in the axial and coronal planes with the guinea pig lying prone, after sedation with intramuscular ketamine (Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (Butler, Columbus, OH) in a 1:1 mixture (0.4 ml/kg of body weight). Images were converted from sunraster files to tiff files and standardized to an internal standard, the fluid-filled globe, using Photoshop (Adobe Systems, Mountain View, CA).

Analysis of the axial images was performed with a Macintosh PowerBook 180 computer (Apple Computer, Cupertino, CA) using the program Image (W. Rasband, National Institutes of Health, version 1.44). Mean signal intensities over regions of interest (ROI) were used to measure the intensity of Gd-DTPA enhancement and T2 weighted signal. Regions of interest from the globe to the optic chiasm were evaluated by an observer masked to the treatment for each optic nerve. Images of poor quality or inadequate fat suppression, or those outside the field of view of the optic...
FIGURE 2. Mean signal intensity (and standard error boxes) of T2 weighted signal intensity is similar 3 days after antigenic sensitization but before commencement of PEG-catalase. Mean values are lower with PEG-catalase treatment at 7 days (4 days of PEG-catalase treatment), 10 days (7 days of PEG-catalase treatment), and 14 days (11 days of PEG-catalase treatment) after antigenic sensitization.

nerve that precluded measurement of the ROI, were excluded from analysis.

Differences for the right and left ROIs of the optic nerves were obtained by subtracting baseline values (obtained 3 days after antigenic sensitization and before PEG-catalase administration) from each of the follow-up values obtained after PEG-catalase administration for treated animals and at corresponding time points for untreated animals. Multivariate analyses of covariance were used to compare these differences for treatment effect at each follow-up time point. For each analysis, the vector of right and left optic nerve differences is the outcome of interest, baseline values are adjusted for, and the effect of treatment is assessed using the Wilks' lambda test statistic. A P value of less than 0.05 was considered statistically significant. Baseline values were compared in a similar manner. Paired analyses were not performed because data were not available at all time points for all animals.

### TABLE 1. Gd-DTPA Enhanced MRI

<table>
<thead>
<tr>
<th>Number of Days Post ag</th>
<th>Number of Days of Treatment</th>
<th>Number of Eyes</th>
<th>Gd-DTPA Untreated</th>
<th>Difference From Initial</th>
<th>Number of Eyes</th>
<th>Gd-DTPA Catalase</th>
<th>Difference From Initial</th>
<th>Statistical Significance (P value)*</th>
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<tbody>
<tr>
<td>3</td>
<td>0</td>
<td>14</td>
<td>160.62 ± 34.21</td>
<td>—</td>
<td>14</td>
<td>169.35 ± 16.92</td>
<td>—</td>
<td>Yes (0.0226)</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>14</td>
<td>164.32 ± 16.40</td>
<td>−3.70</td>
<td>14</td>
<td>135.66 ± 27.03</td>
<td>33.66</td>
<td>Yes (0.0471)</td>
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<tr>
<td>10</td>
<td>7</td>
<td>8</td>
<td>157.20 ± 23.90</td>
<td>13.50</td>
<td>8</td>
<td>138.56 ± 16.66</td>
<td>26.21</td>
<td>Yes (0.0428)</td>
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<tr>
<td>14</td>
<td>11</td>
<td>8</td>
<td>179.05 ± 19.10</td>
<td>−21.45</td>
<td>8</td>
<td>146.59 ± 16.84</td>
<td>44.66</td>
<td>Yes (0.0471)</td>
</tr>
</tbody>
</table>

Gd-DTPA values are mean ± SD. ag = Antigen.

* Comparing change from initial, untreated vs catalase, controlling for initial values.
RESULTS

Magnetic Resonance Imaging

There were no differences in Gd-DTPA enhancement ROI (Fig. 1) and T2 weighted intensity ROI (Fig. 2) measurements of the optic nerves obtained 3 days after antigenic sensitization, but before administration of either PEG-catalase or saline.

Gd-DTPA

Treatment with PEG-catalase reduced Gd-DTPA enhancement (Fig. 1, Table 1) of the optic nerves. After 4 days of PEG-catalase treatment (day 7 after antigenic sensitization), differences in ROI measurements of Gd-DTPA enhancement of the optic nerves between time point 2 and time point 3 were lower in the treated group with a mean value of 33.69 ± 6.26 (mean ± standard error, based on combined right and left optic nerve measurements), in comparison to the untreated littermates with a value of −3.70 ± 6.89. The effect of treatment was statistically significant (P = 0.0226). Figure 3 illustrates a marked reduction in Gd-DTPA enhancement after 4 days of PEG-catalase administration. In contrast, the untreated mate exhibits an increase in Gd-DTPA enhancement during this time interval (Fig. 4).

After 7 days of PEG-catalase treatment (day 10 after antigenic sensitization), differences in ROI measurements of Gd-DTPA enhancement of the optic nerves between time point 2 and time point 4 were lower with PEG-catalase treatment with a mean value of 24.66 ± 9.58, in comparison to the untreated mates with a value of 13.50 ± 3.21. The effect of treatment was statistically significant (P = 0.0471).

Values for Gd-DTPA enhancement were at their highest in the untreated group during the late stage of EAE, on day 14. After 11 days of PEG-catalase treatment (14 days after antigenic sensitization), differences in ROI measurements of the optic nerve between time point 2 and time point 5 were lower with PEG-catalase treatment with a mean value of 26.21 ± 6.02, in contrast to a value of −21.45 ± 19.81 in the untreated group. The effect of treatment was statistically significant (P = 0.0428).

T2 Weighted Signal Intensity

PEG-catalase treatment reduced T2 weighted signal intensity (Fig. 2, Table 2) aberrations in the optic nerve and protected against further changes seen in the late stages of EAE in the untreated group. After 4 days of PEG-catalase treatment, day 14 untreated, and day 14 PEG-catalase-treated). A P value of less than 0.05 was considered statistically significant, and pairwise Wilcoxon rank sum tests were used to determine which groups differed.
### TABLE 2. T2W Enhanced MRI

<table>
<thead>
<tr>
<th>Number of Days Post ag</th>
<th>Number of Days of Treatment</th>
<th>Untreated</th>
<th>Catalase</th>
<th>Statistical Significance (P value)*</th>
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<tr>
<td>3</td>
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<td>16 103.65 ± 27.48</td>
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<tr>
<td>10</td>
<td>7</td>
<td>10 125.66 ± 28.21</td>
<td>14 89.49 ± 30.22</td>
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<tr>
<td>14</td>
<td>11</td>
<td>8 126.64 ± 12.28</td>
<td>10 88.65 ± 29.94</td>
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</table>

T2 values are mean ± SD.
* Comparing change from initial, untreated vs catalase, controlling for initial values.

days of PEG-catalase treatment (7 days after antigenic sensitization), differences in ROI measurements of T2 weighted signal intensity of the optic nerves between time point 2 and time point 3 were lower in the treated group with a value of 4.56 ± 7.06, in comparison to the untreated littermates with a value of -20.51 ± 7.71. The effect of treatment was statistically significant (P = 0.0306).

After 7 days of PEG-catalase treatment (10 days after antigenic sensitization), the differences in ROI measurements of T2 weighted signal intensity of the optic nerves between time point 2 and time point 4 were lower with PEG-catalase treatment with a value of 8.49 ± 5.01, in comparison to the untreated group with a value of -7.12 ± 7.40. However, the effect of treatment was not statistically significant (P = 0.4170).

After 11 days of PEG-catalase treatment (14 days after antigenic sensitization), differences in ROI measurements T2 weighted signal intensity of the optic nerve between time point 2 and time point 5 were lower with PEG-catalase treatment with a value of 28.45 ± 13.02, in contrast to the untreated group with a value of -11.44 ± 5.08. The effect of treatment was statistically significant (P = 0.0035).

Quantitative Ultracytochemical Localization of H2O2

After 4 days of PEG-catalase treatment (7 days after antigenic sensitization), the overall median value of 196 (range, 32 to 198) for total cerium perhydroxide particles counts in the optic nerve head, optic nerve sheath, and myelinated nerve was reduced 69.2%, in comparison to the untreated group median of 637 (range, 347 to 669) (Table 3). The effect of treatment was not statistically significant. After 11 days of PEG-catalase treatment (14 days after antigenic sensitization), the median value of 36.5 (range, 0 to 232) was reduced by 88.5%, compared to the untreated group median of 318.5, (range, 24 to 1149). The effect of treatment was statistically significant (P = 0.002). The median values for the three regions of the optic nerve were also analyzed.

In the optic nerve head after 4 days of PEG-catalase treatment (7 days after antigenic sensitization), median values of 316 (range, 52 to 453) were reduced...
Figure 6. (Top) Axial MRI of the littermate shown in Figure 5 and performed 3 days after antigenic sensitization exhibits increased T2 weighted signal intensity in the orbital segment of the optic nerves (arrows) adjacent to the globes. (Bottom) An increase in T2 weighted signal intensity is evident in the optic nerve (arrows) 14 days after antigenic sensitization.

60% compared to the untreated group with a median value of 789 (range, 588 to 1290). However, the effect of treatment was not statistically significant. After 11 days of PEG-catalase treatment (14 days after antigenic sensitization), the median value of 52 (range, 0 to 453) was reduced by 87% compared to the untreated group median of 404 (range, 128 to 2840) for the optic nerve head. The effect of treatment was statistically significant ($P = 0.015$). Representative electron micrographs of the unmyelinated optic nerve head show that cerium perhydroxide reaction product particles in the lumen of a blood vessel are absent in the perivascular space of an animal treated with PEG-catalase, whereas in the untreated mate, H$_2$O$_2$-derived reaction product is seen in the perivascular and extracellular spaces between axons (Fig. 7).

In the retrobulbar nerve, after 4 days of PEG-catalase treatment (7 days after antigenic sensitization), median values of 0 (range, 0 to 0) showed no difference in comparison to the untreated group with a median value of 0 (range, 0 to 0). After 11 days of PEG-catalase treatment (14 days after antigenic sensitization), the median value of 0 (range, 0 to 3) showed no difference in comparison to the untreated group median of 0 (range, 0 to 354) for the retrobulbar nerve. An electron micrograph of the myelinated retrobulbar optic nerve of a guinea pig treated with PEG-catalase for 4 days shows H$_2$O$_2$-derived reaction product surrounds myelinated axons, some with intracellular edema and vesiculated myelin (Fig. 8). Similar ultrastructural findings were seen in untreated animals.

In the optic nerve sheath, after 4 days of PEG-catalase treatment (7 days after antigenic sensitization), median values of 151 (range, 141 to 635) were reduced 87% in comparison to the untreated group with a median value of 1206 (range, 626 to 2074). Electron micrographs of the optic nerve sheath show a marked reduction in cerium perhydroxide reaction product particles compared with the untreated mate (Fig. 9). After 11 days of PEG-catalase treatment (14 days after antigenic sensitization), the median value of 479 (range, 0 to 1629) was increased 32% compared with the untreated group median of 363 (range, 251 to 1139) for the optic nerve sheath. The effect of treatment was not statistically significant.

### DISCUSSION

Detoxification of H$_2$O$_2$ with exogenous PEG-catalase attenuated, but did not eliminate, endogenous H$_2$O$_2$ in the optic nerves of EAE animals. Although the mode of regulation of reactive oxygen species production by phagocytes is unclear, generation of H$_2$O$_2$ by activated inflammatory cells is not regulated by cata-

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Day 7 Control</th>
<th>Day 7 Treated</th>
<th>Day 14 Control</th>
<th>Day 14 Treated</th>
</tr>
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<tbody>
<tr>
<td>Total*</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Median</td>
<td>637</td>
<td>196</td>
<td>319†</td>
<td>37†</td>
</tr>
<tr>
<td>Minimum</td>
<td>347</td>
<td>32</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>669</td>
<td>198</td>
<td>1149</td>
<td>253</td>
</tr>
<tr>
<td>Optic nerve head</td>
<td>789</td>
<td>316</td>
<td>404†</td>
<td>52†</td>
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<tr>
<td>Median</td>
<td>588</td>
<td>52</td>
<td>128</td>
<td>0</td>
</tr>
<tr>
<td>Minimum</td>
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<td>453</td>
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<tr>
<td>Maximum</td>
<td>1206</td>
<td>151</td>
<td>363</td>
<td>479</td>
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<td>Retrobulbar myelinated nerve</td>
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<td>251</td>
<td>0</td>
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<tr>
<td>Median</td>
<td>2074</td>
<td>635</td>
<td>1139</td>
<td>1629</td>
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<tr>
<td>Minimum</td>
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<tr>
<td>Maximum</td>
<td>363</td>
<td>0</td>
<td>1139</td>
<td>1629</td>
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* Total = Optic nerve head + retrobulbar myelinated nerve + optic nerve sheath particle counts.
† Statistical significance between Control and Treated (Day 14).
FIGURE 7. Electron micrograph of the unmyelinated optic nerve head of a PEG-catalase treated animal shows cerium perhydroxide reaction product particles (arrows) in the lumen (L) of a blood vessel that is absent in the perivascular space (A). On the other hand, in the untreated mate, H₂O₂-derived reaction product (arrows) has a perivascular distribution (B). These particles are also evident in the extracellular space between axons (A). Original magnification, X7500.
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Figure 8. An electron micrograph of the myelinated retrobulbar optic nerve of a guinea pig treated with PEG-catalase for 4 days shows H$_2$O$_2$-derived reaction product (arrow) surrounds myelinated axons (A), some with intracellular edema and vesiculated myelin. Original magnification, ×7500.

Figure 9. Electron micrographs of the optic nerve sheath show a marked reduction in cerium perhydroxide reaction product particles (arrows) with 4 days of PEG-catalase treatment (A) compared with the untreated mate (B). Original magnification, ×7500.
the sites of its generation, and react with myelin lipid contributing to demyelination and amplification of BBB disruption. These effects of H₂O₂-induced injury to the optic nerve were reduced by PEG-catalase.

PEG-catalase reduced demyelination of the optic nerve, as measured by T2 weighted signal aberrations in the ROI. Reactive oxygen species such as superoxide, H₂O₂, and/or metabolites of H₂O₂, may contribute to demyelination by peroxidation of myelin lipid. At foci of demyelination, H₂O₂ may be converted to other more highly reactive species such as the hydroxyl radical, lipid-free radicals, and hydroperoxides amplifying tissue injury. The phospholipids, glycoproteins, glycolipids, glycerides, and sterols present in myelin and axolemma membranes are susceptible to peroxidation by H₂O₂ and reactive oxygen species derived from H₂O₂. By reducing the levels of H₂O₂ in the optic nerve, PEG-catalase may have attenuated peroxidation of myelin lipid, resulting in less demyelination and exerting this protective effect throughout the course of EAE.

Detoxification of H₂O₂ with catalase reduced edema and demyelination of the optic nerve and suppressed BBB permeability. Unlike some other studies of EAE in which agents were administered before or at the same time as EAE induction, administration of PEG-catalase after Gd-DTPA enhancement of the optic nerves as visualized by MRI is logistically possible in patients with acute optic neuritis. The safety of PEG-catalase has been demonstrated in animals showing no effect on the outcome of severe head injury with the oxygen radical scavenger polyethylene glycol-conjugated superoxide dismutase: A Phase II trial.

Key Words
optic neuritis, experimental allergic encephalomyelitis, hydrogen peroxide, free oxygen radicals, multiple sclerosis

References
18. Freeman BA, Turrens JF, Mirza Z, et al. Modulation of oxidant lung injury by using liposome-entrapped...