

TREATMENT OF DISEASES OF THE CENTRAL NERVOUS SYSTEM USING URIC ACID AS A SCAVENGER OF PEROXYNITRITE

FIELD OF THE INVENTION

The field of the invention is the treatment of diseases of the central nervous system using either a nitric oxide scavenger, a peroxynitrite scavenger, or an agent that interferes with the activity or cellular production of the enzyme, inducible nitric oxide synthase (iNOS).

BACKGROUND

The overproduction in the body of nitric oxide (NO) and/or peroxynitrite (ONOO⁻) has been suggested by some to be a contributing factor to diseases of the central nervous system, particular those that are immune-mediated and/or inflammatory.

The enzyme iNOS is responsible for the production of nitric oxide during an immune response. Nitric oxide combines with superoxide (O₂⁻) to form peroxynitrite. Those molecular level considerations are relevant to the present inventions.

An extensively used model system to study multiple sclerosis, an example of a disease treated by the present invention, is experimental allergic encephalomyelitis (EAE) in rats and mice. This model was used for the experiments described below.

BRIEF SUMMARY OF THE INVENTION

The present invention, in a general aspect, is the process of treating a disease diagnosed as a disease of the central nervous system with an agent from one or more of the following three classes of agents: (1) nitric oxide scavengers, (2) peroxynitrite scavengers, and (3) agents that either interfere with the synthesis of iNOS in the cell or the enzymatic activity of iNOS in the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Measurement of local NO levels in EAE using spin-trapping and EPR-spectroscopy.

FIG. 2. Effect of daily administration of c-PTIO (carboxy-PTIO) on EAE in SWXJ-14 mice. EAE was induced in SWXJ-14 mice by two subcutaneous immunizations (d.0 and d.7) of 100 mg PLP in CFA over two injection sites. Mice (N=3) were treated beginning on day 5 post-immunization with 2 mg/mouse c-PTIO twice daily i.p. and was continued until day 16 post-immunization (day 0 being the day of first immunization). Mean severity scores were graded as detailed in Table 1.

FIG. 3. Effect of daily administration of c-PTIO on NO levels in the brain and spinal cord of SWXJ-14 mice immunized with PLP. SWXJ-14 mice were immunized as described below for FIG. 1. Beginning 4 days post immunization, mice (n=7) were treated with 2 mg c-PTIO given i.p. until 16 days post-immunization. Mean severity scores were graded as detailed in Table 1. Mean nitric oxide levels in brain and spinal cord were semi-quantitated using spin-trapping with DETC and EPR spectroscopy at 17 days post-immunization as described elsewhere [2,10]. (For brain or spinal cord results are represented as follows: Left hand column: untreated. Center column: treated with c-PTIO. Right hand column: no EAE control)

FIG. 4. Effect of D609 on the development of EAE in SWXJ-14 mice. SWXJ-mice were immunized with PLP as detailed for FIG. 2. Mice (n=2) were treated i.p. with 1 mg/mouse D609 from day 5 through day 14 post-immunization. Mean severity scores were graded as detailed in Table 1.

FIG. 5. D609 inhibits nitrite production by activated A549 cells in vitro. The accumulation of nitrite over time was measured in the presence and absence of D609. Human A549 cells were activated with IL-1 β (100 units/ml), γ IFN (500 units/ml) and TNF α (10 ng/ml), and the accumulation of nitrite over time was measured using the Griess reaction [15]. The concentration of nitrite was calculated using a standard solution of nitrite in culture media.

FIG. 6. Effects of PTIO on clinical severity of EAE in Lewis rats.

FIG. 7. Comparison of PTIO and carboxy-PTIO on EAE in SJL mice. For each day, the left hand column represent results for PTIO-treated mice, the center column shows results for c-PTIO-treated mice, and the right hand column shows results for untreated mice.

FIG. 8. Mean levels of Nitric Oxide in EAE immunized SJL mice 18 days post-immunization.

FIG. 9. Effect of Carboxy-PTIO on EAE in SWXJ-14 mice.

FIG. 10. Mean levels of Nitric Oxide in EAE-reduced SWXJ-14 mice. (For the results with the brain, and the results for the spinal cord, the left hand column represent results with untreated mice, the right hand column represents results with c-PTIO-treated mice.)

FIG. 11. Effect of carboxy-PTIO on EAE in SWXJ-14 mice.

FIG. 12. Effect of the administration of various doses of uric acid on EAE in SWXJ-14 mice. EAE was induced in SWXJ-14 mice by two subcutaneous immunizations (d.0 and 7) of 1900 mg PLP in CFA over two injection sites. Mice (n=5) were treated once daily, beginning on day 5 post-immunization, with the indicated doses of uric acid. Mean severity scores were graded as detailed in Table 1.

FIG. 13. Effect of the administration of uric acid on brain and spinal cord NO levels and the clinical severity of EAE in SWXJ-14 mice. SWXJ-14 mice were immunized with PLP in CFA as described for FIG. 12 and treated once daily with the indicated doses of uric acid i.p. At day 16 post-immunization the animals were euthanized and NO levels in brain and spinal cord assessed as detailed for FIG. 3. Results are for individual mice. Each central white column represents the spinal cord NO level. The black column to the left of a white column represents the clinical severity score. The cross hatched column to the right of a white column represents the brain NO level.

FIG. 14. Comparison of the survival of SWXJ-14 mice with EAE treated with PTIO and uric acid. SWXJ-mice were immunized with PLP in CFA as described for FIG. 12 and treated once daily with a 2 mg dose of uric acid or PTIO i.p. from day 5 to day 13, followed by two daily doses on day 14 and three daily doses thereafter.

DETAILED DESCRIPTION

Glossary and Definitions

"PLP" is proteolytic protein from the myelin sheath, specifically PLP 139-151 (8) (The "8" in parenthesis refers to reference 8, below.)

"MEP" is myelin basic protein, an autoantigen from the myelin sheath of nerves, a target of much damage in multiple sclerosis.

"pMBP" is a peptide with an amino sequence found in MBP.

"MS" is multiple sclerosis.

"PTIO" is 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide.

"Carboxy-PTIO" is 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide.

"Uric acid" is 2,6,8-trihydroxypurine.

"D609" is tricyclodecan-9-yl-xanthogenate. D609 is believed to block the activation of PC-PLC by blocking PC-PLC activation.