

**Nitric oxide synthase is present in the cerebrospinal fluid of patients with active multiple sclerosis and is associated with increases in cerebrospinal fluid protein nitrotyrosine and S-nitrosothiols and with changes in glutathione levels**

Vittorio Calabrese , Giovanni Scapagnini , Agrippino Ravagna , Rita Bella , Roberta Foresti, Timothy E. Bates , Anna-Maria Giuffrida Stella , Giovanni Pennisi

Correspondence to Vittorio Calabrese, Biochemistry and Molecular Biology Section, Department of Chemistry; Faculty of Medicine, University of Catania, Viale Andrea Doria No. 6, 95100 Catania, Italy

Nitric oxide (NO) is hypothesized to play a role in the immunopathogenesis of multiple sclerosis (MS). Increased levels of NO metabolites have been found in patients with MS. Peroxynitrite, generated by the reaction of NO with superoxide at sites of inflammation, is a strong oxidant capable of damaging tissues and cells. Inducible NO synthase (iNOS) is up-regulated in the CNS of animals with experimental allergic encephalomyelitis (EAE) and in patients with MS. In this study, Western blots of cerebrospinal fluid (CSF) from patients with MS demonstrated the presence of iNOS, which was absent in CSF from control subjects. There was also NOS activity present in both MS and control CSF. Total NOS activity was increased (by 24%) in the CSF from MS patients compared with matched controls. The addition of 0.1 mM ITU (a specific iNOS inhibitor) to the samples did not change the activity of the control samples but decreased the NOS activity in the MS samples to almost control levels. The addition of 1 mM L-NMMA (a nonisoform specific NOS inhibitor), completely inhibited NOS activity in CSF from control and MS subjects. Nitrotyrosine immunostaining of CSF proteins was detectable in controls but was greatly increased in MS samples. There were also significant increases in CSF nitrate + nitrite and oxidant-enhanced luminescence in MS samples compared with controls. Additionally, a significant decrease in reduced glutathione and significant increases in oxidized glutathione and S-nitrosothiols were found in MS samples compared with controls. Parallel changes in NO metabolites were observed in the plasma of MS patients, compared with controls, and accompanied a significant increase of reduced glutathione. These data strongly support a role for nitrosative stress in the pathogenesis of MS and indicate that therapeutic strategies focussed on decreasing production of NO by iNOS and/or scavenging peroxynitrite may be useful in alleviating the neurological impairments that occur during MS relapse. © 2002 Wiley-Liss, Inc.

