Cyclic Guanosine Monophosphate (cGMP), Nitrite and Nitrate in the Cerebrospinal Fluid in Meningitis, Multiple Sclerosis and Guillain-Barre Syndrome

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Recent evidence suggests the involvement of nitric oxide (NO) in inflammation and demyelination in the brain. To test this hypothesis, we measured NO markers in the cerebrospinal fluid from patients with bacterial meningitis (BM), aseptic meningitis (AM), multiple sclerosis (MS), and Guillain-Barre syndrome (GBS). Subjects with non-inflammatory neurologic diseases served as the controls. NO markers were cyclic guanosine monophosphate (cGMP) measured with an enzyme immunoassay, and nitrite and nitrate measured with the Griess reaction. Except for BM, cGMP was not increased in AM, MS or GBS compared with the controls. Nitrite and nitrate were unaltered in any of the groups studied. These results do not support the hypothesis that NO is increased in the brain in meningitis, MS or GBS. Otherwise cGMP, nitrite and nitrate in the cerebrospinal fluid do not reflect the increase in NO in the brain.

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Introduction

Nitric oxide (NO) plays multiple roles in the brain: as a neurotransmitter (1), as a vasodilator (2), and as an immune mediator (3). In the immune system, NO has both protective (4) and injurious actions (5, 6) but its role in brain diseases remains unknown. In the animal models of meningitis (3) and encephalitis (7), the mRNA expression of inducible NO synthase (iNOS) is increased. In humans, Visser et al (8) reported that patients with meningococcal meningitis have greatly increased concentrations of NO metabolites in the cerebrospinal fluid (CSF) while Milsen et al (9) observed only a mild increase in patients with meningitis.

NO is implicated also in multiple sclerosis (MS). NO mediates toxicity toward oligodendrocytes, resulting in demyelination in vitro (6). NO is localized in the spinal cord of mice with experimental allergic encephalomyelitis (EAE) (10), the animal model of MS. mRNA expression of iNOS is increased not only in EAE (7), but also in the brain of patients with MS (11). Guillain-Barré syndrome (GBS) is another inflammatory neurologic disease which is different from meningitis and MS. Although NO-inducible cytokines are increased in the CSF (12) and serum (13) in GBS, there has been no study on NO in GBS.

The present study was undertaken to determine if NO is increased in meningitis, MS and GBS and if there is any difference in NO production in these diseases. We measured cyclic guanosine monophosphate (cGMP), nitrite (NO₂⁻) and nitrate (NO₃⁻), common markers of NO (14), in CSF from patients with bacterial meningitis (BM), aseptic meningitis (AM), MS and GBS.

Patients and Methods

The patients were divided into five groups. (i) The control group consisted of 29 patients (age 54.0±3.8 years) with one of the following non-inflammatory neurologic diseases: tension headache, cervical spondylosis, myotonic dystrophy, amyotrophic lateral sclerosis, spino-cerebellar degeneration or Parkinson’s disease. (ii) BM: age 48.7±5.9 years; n=9. The patients had severe febrile illness, meningeal signs and polymorphonuclear pleocytosis (650–12,000/µl) in CSF. The pathogenic bacteria were Neisseria meningitidis (n=2), Streptococcus
pneumoniae (n=2), Streptococcus viridans (n=1), Staphylococcus aureus (n=1), unknown (n=3). (iii) AM: age 35.3±2.0 years; n=15. The patients had fever, headache and CSF pleocytosis (50–900/μl) with mononuclear predominance. The clinical course was benign. (iv) MS (Relapsing-remitting type) (15): age 34.6±4.1 years; n=7. All had rapidly progressive motor weakness of lower and upper extremities, areflexia and mild sensory signs. CSF showed normal cell counts in all subjects. The concentration of protein was increased in five patients. Two patients had normal CSF protein initially with a later rise in subsequent lumbar punctures.

CSF samples, obtained by lumbar puncture, were frozen immediately and stored at −80°C. Total protein, glucose, white blood cell counts and the presence of bacteria were tested by routine laboratory methods. The cGMP concentration was assayed in duplicate with a commercial enzyme immunoassay kit (Amersham). The samples (50 μl), acetylated in an acetic anhydride-triethylamine mixture, was incubated with 100 μl of rabbit anti-cGMP serum at 4°C for 2 hours. Then 100 μl of cGMP-peroxidase conjugate was added and the samples were incubated at 4°C for 1 hour. After aspiration of unbound conjugate, 200 μl of enzyme substrate was added. A blue color, which can be read at 630 nm, developed after the incubation at room temperature for 30 minutes.

NO2− and NO3− (the latter after reduction to NO2−) were measured by a microplate version of the Griess reaction (14). NO3− was reduced to NO2− in the presence of 0.1 U/ml nitrate reductase (Aspergillus species; Boehringer-Mannheim). Then 2% sulfanilamide (150 μl) in 1M H3PO4 was mixed with 100 μl of the sample. After 10 minutes, 150 μl of 0.2% naphthylenediamine in water was added. The absorbance at 546 nm was measured after incubation at room temperature for 10 minutes.

Statistical analysis was performed by means of Kruskal-Wallis test for multiple comparisons. Two individual mean values were compared post hoc with Dunnett’s t-test. Regression analysis was done by the method of least squares.

Results

Figure 1 shows that the markers of NO were generally unaltered in the patient groups studied. The cGMP concentration in CSF in the control group was 2.4±0.4 nM. With the exception of AM (10.4±2.6 nM, *p<0.05), cGMP was not increased in AM (2.5±1.1 nM), MS (2.3±0.6 nM) or GBS (1.8±0.6 nM). NO2− and NO3− were unaltered in AM (0.56±0.08, 7.4±0.6 μM respectively), MS (0.58±0.06, 8.7±1.6 μM), and GBS (0.58±0.06, 7.4±2.0 μM) compared to those of the controls (0.63±0.04, 7.6±0.7 μM). cGMP, NO2− and NO3− did not correlate with the number of white blood cells in CSF or the age of the subjects in any of the groups studied (−0.305<r<0.259). There was no correlation between cGMP and NO2− or NO3− (r=−0.006, −0.025, respectively).

Discussion

In the present study, cGMP, NO2− and NO3−, were generally unaltered in the groups studied. The only significant change was the increase in cGMP in BM.

In the animal model of meningitis, Campbell et al (3) reported that the expression of iNOS mRNA is increased in the glia of the inflammatory lesions. Visser et al (8) reported that NO2− and NO3− are increased in CSF in patients with meningococcal meningitis while Milstien et al (9) observed only a mild increase in CSF NO2− in patients with BM and AM. In the present study, NO2− and NO3− in BM and AM remained similar to those in the controls. Multiple factors, i.e. pathogens, treatment and the status of the patients, could explain these discrepant results. For example, Visser et al (8) focused on meningococcal meningitis and studied 94 patients whereas our BM group included only two patients with meningococcal meningitis. It may be that only particular bacteria increase NO production in the brain.

Milstien et al (9) question if NO2− and NO3− in CSF originate in the brain in meningitis. They indicated that the serum nitrate concentration, much greater than that in CSF, may contribute to the increase in CSF nitrate through the damaged blood-brain barrier. Another problem with NO2− and NO3− as NO markers is that they derive not only from the metabolism of NO but also from other sources, such as food (16).

cGMP may not be a specific marker of NO in vivo as it is in vitro (14). The increase in cGMP without an increase in NO2− and NO3−, as in BM in the present study, suggests that the activation of guanylate cyclase is caused by another molecule (17). The lack of correlation between cGMP and NO2− and NO3− in the present study is additional evidence for the activation of guanylate cyclase without NO.

As in inflammatory diseases in other tissues, NO production is considered to be increased in meningitis (3) but previous studies have not all supported this hypothesis. First, investigators are not necessarily successful in inducing high-output NO synthesis from human cells cultured in vitro by treatment protocols with cytokines that are highly effective with rodent cells (18, 19). Secondly, some inflammatory cytokines decrease constitutive NOS (20) but increase the other type, iNOS. Moreover, other cytokines which are increased in the CSF from patients with BM inhibit even iNOS. Therefore, the effect of other cytokines cannot be discounted.

Lin et al (10) showed that NO is localized in the spinal cord of mice with EAE. iNOS mRNA is increased both in the mouse brain with EAE (7) and in the brain of patients with MS (11). Although these studies suggest that NO is increased in the MS brain, NO markers in CSF were not increased in the present study. One explanation is that the increase in NO is sequestered in a closed compartment of the brain and is not reflected to the CSF space. This is not the case with GBS in which the demyelinating lesion is exposed to the CSF space. To our knowledge, there is no study on NO in GBS although NO-inducible...
cytokines are increased in CSF (12) and serum (13) in GBS.

In conclusion, the present study does not indicate an increase in NO in the brain in meningitis, MS or GBS; or cGMP, \( \text{NO}_2^- \) and \( \text{NO}_3^- \) in the CSF are not reflective of the increase in NO in the brain.

References

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