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Presumed Hydrogen Sulfide-Mediated Neurotoxicity Following *Streptococcus Anginosus* Group Meningitis

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Abstract

Hydrogen sulfide (H₂S) is an environmental toxicant and gaseous neurotransmitter. It is produced enterically by sulfur-reducing bacteria and invasive pathogens including *Streptococcus anginosus* group, *Salmonella* and *Citrobacter*. We describe putative focal H₂S neurotoxicity following *S. constellatus* meningitis, treated with adjunctive sodium nitrite and hyperbaric oxygen therapy.

Keywords

hydrogen sulfide; meningitis, bacterial; *Streptococcus milleri* group; sodium nitrite; hyperbaric oxygenation

Detrimental outcomes following bacterial meningitis remain despite improvements in supportive care. Alternative conceptual approaches to meningitis and novel therapies are needed.

Salmonella and *Citrobacter* meningitis, usually restricted to neonates and the immunocompromised, are sometimes associated with prolonged progression under therapy. We report our recent experience treating meningitis caused by a member of the *Streptococcus anginosus* group (SAG), also with prolonged progression despite therapy. The unusual features of meningitis in our patient suggested that a major metabolite, hydrogen sulfide (H₂S), might be involved in pathogenesis.(1) H₂S production is shared with *Salmonella*, *Citrobacter*, and other bacterial pathogens of epidemiologic importance.

H₂S occurs in significant concentrations in livestock wastes and petroleum. Farmers and oil industry workers lose consciousness after inhalation of the gas. Exposure is often lethal.(2) H₂S is produced as a gaseous neurotransmitter and enterically by sulfate-reducing anaerobic bacteria.(3;4) It is less well recognized that many pyogens produce H₂S from organic substrates such as cysteine.(5) Localized H₂S neurotoxicity from bacterial infection and its therapy have not been previously described .

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Case Report

A 4 year old boy had 2 weeks of fever, lethargy and neck stiffness. He was hospitalized with a temperature of 103F, encephalopathy, brisk deep tendon reflexes, meningismus and intermittent posturing suggestive of raised intracranial pressure. White blood cells (WBC) were 36,000/mm³ and C-reactive protein was 13.0.mg/dl Contrast enhanced computed tomography of the head demonstrated acute hydrocephalus with transependymal cerebrospinal fluid (CSF) flow and basilar enhancement. An external ventricular drain was placed. CSF showed WBC count of 1515 (72% neutrophils), protein of 83 gm./dl, and glucose of 50 mg/dl with negative Gram stain. Vancomycin, cefotaxime and metronidazole were started. No vasoactive infusions were needed. CSF cultures grew *Streptococcus intermedius*. A detailed immunologic evaluation was negative.

The child became interactive, but with poor tracking, upper motor neuron signs and tonic stiffening. Electroencephalogram indicated diffuse slowing without any electrographic seizures. Magnetic resonance imaging (MRI) of the brain showed fluid levels in the dependent part of the lateral ventricles that were hypointense on T2, isointense on T1 weighted sequences and demonstrated restricted diffusion on diffusion weighted MRI (DWI), which was confirmed by the derived apparent diffusion coefficient (ADC) maps. Restricted diffusion, apparent as areas of low ADC, is consistent with cytotoxic edema, such as that observed early on with ischemic stroke. Restricted extraaxial diffusion was also seen in the suprasellar cistern and left cerebellopontine angle, consistent with pus. Foci of restricted diffusion were present in the posterior aspect of right basifrontal region and along the lateral wall of the anterior third ventricle. Cerebral parenchyma otherwise showed normal morphology and signal intensity with normal intracranial vascular flow voids. Transcranial Doppler (TCD) measured elevated mean arterial velocities and low resistive indices in the middle cerebral arteries. Intracranial pressure was well controlled. Seizure prophylaxis (Levetiracetam) and spasticity management (Baclofen) were begun. In spite of falling inflammatory markers, the child's neurological exam worsened. He exhibited severe agitation and spasticity and could no longer follow commands. Repeat DWI verified expansion of the affected areas to include occipital, parietal and frontal lobes, corpus callosum and basal ganglia (See Figure, Supplemental Digital Content 1, <http://links.lww.com/INF/B359>). In view of continued deterioration, a brain biopsy was performed in the third week of illness. No organisms were isolated, and vasculitis or other neurodegenerative processes were ruled out. Electron microscopy showed no dilatation of the mitochondrial cristae.

A detailed literature search identified the propensity for H₂S production by SAG.(1) H₂S-mediated inhibition of the cytochrome c oxidase (CCO) enzyme involved in ATP generation in affected neurons was plausible.(6) We were initially concerned that an acute intoxication by H₂S, elaborated by bacteria that were rapidly killed, would not be amenable to directed therapy weeks later. Fortunately, mitochondrial CCO has a very long half-life, such that sulfide bound tightly to CCO would result in dysfunction persisting for weeks.(7) We hypothesized that treatments directed at disinhibiting CCO might also be effective after several weeks.

Dipyridamole was started to increase serum nitrite and cause mild methemoglobinemia (metHb).(8) Plasma metHb was 1.1-1.2 mg% (normal 0-2.0) on 4 mg/kg/day of dipyridamole; plasma or urinary nitrites were not measured. He received six hyperbaric oxygen (HBO) treatments over two weeks.(9) Each Table 6 dive consisted of 100% oxygen for 4 hours 45 minutes with maximum pressure at 3 atm under conscious sedation. TCD velocities and resistance, unchanged for weeks, improved. He improved in alertness, visual tracking, and slightly in tone. He was able to swallow pureed food. HBO was continued

twice weekly, with incremental effect, until surgery on HD 59 to evacuate a subdural fluid collection, when no further effect was noted. He was discharged home at 12 weeks.

On follow up six months later he has cognitive and motor impairments with stable brain volume loss by MRI. He is on anti-seizure medications for clinical tonic seizures and has a ventriculoperitoneal shunt and a baclofen pump.

Discussion

This case suggests a novel mechanism for neurologic injury following infection, with therapeutic implications. This boy developed severe basilar meningitis caused by *S. anginosus* group (SAG). A rolling progression of cytotoxic edema and laminar necrosis passed through contiguous regions of cortex during a period of 4 weeks. Laminar necrosis is the product of severe hypoxia-ischemia or mitochondrial intoxication and is not associated with bacterial meningitis. SAG bacteria are prolific producers of H₂S.(1) Our patient's biphasic course of initial improvement, then severe encephalopathy and spasticity, with laminar necrosis by MRI, is consistent with possible non-fatal H₂S intoxication.

H₂S production by bacteria may involve 3 distinct pathways.(5) The volume of H₂S produced varies by species and is determined post-transcriptionally.(1) SAG synthesizes large volumes of H₂S by the action of beta-C S lyase on its substrate, cysteine. Interconvertible at physiological pH, cysteine (10μM) and cysteine (300μM) are present in CSF.(10;11) Extracellular cysteine is secreted by activated phagocytes.(12) H₂S inhibits phagocytic killing. The direct mitochondrial toxicity of H₂S diffusing into tissues may explain in part the propensity for abscess formation by SAG relative to most viridans streptococci.(13) Other organisms producing copious H₂S include *Citrobacter* and *Salmonella*, notorious for abscess formation after neonatal meningitis.(14)

H₂S is a reversible CCO inhibitor producing effects similar to cyanide intoxication, characterized by instant coma, recovery within 24 hours, followed by abrupt irritability, agitation, confusion and spasticity 2-10 days later.(2) Sulfide is a noncompetitive inhibitor of oxygen kinetics in CCO (6) Whereas the binding site for O₂ is the Fe³⁺ of the heme a₃-Cu_B site, sulfide enters the catalytic cycle by binding to and reducing the Cu_B.(6) (See Figure, Supplemental Digital Content 2, <http://links.lww.com/INF/B360>) In this way, both sulfide and O₂ act as substrates for CCO, with downstream increases in the membrane potential and subsequent ATP production.(6) Yet at any concentration of H₂S significantly above its K_i of 0.2μM, the enzymatic activity of CCO is insignificant.

Accepted treatment goals for H₂S toxicity include eliminating the exposure, inhibiting free sulfide binding to cytochrome oxidase, and promoting detoxification. It is trivial to eliminate microbial exposure through appropriate use of antimicrobials but local tissue sources for H₂S may persist until drained. Previous reports on inhalational intoxications recommend amyl or sodium nitrite and HBO therapy. Nitrite reacts with sulfide to release NO and also induces metHb, which combines with hydrogen sulfide to form sulfmethemoglobin. (See Figure, Supplemental Digital Content 2, <http://links.lww.com/INF/B360>) Metabolism of H₂S occurs by 2 oxygen-dependent enzymatic pathways involving mitochondrial enzymes sulfur dioxygenase (SDO), affected in ethylmalonic encephalopathy, and cytochrome c oxidase (CCO).(7) At low partial pressures of oxygen, SDO activity slows and sulfide accumulates to act as a powerful inhibitor of CCO at micromolar concentrations. An increase in dissolved oxygen during HBO should increase the activity of SDO and CCO. As sulfide is both inhibitor and substrate of CCO, supraphysiologic oxygen tension will accelerate sulfide consumption. (See Figure, Supplemental Digital Content 2, <http://>

links.lww.com/INF/B360) Increased enzymatic rates of CCO and SDO will also provide energy to a system with compromised respiration.

Because we had no direct evidence for H₂S neurotoxicity, induction of 7% methHb after several weeks of illness seemed too aggressive. Dipyridamole subtly increases serum nitrite in animal models, and has a favorable safety profile.(8) While dipyridamole is a potent antioxidant, the extent of its interaction with H₂S is unknown.(15) Dipyridamole appeared to improve the patient's alertness and oral skills. We then administered HBO, with favorable results on alertness and spasticity. Improvement ceased with evacuation of a subdural collection, suggesting that parameningeal sinks for sulfide may diffuse H₂S into tissues for up to 59 days. Similar beneficial effects of HBO after significant delays have been reported by others.(16)

Our report of a single patient limits generalization. The pathogenesis of bacterial meningitis is complex, while critical care, neurosurgical procedures, and use of baclofen introduce additional confounding regarding temporal causality or mechanism. We provide no direct evidence for H₂S toxicity in the patient. Assays for bacterial metabolism of organic sulfate are not standardized, so quantitation of H₂S production by this specific isolate was not done. Brain biopsy did not include sufficient frozen tissue to quantify mitochondrial respiration in retrospect. Indirect evidence includes a prolonged, atypical disease course without other explanation by brain biopsy; laminar necrosis by imaging; identification of a pathogen established as a potent H₂S producer, and clinical and ultrasonographic response to therapies directed at H₂S intoxication. Unlike neonatal meningitis caused by *Salmonella* or *Citrobacter*, there was no parenchymal cavitation; like these entities, contrast enhancement was minimal.

Conclusion

We propose H₂S toxicity as a plausible, and possibly common, mechanism for complicated meningitis or brain abscess involving *S. anginosus* group or *Enterbacteriaceae*, particularly *Salmonella* or *Citrobacter*. Treatment of H₂S-mediated neural damage would require specific medical interventions in addition to antibiotics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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