Tremorgenic Encephalopathy: A Role of Mycotoxins in the Production of CNS Disease in Humans?

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ABSTRACT: We describe a young man who, shortly after exposure to moldy silage, developed a neurological syndrome consisting of dementia and a remarkable tremor which was precipitated by movement. All symptoms resolved within one week. Despite investigation, no definitive diagnosis was reached. We propose that this patient's illness may have resulted from inhalation exposure to a tremorgenic mycotoxin.

RÉSUMÉ: Encéphalopathie toxique provoquant de tremblement: rôle des mycotoxines dans la genèse des maladies du SNC chez l'humain. Nous décrivons le cas d'un jeune homme qui, peu de temps après avoir été exposé a de l'ensilage moisi, a développé un syndrome neurologique caractérisé par une démence et un tremblement sévère qui était déclenché par le mouvement. Tous les symptômes se sont résolus en une semaine. Malgré l'investigation, aucun diagnostic précis n'a été posé. Nous proposons que la maladie de ce patient a pu résulter de l'exposition par inhalation à une mycotoxine provoquant du tremblement.

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The recognition of neurotoxin-produced disease is often difficult and, as a result, many neurotoxicological diseases may go unrecognized.[1] We describe a patient who presented with a clinical syndrome consisting of muscular tremor and encephalopathy that has not been described previously. Tremorgenic compounds are produced by several fungal species, and produce a recognizable clinical syndrome in animals. Because of the circumstances of our patient's exposure and the similarity between his syndrome and that of the animal model, we propose that he may have been exposed to a tremorgenic mycotoxin. This may be the first description of mycotoxin disease involving the central nervous system of children.

Clinical History

A 16-year-old young man had assisted his older brother and father in removing moldy fodder from the silo of the family's dairy farm. This silage had been stored over the winter and consisted of red clover, timothy grass and alfalfa. No pesticides had been applied to this crop. While his father and brother worked within the silo removing the moldy silage, the patient worked at ground level clearing the removed material from the end of a discharge chute. As the patient's work area was enclosed by the barn, it became quite dusty. No respiratory protection was worn by any of the workers.

Within hours of completing the job, all three workers felt unwell, complaining of malaise, fatigue, and headache. The patient and his father became febrile with the patient's temperature reaching a high of 41.1°C in the course of the next two days. They also experienced chills, nausea and vomiting. None of the three experienced any respiratory symptoms. By 48 hours, the patient's father and brother had recovered and his flu-like symptoms had resolved. However, by this time he had developed progressive somnolence, slowness of thinking, and an incapacitating tremor, which prompted his admission to hospital for assessment on the fourth day of his illness.

Prior to this illness, the patient was healthy and an above average student. There was no history of tobacco or any illicit drug use.

Physical examination of the patient demonstrated normal findings outside the neurological system. Notably, his chest was clear with a respiratory rate of 20/min. He held himself rigidly and his movements were slow. Muscle twitches were seen to occur continuously in all the major muscle groups of the body, including those of the face and tongue. These were initially interpreted as coarse fasiculations. He had a large amplitude 2-3 Hz tremor with movement that did not vary with posture, but did increase with anxiety. He walked on a broad base and only with the support of two people. He was unable to stand unassisted. When sitting, he required one arm support. During mental status examination, he was slow in responding to questions. He was not oriented to place or time. His attention span was reduced. His fund of knowledge was inadequate for his level of schooling. He was unable to add two digit numbers.

Blood counts included a normal hemoglobin, with a WBC of 3.7 x 10^9 cells/l and normal differential. Electrolytes, creatinine, and liver function tests were normal. Ammonia was normal. Random glucose was 4.4 mmol/l. Creatinine kinase was normal. His ESR was 34 mm/hr. Both Rheumatoid factor and VDRL were negative. Serum immunoglobulins were normal.

Cerebrospinal fluid analysis showed 144 x 10^6 cells/l RBC (felt to be traumatic), and 3 x 10^6 cells/l WBC , all monocytes. CSF glucose was 2.6 mmol/l and protein was elevated at 61 mg/dl. CSF immunoglobulins were within normal limits. Gram stain was negative, as were bacterial cultures. Viral culture was negative including Eastern equine, Western equine, California, Powassan and St. Louis agents.

Urine toxicology, four days after initial symptoms, demonstrated an unidentified compound which was interpreted as a possible nicotine degradation product. The sample was discarded because of the lack of a

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specific syndrome related to nicotine, and the frequency of nicotine degradation products as a contaminant using this assay.

The electroencephalogram showed a generalized dysrhythmia, felt to be consistent with a toxic encephalopathy. A CT scan of the head was normal. The chest x-ray was clear. Electromyography, obtained on day 6 of the illness was normal.

Precipitin tests in both concentrated and unconcentrated serum were negative for Thermoactinomyces vulgaris, Micropolyspora faeni, Aspergillus fumigatus, Aureo pullulans, pigeon serum and farmer's lung. Antibody and antigen tests for Aspergillus flavus, fumigatus and niger were negative when sent on day 6 of the illness. Serology by complement fixation for influenza A and B, measles, mumps, CMV, herpes, varicella zoster, mycoplasma, and Q fever demonstrated no increase.

Samples of the moldy silage were subsequently cultured, yielding Aspergillus fumigatus, niger, flavus, and clavatus, and the species Rhizopus, Mucor, Pnecilomyces, Penicillium, and Cephalosporum.

An occupational medicine consult was obtained. There had been no insecticide applied to the silage crop. Sevin, an organophosphate insecticide, had been aerially sprayed in an adjacent area the previous summer, but this pesticide is rapidly biodegraded. Air was sampled from within the silo with no excessive nitrogen dioxide or carbon dioxide being found. Modest amounts of white mould were still present in the upper surface of the silage and fine white dust was easily aerosolized when the surface of the silage was disturbed. When the silo unloading machinery was activated, a cloud of dust formed at the discharge chute and this was not effectively dissipated by the ventilation fans of the barn.

Within three days of admission (seven days after symptom onset), the patient's trembling and encephalopathy had cleared entirely. He was discharged six days after admission and has remained well since. He has successfully returned to school.

Discussion

Various fungal species including Penicillium, Claviceps, and Aspergillus flavus, fumigatus, and clavatus, are known to be associated with the production of tremorgenic toxins. These fungi are frequent contaminants of cereal grains[2] and silage.[3] The production of tremorgenic mycotoxins is dependent upon the cultural conditions of the fungi. For example, production of fumitremorgins (a tremorgenic mycotoxin) increase markedly with the availability of L-tryptophan in the culture media.[4]

Rye grass staggers (Bermuda grass staggers, Marsh staggers), a disease of cattle and sheep, is produced by exposure to tremorgenic mycotoxins.[5,6] Animals with this condition exhibit tremor and an uncoordinated rocking gait. The affected animals may collapse in the field but mortality is rare. Their exposure to mycotoxins comes through direct consumption of fungally infected foodstuffs, or consumption of grass grown in infected soil.[6]

Tremorgenic mycotoxins were first isolated in 1964.[7] A toxin was extracted from Aspergillus flavus which produced tremors and stiffness in rodents. Tremor developed within 30 minutes of gastric exposure and persisted for up to two days. A tremorgen was subsequently isolated from Penicillium cyclopium.[8] Small doses of this toxin in mice produced a muscle tremor which persisted for hours. With increased doses, the animals were tremorous, irritable, weak and demonstrated loss of grasp. The highest doses were associated with clonic and tetanic seizures and sometimes with death. Survivors at the high doses continued to tremble for 24-72 hours. A number of other tremorgens, have shown similar findings of irritability, ataxia and sustained tremors. For some tremorgens (verruculogen), tremor was only evident with movement.[9] There has not been any comment about cortical function of affected animals during the symptomatic period. Intraperitoneal administration of the tremorgen verruculogen is associated with a reduction in the effective dose and LD_{50}, when compared to oral administration,[10] suggesting that pulmonary exposure could be associated with increased toxicity. The effects of tremorgenic mycotoxins appear to be temporary and fully reversible as repeated exposure to tremorgens in rodents have been associated with complete recovery without any subsequent pathological findings.[11]

The mechanism of tremor production is unclear. Penitrem A, a tremorgenic mycotoxins increased the endplate potential and duration, and increased the frequency of miniature endplate potentials in rat phrenic nerve-diaphragm preparations.[12] These changes had returned nearly to control levels in a single animal who was studied at 24 hours. Penitrem A increased the spontaneous release of glutamate, GABA and aspartate from cerebrocortical synaptosomes while verruculogen, another tremorgen increased the spontaneous release of glutamate and aspartate, but not GABA.[9] These data suggest that the actions of tremorgenic mycotoxins are presynaptic, involving increased release of amino acid neurotransmitters.

Our patient was investigated for a number of disease processes. Many of the occupational diseases associated with farming are associated with pulmonary symptoms and signs. Our patient had no chest complaints, a normal physical examination, and a normal chest x-ray. Our patient's disease was characterized by an initial flu-like illness which was followed by tremor and encephalopathy. The disease resolved without treatment or sequelae in about one week and does not appear to have been described previously.

All three workers were exposed to the moldy silage but dust conditions were probably worst at the end of the discharge chute. Such dust can contain huge numbers of micro organisms and their metabolic by-products, including fungal elements and mycotoxins. All three workers became sick within hours of completing their work. The two with the least exposure gradually recovered over 48 hours. But our patient went on to develop a prominent tremor which suggests a disease process similar to Rye grass staggers. Despite extensive testing, we were unable to demonstrate any alternative explanation for this patient's illness. We were able to recover from the offending silage, fungal species capable of production of tremorgenic toxins. At the time the patient presented, we could not find a lab capable of analyzing for the presence of specific mycotoxins. If such assays had existed, we still might not have been able to recover toxin from the patient as his presentation was 96 hours after exposure.

There were some features of our patient's disease that differed from the animal model, including the early presence of fever, and the latency between the presumed exposure and the development of tremor. Other authors[1] have commented upon the production of multiple syndromes from a single toxin, suggesting that there may be different but related syndromes in different species.

Epidemics of human mycotoxicosis have occurred where a food source was suspected as the causative agent. Recently, in one such epidemic, an appropriate mycotoxin was isolated from the food.[13] Tremorgenic mycotoxins have also been implicated in Woodtrimmers' disease.[14]

Conclusions

Generally it is difficult to establish causation where mycotoxin exposure is suspected.[1] In our case, there was a very short latency after exposure and a crude dose response. The development of disease was followed by gradual improvement in all affected individuals with the passage of time. Fungus, capable of producing tremorgenic toxins, was recovered at the site of exposure. An animal model of a similar disease exists. A mycotoxin was not recovered directly from the patient because a specific assay was not available.

This case is important because "recognition of individual cases has been of considerable value in identifying neurotoxins ... ".[1] Although causation has not been irrefutably demonstrated, it is not unreasonable to assume that our patient represents a case of human disease due to exposure to tremorgenic mycotoxins.

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