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Hypothesis: *Mycotoxins Causing Amyotrophic Lateral Sclerosis*

Reviewer's questions:

1. **Pattern of Cascade through the Neuroaxis in ALS**

2. **Biomarkers for ALS & for Mycotoxins**

I. **ALS Cascade Upper & Lower Motor Neurons** :

It is intriguing to relate the available data on neurotoxic mycotoxins with the clinical findings in ALS. ALS is a motor neuron disease with insidious, progressive weakness and muscle wasting. One of the essential clues to the diagnosis of ALS is the involvement of both upper and lower motor neuron disease. The diagnostic strategy presented by Statland et al 2015[1] defines upper motor neuron deficits with spasticity and hyperreflexia and lower motor neuron disease with muscle wasting, weakness, fasciculations, fibrillations and positive sharp waves. In ALS there is a mixture of both upper and lower motor neuron deficits. The pathology of the neurotoxic mycotoxins, especially the trichothecenes(Dai, C. et al 2019[2]), closely mimics the pathology of ALS with oxidative stress, mitochondrial dysfunction and respiratory chain damage.

Intranasal Pathways Bypass of Blood Brain Barrier & CSF :

The trichothecenes, T-2 Toxin and Deoxynivalenol, are major contaminants of the world food supply. If the opportunistic fungi responsible for these secondary metabolites develop a niche in the body by colonization or infection, these potent, lipophilic mycotoxins are able to cross barriers, especially the blood brain barrier and accumulate in the brain and spinal cord. Marcesca, M. 2013[3] reports on of transport of food-associated trichothecenes from the gut to the brain.

There is a more intriguing possibility if there is a focus of a fungal infection in the upper airway, especially the upper sinus cavity. There is a growing body of research documenting intranasal delivery of compounds and drugs to the brain and spinal cord. Thorne, R.G. et al 2004 [4]report on transport of IGF-1 in the nose of rats. Thorne, R.G. et al 2008[5] report on transfer of interferon-beta in a monkey's nose to brain. There are extensive studies documenting intranasal absorption of insulin in the objective of replacing injection requirements. Lochhead, J.J. et al 2019[6] found insulin had neuroprotective effects traveling along the trigeminal nerve. Avgerinos, K.L. et al 2018[7] found intranasal insulin in 293 patients improved memory in patients with Alzheimer's or mild cognitive deficits. Intranasal glucagon was effective in adults with type 1 diabetes and insulin-induced hypoglycemia (Rickels, M.R. et al 2016[8]). What these studies discovered are pathways bypassing the blood brain barrier as well as the CSF.

Pardridge, W.M. in 2011[9] and 2012[10]UCLA pointed out misconceptions surrounding drug and compound levels in the CSF, blood and brain. His studies showed that CSF drug levels were the same as systemic

blood levels and were not equivalent to brain or spinal cord levels. Over the past 10 years the literature has defined two intranasal pathways that bypass both the CSF and the blood brain barrier.

The upper sinus cavity is lined by olfactory mucosa that has direct passage of olfactory nerve endings exposed to the external environment. There is a second lining of respiratory mucosa fed by the ophthalmic and maxillary branches trigeminal nerve. Djupesland, P.G. et al 2014[11] describes the delivery of drugs from the nasal cavity directly into the brain. Passage is rapid in 5 minutes to 60 minutes passing by convective or bulk flow. Drugs bypass the blood brain barrier and the CSF.

The olfactory pathway passes compounds of significant size into the olfactory bulb. The drugs move outside of the neurons with movement by pulsatile flow extracellular pathways. The trigeminal pathways from nasal cavity to brain moves to the midbrain, pons and the lower motor neurons of the spinal cord. The olfactory pathway feeds into the limbic system and forebrain.

If there is an infection of the upper nasal cavity involving opportunistic fungi, such as Fusarium species that release lipophilic, neurotoxic mycotoxins, it could poison the brain and spinal cord in an insidious fashion. The pathway from the olfactory bulb to the upper brain would involve the upper motor neurons. The pathway from the trigeminal nerve branches would transport drugs to the brainstem and lower motor neurons (Djupesland, P.G. et al 2014[11], Lochhead, J.J. et al 2015[12], Ganger, S. & Schindowski, J. 2018[13]). There are studies of giving chemotherapy intranasally for glioblastoma multiforme (Bruinsmann, F.A. et al 2019[14], van Woensel, M. et al 2013[15]).

Pseudobulbar Affect :

Intranasal passage of neurotoxins into the brain and spinal cord would explain the dual injury to upper and lower motor neurons. The olfactory intranasal pathway leads to the olfactory bulb and limbic system. This pathway could help to explain pseudobulbar affect. Thakore, N.J. & Pioro, E.P. 2017[16] in Cleveland reported on pseudobulbar affect found in 209 out of 735 ALS patients. They found an association with bulbar onset and dysfunction with predominantly upper motor neuron disease. The ALS patients with pseudobulbar affect were younger in age with a shorter duration of disease. They found an association with worse bulbar findings, dysarthria and dysphagia. There was an association with the use of Baclofen, a surrogate for upper motor neuron dysfunction.

Misconceptions of Blood Brain Barrier & CSF :

Pardridge 2011[9] and 2012[10], report on the literature before 2011 with the misconceptions about CSF drug and metabolite levels. The intranasal passage of drugs and metabolites to the brain did not feed into the CSF. This leads to misconceptions when studies assume CSF levels are equivalent to brain and spinal cord levels. In order to get accurate brain levels requires Intracerebral Microdialysis, PET scans or functional MRI (Chefer, V.I. et al 2009[17], Lasley, S.M. 2019[18]).

Immunotoxicity of Mycotoxins-Immune Evasion:

The literature on trichothecenes list their immunotoxicity as possibly their most significant pathology. T-2 Toxin appears to represent the most toxic of the trichothecenes. Studies show T-2 toxin impairs the mouse response to Reovirus with suppressed immunoglobulin and interferon (Li, M. et al 2006[19]). Pestka, J.J. et al 2004[20] found immune suppression by Deoxynivalenol and other trichothecenes with initial immune stimulation then suppression as the dose increased. Studies by Obremski, K. et al 2013[21] showed T-2 Toxin suppressed CD4, CD8 and CD21 in pig ileal Peyer's patches. Wu, Q. et al 2018[22]report downregulation of interferon, TGF-beta and Toll-like Receptors(TLR) with immune evasion. This could explain the recalcitrance of treatment against fungi. It also opens up the possibility to the use of the new PD-1/PD-L1 pathway inhibitors in ALS patients.

II. **Biomarkers for ALS and Mycotoxins** :

Biomarkers for ALS :

There are extensive lists of biomarkers for ALS. Lucas, T.V. & Bowser, R. 2017[23] reviewed the fluid-based biomarkers for ALS divided into neurofilament proteins, inflammatory markers, mRNA such as C0orf72 and metabolic biomarkers. It is interesting to compare the biomarkers for ALS with the biomarkers listed for common mycotoxins. The pattern of inflammatory biomarkers and metabolites are similar in mycotoxin toxicity compared to the pathology with ALS.

Biomarkers of Mycotoxins :

Mycotoxins have garnered increasing scrutiny given their prevalence in food and human environmental exposure. Vidal, A. et al 2018[24] did a comprehensive review of mycotoxin biomarkers. The development of ultra-high-performance liquid chromatography-high resolution mass spectroscopy has improved the accuracy and sensitivity of detecting food borne mycotoxins. The main mycotoxins found were trichothecenes from Fusarium species, Deoxynivalenol, one of the most frequently occurring fungal contaminants in food and feed worldwide. Nivalenol, Fusarenon-X, T-2 Toxin and Diacetoxyscirpenol are biomarkers of Fusarium species in food.

Fumonisins are secondary metabolites of Fusarium species found mainly in maize. They cause hepatotoxicity, nephrotoxicity and are possibly carcinogenic. They are notable for causing neurologic disease such as Equine Leukoencephalopathy as well as swine pulmonary edema, esophageal cancer and neural tube defects.

Ochratoxin A and Citrinin are products of Penicillium and Aspergillus found in cereals. They are possibly carcinogenic, but notable for nephrotoxicity along with neurotoxicity and immunotoxicity.

Zearalenone is a Fusarium metabolite known as a powerful estrogen mimic causing birth defects and breast cancer.

Aflatoxins are secondary metabolites of Aspergillus species. Of all the mycotoxins in human food, aflatoxins have the strongest association with cancer. Sterigmatocystin is precursor of aflatoxin produced by Aspergillus species. It has been associated with cancer in humans.

Mitochondrial Dysfunction & Biomarkers :

Mitochondrial dysfunction is a major finding in both ALS and with poisoning by trichothecenes neurotoxins. Kreb cycle intermediates in urine rise. Erythrocyte Protoporphyrins and Urine Fractionated Porphyrins are abnormal in a pattern suggesting a toxic exposure.

Immunologic Biomarkers :

One of the most prominent abnormal findings in both ALS and mycotoxin exposure is the suppression of the immune system. Immune deficits would be potential early clues to help follow the course of ALS or mycotoxin exposure. Suggested blood tests would be, IgG Subclass, IgA, IgM, IgE

Lymphocyte Mitogen Proliferation

Cytokines, IL-6, IL-2, Interferon-gamma

Immune Evasion :

Studies for PD-1/PD-L1 pathway abnormalities could uncover evidence of immune evasion. This could explain the recalcitrance of ALS to treatments.

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