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RESEARCH ARTICLE

Neurodegenerative Diseases due to Neurotoxins passing through the Nose-to-Brain Pathway

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Abstract

Three neurodegenerative disorders- Alzheimer's dementia (ALZ), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS)- share a common feature in their pathogenesis: evidence of mitochondrial dysfunction and reactive oxygen stress. Their pathologic classifications are based on the findings at autopsy based on patterns of protein aggregates in neurons and glial cells. This pathology supports the concept that neurotoxins are a major factor in the etiology of these disorders. There is value in exploring the similarities in the pathogenesis of ALS, Parkinson Disease and Alzheimer Dementia based on non-genetic etiologies.

The nose to olfactory pathway feeds sensory input from the nasal cavity to the olfactory bulb and the entorhinal lobe. Another component of these pathways involves two branches of the trigeminal nerve with sensory input to the pons and midbrain. A key factor is their capacity to bypass the blood-brain barrier (BBB). The protection of the brain by the BBB diminishes with age and can be lost with damage from insults as with viral infections. The olfactory nerve is the only cranial nerve with direct exposure to the ambient environment and has a high rate of turnover of sensory receptors.

The nasal cavity is being studied for drug delivery and can be used to deliver medications into the central nervous system (CNS. The nose-to-brain pathway may represent a critical avenue of exposure to oxidative neurotoxins. Neurotoxic mycotoxins are a major risk to humans. Neurotoxins may be amplified by the nose-to-brain pathway. The pathology shows similarities to prion disease. These neurotoxins are highly fat-soluble and tend to accumulate in mitochondria and synaptic vesicles. Neurotoxins in synaptic vesicles can migrate from neuron to neuron.

There is evidence of chronic fungal infections in ALS patients that secret neurotoxic and immunotoxic mycotoxins leading to progressive immune suppression. The nose-to-olfactory pathway may amplify neurotoxins levels in the brain. If Parkinson Disease and ALZ are due to systemic poisonings, the source of neurotoxins may be episodic and lead to autoimmune disease.

Introduction

Three neurodegenerative disorders- Alzheimer's dementia (ALZ), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS)- share a common feature in their pathogenesis: evidence of mitochondrial dysfunction and reactive oxygen stress. Their pathologic classifications are based on the findings at autopsy and are focused on patterns of protein aggregates in neurons and glial cells. This is distinct from the pathologic findings due to viral infections. According to Dugger, B.N and Dickson, D.W. 2017[1], these protein aggregates are derived from normal proteins in neurons and glial cells that have been damaged by "proteotoxic stress" due to ubiquitin-proteosomal, autophagosomal, lysosomal-proteasomal systems, oxidative stress, programmed cell death and neuroinflammation. The main protein aggregates that define the pathologic classification of these disorders are amyloid, tau, a-synuclein and transactivation response DNA binding protein 43 (TDP-43). This pathology supports the concept that neurotoxins are a major factor in the etiology of these disorders. In research over the past decade, the focus has shifted to genetic etiologies. Although this approach has been productive, genetic factors account for only 10% or less of the etiologies. There is value, therefore, in exploring the similarities in the pathogenesis of ALS, PD and ALZ[2] based on non-genetic etiologies.

The Braak hypothesis for Parkinson Disease[3,4] proposes progressive stages of pathology starting in the olfactory bulb, "brain-first" or the vagal nerve of the GI tract, "body-first". The nose to olfactory pathway feeds sensory input from the nasal cavity to the olfactory bulb and the medial temporal lobe of brain, entorhinal lobe. Another component of these pathways involves two branches of the trigeminal nerve with sensory input to the pons and midbrain. A key factor is their capacity to bypass the blood-brain barrier (BBB). The protection of the brain by the BBB diminishes with age and can be lost with damage from insults as with viral infections such as Covid-19. The olfactory nerve is the only cranial nerve with direct exposure to the ambient environment and has a high rate of turnover of sensory receptors[5]. This creates gaps in the olfactory receptors and the BBB that are not always replaced.

Relevant to the study of these pathways is research studying the use of the nasal cavity for drug delivery[6-8]. For example, there are studies of intranasal insulin delivery aimed at avoiding the need for injections. These studies have shown limited success in treating diabetes but have found some value in delivering medications into the central nervous system (CNS). In the studies of drug delivery, the movement of drugs into the CNS is remarkably rapid. Insulin enters the brain in 5-30 minutes via the perivascular space by bulk flow following arterial pulsations[9,10].

The nose to brain pathway may represent a critical avenue of exposure to oxidative neurotoxins. The exposure to environmental toxins is extensive including inhaled pesticides such as dichlorodiphenyltrichlorethane (DDT), water borne-toxins, toxic metals, viral infections and mycotoxins[3,5]. Mycotoxins are major sources of risk due to globalization and

climate change[11]. The exposure to these environmental toxins is inherently systemic. This prompts the question: why does the pathology predominate in the CNS? One possibility is that neurotoxins are amplified by the nose to brain pathway. The pathology in neurodegenerative disorders has similarities to prion disease[1,12]. These neurotoxins tend to be highly lipophilic and may accumulate in the lipid layers of plasma membranes including the membranes of the mitochondria, lysosomes and synaptic vesicles. The neurotoxin would accumulate in synaptic vesicles and in mitochondria migrating caudal and rostral, from neuron to neuron.

In comparing neurodegenerative disorders, it is important to consider the chronologies. ALS follows a progressive time course spanning 3 to 6 years to eventual death. By contrast, there is evidence that Parkinson Disease and ALZ have long asymptomatic periods before the onset of clinical symptoms with time courses spanning decades.

In ALS, there is evidence of chronic fungal infections accentuated by immune suppression releasing neurotoxic mycotoxins. If the fungal infection is localized in the nasal cavity, then it could concentrate mycotoxins along the nose-to-olfactory pathway, effectively amplifying neurotoxins and their impact on the brain. If Parkinson Disease and ALZ are due to systemic poisonings, the source of neurotoxins may be due to episodic exposures derived pulmonary or dietary sources. The neurotoxins would enter the brain along olfactory or vagal pathways. These exposures would be accentuated by the damage to the BBB by age and past insults.

Olfactory Vector Hypothesis

Prediger, R.D.S. et al 2012[5] presented the "olfactory vector hypothesis" that proposes that intranasal neurotoxicants are responsible for Parkinson Disease. They were able to show methyl-phenyl-tetrahydropyridine (MPTP) readily damaged the nigrostriatal dopaminergic system causing Parkinson Disease-like findings. Arce-Lopez, B. et al 2021[11] attempted to measure mycotoxins in patients with Parkinson Disease and ALZ. Using liquid chromatography and mass spectroscopic analysis (LC-MS/MS), they measured 19 mycotoxins in plasma including aflatoxin, ochratoxin, the trichothecenes deoxynivalenol and T-2 toxin. They found evidence of elevated levels for ochratoxin and sterigmatocystin, but none of the other mycotoxins that were predicted. The fact that so many of the mycotoxins expected were not found prompts the question whether exposures are under-the-radar of detection. These toxins may be able to accumulate in small pockets along the BBB as well as in mitochondria membranes and synaptic vesicles. In addition, there is a longtime span in the course of Parkinson Disease and ALZ before the onset of overt clinical findings, and this could tend to mask exposures.

Olfactory Dysfunction and All-Cause Mortality

Choi, J.S. et al 2021[13] objectively measured olfactory function with a Pocket Smell Test studying 3503 individuals 47.7% men. At 5 years, in ages 40 to 64 years old, there was no association with human disease; by contrast, in those >65 years old, there was a statistically significant increase of 53% in all-cause mortality in those with olfactory dysfunction.

They found objective olfactory dysfunction associated with an increase in all-cause mortality. Olfactory dysfunction may precede by many years the emergence of neurologic symptoms in ALZ and Parkinson disease. Neurodegenerative diseases account for 22% of the increased risk of 10-year mortality in those with poor olfaction. Pang, N.Y.L. et al 2022[14] reviewed 21,601 cases finding a 52% increase in mortality in those with olfactory dysfunction. The olfactory nerve is the only cranial nerve in direct contact with the environment. The author pointed to studies finding an increase in interleukin-6 (IL-6) levels in older adults with olfactory dysfunctions, suggesting an ongoing inflammatory mechanism.

The Parameters for Passage through the Blood Brain Barrier (BBB)

Pardridge, W.M. 2011[15] and 2012[16] at UCLA studied the permeability of small molecules across the BBB. They defined the parameters for passage of molecules as those that were fat soluble (<8 hydrogen bonds) with molecular weights below 400 Da. A major exception was in the upper sinus cavity where molecules moved rapidly by bulk flow along the olfactory nerve and trigeminal nerve into the olfactory bulb to entorhinal cortices or into the pons and midbrain. The anatomic explanation for this increased permeability across the BBB was the unusually rapid turnover of the sensory receptor cells in the upper sinus cavity and the exposure to the ambient environment. When the mucosal cells lining the sinus cavity die, they leave gaps accentuating permeability until the cells can be replaced. Studies of ALS patients[17] using magnetic resonance spectroscopy for P31 showed pathologic findings limited to the midbrain at the level of the pons. PET scans of ALS patients showed hypermetabolism limited to the midbrain which was highly specific for ALS. There is a proliferation of astrocytes and selective sensitive of astrocytes to the mycotoxin, such as T-2 toxin. The hypermetabolism was reported to localize in the midbrain, pons, hippocampus, superior temporal gyrus and cerebellum[18],[19].

Nose-to-Brain Pathway for Drug Delivery

Studies of the nose-to-brain pathway have explored the delivery of drugs such as insulin into the systemic circulation[6-8]. There was no evidence of hypoglycemia, suggesting poor entry into the systemic circulation. Instead, the drugs were passing rapidly into the CNS bypassing the systemic circulation. Lochhead, J.J. & Davis, T.P.[10,20] studied the delivery of neurotherapeutics into the CNS via an IV route. They found markedly diminished drugs entering the brain across the BBB. Under physiological conditions, only small molecules under 600 Da and highly lipophilic entered the CNS by the IV route. For comparison, Pardridge 2012[16] defined the BBB cutoff as under 400 Da. The approach of delivering chemotherapy via the nose-to-brain pathway showed promise for treating brain gliomas. Studies of the nose-to-brain pathway in mice found AUC brain/AUC plasma for insulin (5.8 kDa) at nearly 2000/1, which would mean that drugs are entering the brain not the plasma except after the drugs exit the brain. The relevant areas of the nasal cavity for drug delivery are the olfactory epithelium and a respiratory epithelium. The olfactory nerve and two branches of the trigeminal nerve,

ophthalmic and maxillary, supply sensory endings to the olfactory epithelium. The trigeminal nerve supplies sensory receptors to both the olfactory and respiratory epithelium. Drugs can be directed to favor the olfactory epithelium for entry into the CNS bypassing the systemic circulation and the cerebrospinal fluid (CSF). Drugs directed to the respiratory epithelium were found in the systemic circulation and not the CNS. There are pharmacologic strategies to target the olfactory region of the nasal cavity using pressurized devices. The use of tracers on Insulin-like-Growth Factor and Interferon-B in rats and monkeys, found uptake in 30-60 minutes into the olfactory bulb and into the trigeminal nerve passing into the pons and midbrain. Movement occurred predominantly in the perivascular spaces with entry into the CNS at the level of the olfactory bulb and pons by bulk flow based on arterial pulsations. Drug entry into cells was present but very slow. Entry into the CSF did not precede entry into brain suggesting that blood and CSF entry occurred after the drugs exited the brain. The study of the use of the nasal cavity for drug delivery is exemplified by the publications of Thorne, R.G. et al 2004[21] studying IGF-1; Thorne, R.G. et al 2008[22] studying interferon; Lochhead, J.J. et al 2019[20] studying insulin along with Avgerinos, K.L. et al 2018 [23] and Rickels, M.R. et al 2016[24] studying glucagon. Many studies found that drugs and molecules with significant molecular weights such as Insulin (mol wt 5.8 kDa), could rapidly pass from the sinus cavity into the CNS along the olfactory nerve and trigeminal nerves. Insulin moved from the sinus cavity into the brain in 5-30 minutes. In another key finding in these studies, drugs were bypassing the systemic blood and CSF. This limited the value of using the nasal cavity to deliver insulin to control diabetes. It also points out the danger of using drug or toxin levels measured in the CSF as equivalent to brain levels. The drug levels in the CSF were more representative of the systemic blood levels, not the brain levels. This supports the need to use intracerebral microdialysis to assess drug and toxin levels in the CNS, not CSF[25].

Mitochondrial Dysfunction in Neurodegenerative disease

Neurodegenerative diseases have mitochondrial dysfunction in common[26]. Sassani et al 2020[17] is one of the first studies of the brain of patients with ALS using 31P-MRS scans. In ALS there is both upper and lower motor neuron damage. It was expected that there would be mitochondrial disease in both the cortices of the brain and the lower spinal cord, but instead, all the evidence of mitochondrial damage was localized in the pons and midbrain. In the peripheral skeletal muscles, the findings were consistent with denervation, not a direct toxic insult. There was no evidence of mitochondrial disease in the cortices of the brain. The authors concluded that upper motor neuron disease might be due to damage to the corticospinal and corticobulbar tracts as they passed through the pons and midbrain. This gives substantial support to the prospect that the pathology in ALS begins in the pathways from olfactory and trigeminal nerves. From the olfactory bulb, neurotoxins can pass to the entorhinal

cortices of the temporal lobe and from the trigeminal nerve passing to the pons and midbrain. The neurotoxins travel in the perivascular space caudal and rostral. Studies of ALS patients using 18F-deoxyglucose (FDG) PET scans found hypermetabolism localization in the pons and midbrain followed by the hippocampus, superior temporal gyrus and cerebellum suggesting a toxic insult with astrocyte activation. Midbrain hypermetabolism appears to be specific to ALS. These pathologic findings implicating mitochondrial disease seen in ALS extrapolate to similar findings in PD and AD.

Immune Dysfunction in ALS and Neurodegenerative Disease

Patients with ALS develop a progressive immune dysfunction that involves both humeral and cellular immunity[27,28]. Thonhoff, J.R. et al 2018[29] describes the neuroinflammatory mechanisms in ALS as an early slow phase with "anti-inflammatory" mediators and a later fast phase with "pro-inflammatory" mediators. The anti-inflammatory early phase includes immune suppressive regulatory T lymphocytes (Tregs). Gustafson, M.P. et al 2017[30] performed extensive phenotyping of 80 ALS patients. They found two distinct immune profiles. Profile 1 patients lived an average of 3 years and Profile 2 patients lived an average of 6 years. A key finding in profile 2 was the elevated immune suppressive T reg cells, CTLA4+ cells and PD1+CD4 T cells. There was a loss of HLA-DR monocytes and a gain of CD3+CD56+ T cells associated with the loss of CD28 markers. In all the ALS patients, there were prominent changes in T regulatory cells. There are similar patterns of immune toxicity seen with mycotoxins especially T-2 toxin.[31] In poisoning by T-2, there is a progressive loss of immunity. Eventually, the patient develops invasive opportunistic or nosocomial infections. There is a balance between inflammatory and immunosuppressive biomarkers.[32,33] The treatment paradigm is to address both the inflammation and the immune suppressants with immunotherapy[34,35]. In Parkinson Disease and ALZ, there is a long history of an inflammatory process along with autoimmune disease.[36-38]

Fusarium Fungal Infections as Source of Mycotoxins

Mycotoxins have become a major risk to human health and Fusarium species are a major source of mycotoxins contaminating the human environment. Fusarium infections are opportunistic[39], unable to invade except in patients with severe immune deficits such as prolonged neutropenia, cellular immune dysfunction, use of glucocorticoids, diabetes, liver cirrhosis and post hematologic transplant. None of the ALS patients examined had a level of immune suppression that would suggest an invasive fusarium infection[40], at least until late in their course of the disease. In the research studies in Spain, Alonso, R. et al 2017[41,42] reported finding Fusarium, Cryptococcus, Malassezia, Botrytis, Trichoderma and Candida in the brains of eleven ALS patients using next-generation DNA sequencing. Invasive Fusarium, Fusariosis, is the second most common invasive mould infection after Aspergillosis.

Fusarium infections are some of the most resistant to available antifungals. In textbook reviews, Fusarium species are listed as unresponsive to all available antifungals. Recent publications report Fusarium infections are sensitive to Voraconazole[39]. Fusariosis is being reported as a significant pathogen in patients with cancer, HIV, diabetes, liver cirrhosis and bone marrow transplant with reports of unusually high mortality rates approaching 75%.[39] Despite the ubiquitous nature of Fusarium in the environment, there has been a low rate of detection in human samples. This could be due to misdiagnosis as Aspergillosis. As of the 2020 publication Thornton, C.R.,[43] there were no nucleic acid-based detection systems commercially available nor any FDA approved testing systems for Fusarium. There are methods for diagnosis of Fusarium from paraffin-embedded human tissue distinguishing Fusariosis, Aspergillus, Mucormycosis and Scedosporiosis, Salehi et al, 2016[44] used PCR probes. These PCR probes were too often in-house creations. Due to the lack of available FDA approved systems, detection requires the time-consuming use of fungal cultures[45,46]. In conclusion, Fusarium is found throughout the human environment including colonization of skin, nails, the upper airway and the sinus cavity. Fusarium infections could be a source of neurotoxic and immunotoxic mycotoxins that have the potential to be causative in ALS.

Spectrum of Mycotoxins produced by Fusarium including T-2 Toxin

There are three major classes of mycotoxins secreted by fusarium species that cause animal disease- trichothecenes, fumonisins and zearalenones[47]. Of these, fumonisins and trichothecenes have been implicated in outbreaks of human disease. They both cause significant neurotoxicity. The trichothecenes are some of the most toxic of the mycotoxins commonly found in the human environment. There are four types of trichothecenes, type A, B, C and D. Fusarium secretes smaller, non-macrocytic trichothecenes such as T-2 Toxin (type A mol wt 466 Da) and Deoxynivalenol (type B mol wt 296 Da) (Thornton, C.R. 2020, Tortorano, A.M. et al 2014).[43,46] The pathology of the type A and B trichothecenes have similarity to the pathology seen in ALS (Dai, C. et al, 2019, Wu, Q. et al 2020)[31,48]. They readily cross the blood brain barrier (BBB) and accumulate in the brain even at low doses. Astrocytes have a heighten sensitivity for T-2[49]. Satratoxin is a type C trichothecene mol wt 544 Da secreted by the black mold, Stachybotrys. Sick Building Syndrome has been tied to Stachybotrys. Satratoxin could be pathogenic to the brain with significant damage to the BBB despite its molecular weight of 544 Da. [50,51] Trichothecenes are sesquiterpenes with an epoxide ring moiety[47] at the 12,13-positions. The epoxide ring moiety generates highly reactive free oxygen species and creates covalent bonds to proteins, DNA, RNA and lipids. The reactive oxygen species (ROS) induce oxidative stress reactions that damage signaling pathways including p53, MAPK, Akt/mTOR, PKA/CREB and NF-kB. They cause severe mitochondrial dysfunction with perturbation of the mitochondrial respiratory chain and reduction of available ATP. They target the 60S

ribosome and peptidyl transferase shutting down protein synthesis.

Permeability of T-2 Toxin, HT-2 and Deoxynivalenol through the BBB

By all accounts, T-2 Toxin is the most toxic of the trichothecenes secreted by fusarium[48] species. Weidner, M. et al 2013[52] studied T-2 and its main metabolite, HT-2, using *in vitro* techniques. They found that both T-2 and HT-2 readily crossed the BBB. As noted above, Pardridge predicted molecules under 400 Da with high lipophilicity could pass through the BBB. The type B trichothecene, deoxynivalenol, with a molecular weight of 266 Da, readily crosses through the BBB. T-2 toxin, molecular weight of 466 Da, was initially excluded, then there was an abrupt increase in permeability due to damage to the tight junctions.

Magnetic Resonance Spectroscopy-P31 of ALS Patients- Mitochondrial Dysfunction limited to the Pons

One of the major pathologic abnormalities in ALS is mitochondrial dysfunction. Sassani, M. et al, 2020[17] performed one of the first use of P31-magnetic resonance spectroscopy(P31-MRS) on the brain and spinal cord of ALS patients. The study scanned 20 patients with ALS compared to 10 normal controls. The initial sites of onset of ALS were bulbar in four, upper limb in six and lower limb in ten. Their El Escorial status at diagnosis had 17 probable, two possible and one definite. They scanned the precentral gyrus, the descending corticospinal and corticobulbar tracts and the midbrain including the pons. They compared these scans with scans of the proximal tibialis anterior muscle. The results found no difference between the ALS patients and controls in the scans of the brain except at the pons, where they found lower phosphocreatine, higher ADP and depressed Gibbs free energy of hydrolysis for ATP. ATP levels were unchanged. The fact that both corticospinal tract and most corticobulbar tract axons pass through the midbrain at the level of the pons might help to explain upper motor neuron deficits due to injuries at the level of the pons. The scans of the tibialis anterior muscle were consistent with denervation with reduced Gibbs free energy for ATP, unchanged ATP levels, higher inorganic phosphate, higher ADP, lower free magnesium in comparison to controls. These findings would be consistent with pathologic events in the mitochondria localized at the level of the pons and midbrain. The upper motor neuron deficits were presumed due to injuries of the corticobulbar and corticospinal tracts in the midbrain. This would imply that key pathologic events in ALS are limited to the midbrain at the level of the pons. Neurotoxins passing out of the sinus cavity along the olfactory and trigeminal nerves would support these findings.

Treatment of Immune Dysfunction with Immunotherapy

Patients with ALS develop progressive immune dysfunction. There are reports of T-cell immune deficiency[28] and subclass IgG deficiency[27] in ALS patients. Comparison of the immune toxicity of T-2 toxin with that reported in ALS shows similarities that have been well documented in the review by Wu, Q. et al 2020[31]. The immunotoxicity of T-2 could be the key pathologic event

undermining therapy of ALS patients. Immunotherapy with T regulatory lymphocytes has been proposed in ALS as immune suppressants.[53] Treatment has been used successfully to support the immune system using monoclonal antibodies against check-point inhibitors in fungal sepsis due to mucormycosis[54]. In addition, patients with fungal sepsis showed improvement when given interferon, interleukin 7, interleukin 15 and GM-CSF.[54-57]

Stratification of Immune Status-Inflammation vs. Anti-Inflammation

There is a need to stratify the patient's immune status as to the predominance of inflammation versus anti-inflammation[34]. In the past, there was a tendency to suppress the immune system with glucocorticoids or infusions of T-reg cells[53]. There has been the realization that immune suppressants are active from the start of many chronic infections. Tracking both inflammatory and anti-inflammatory biomarkers could help establish the balance. The level of immune suppression includes check-point inhibitors, PD-1 and CTLA-4[35,58]. The monoclonal antibody against PD-1, Nivolumab, has been successfully used to treat chronic fungal infections with reduced mortality rates[55]. The addition of GM-CSF, Interferon and Interleukins 7 and 15 would tend to reduce the immune suppression.

T-2 Toxin Induces Oxidative Stress at Low Doses via Activating Transcription Factor-3 (ATF3) Ubiquitination and Nuclear Factor Erythroid-2 (Nrf2) Degradation

Could T-2 toxin be responsible for the cytoplasmic aggregates such as TAR DNA-Binding Protein (TDP-43) seen in ALS? Chen, X. et al, Deng, Y. 2021[59] studied T-2 toxin poisoning of human breast adenocarcinoma cell line MCF-7. They titrated the dose in the cell cultures to maintain 80% viability. T-2 toxin promoted accumulation of reactive oxygen species (ROS) in a dose dependent manner even at low doses. T-2 caused a 4 to 6-fold downregulation of Nrf2, the master regulator of antioxidant genes. T-2 toxin, as well as deoxynivalenol, induced an increase in the oxidative stress transcription factor, ATF3. The rise of ATF3 appeared to be involved in accentuating the accumulation of ROS in large part by the reduction of the opposing Nrf2 pathway. It did this to a great extent by the promotion of ubiquitination and degradation of Nrf2. The transcription factor, ATF3 appears to play a complex role. The ATF3 pathway accentuates inflammatory processes. The T-2 stimulated rise of ATF3 appears to undermine the homeostatic, anti-oxidative Nrf2 pathway making oxidative stress worse even with "subclinical" T-2 poisonings. Astrocytes appear to be hypersensitive to ATF3 and T-2 toxin. There was a 30 fold increase in uptake of T-2 in astrocytes compared to surrounding neurons in studies by Zhang, J. et al 2020.[60] In PET scans of ALS patients, there is hypermetabolism of the glucose marker in the midbrain, pons, hippocampus, superior temporal gyrus and cerebellum.[61] This was attributed to neuronal hyperexcitability secondary to astrocytic proliferation Pagani, M. et al 2014.[18] Could low doses of T-2 toxin be responsible for inducing aggregates of ubiquitinated proteins

in the cytoplasm that mimic the protein aggregates reported in ALS such as TDP-43?

Targeting the Motor Neurons

There has not been a clear explanation why the motor neurons are the main target in ALS. Consistent with a generalized poisoning, there is ample evidence that ALS is a systemic process. Studies of fibroblasts and peripheral lymphocytes find evidence of the same pathologic defects found in the CNS. The pathologic defects in ALS appear to be generalized and not selective for any one cell type.[62,63] ALS is not "cell-autonomous". Lastres-Becker, I. et al 2021[63] established lymphoblastic cell cultures using Epstein-Barr infected lymphocytes[64] from ALS patients and controls. They were able to show molecular abnormalities of oxidative stress, mitochondrial dysfunction, Nrf2 pathway, inflammatory and autophagic flux all consistent with ALS. They were able to distinguish the lymphocytes from sporadic ALS from the lymphocytes of super oxide dismutase type 1 (SOD1) ALS patients. Both sporadic and SOD1 ALS lymphocytes had reduced ATP production and elevated lactate. The mRNA from sporadic and SOD1 lymphocytes did not show any distinct difference compared to controls. The protein products of mRNA using immunoblot (Western blot) analysis showed that Kelch-like ECH-associated protein 1 (KEAP1) ubiquitination control of the NRF2 pathway were distinct. Lymphocytes from sporadic ALS patients had elevated IL-6 and those from SOD1 ALS patients had elevated tumor necrosis factor (TNF). If ALS is a systemic process, then how are the motor neurons selectively targeted? The trichothecenes, T-2 toxin, HT-2 and deoxynivalenol selectively accumulate in the CNS. Studies of astrocytes in culture found them selectively sensitive[49]to T-2 toxin. If ALS is due to a poisoning by a mycotoxin such as T-2, then the anatomy of the sinus cavity juxtaposed to the midbrain could allow the toxin to selectively accumulate. Movement of the neurotoxins into lipid layers such as plasma membranes of mitochondria and synaptic vesicles could migrate neuron to neuron mimicking prion disease. Even when T-2 is generated outside the CNS, it tends to accumulate in the CNS due to the intranasal anomalies with the olfactory and trigeminal nerves. The molecular weight of T-2 toxin at 466 Da makes it unable to cross the BBB initially, but it would accumulate at gaps in the BBB causing damage and increased permeability. This results in amplification of the neurotoxin at gaps in the olfactory to brain pathway.

Can the Findings in ALS be generalized to address Parkinson Disease and Alzheimer Dementia (ALZ)

The pathologic findings in all three neurodegenerative disorders suggest a poisoning by neurotoxins. Olfactory dysfunction is common in a high percentage of the patients with neurodegenerative disorders and monitoring toxins in the nasal-olfactory system might establish the significance. Intracerebral microdialysis of the CNS could be an important technique if neurotoxins are using the olfactory pathway to bypass the BBB and accumulate in plasma membranes, synaptic vesicles and mitochondrial membranes. If the same or similar neurotoxins are involved in Parkinson's

Disease and Alzheimer's Dementia, it could explain the different chronologies. If ALS is due to mycotoxins produced by fungi colonizing the upper airway and nasal cavity, then the levels of toxins may allow them to accumulate in the olfactory bulb, pons and midbrain, then migrate rostral and caudal. In Parkinson's Disease and ALZ, the source of neurotoxins may be intermittent with episodic exposures spanning decades.

Conclusion

Neurodegenerative disorders have in common evidence of oxidative stress and mitochondrial injury. The nose-to-brain pathway may be an avenue allowing CNS exposure to environmental toxins such as neurotoxic and immunotoxic mycotoxins. The pathology has similarities to prion disease. This could be due to the lipophilicity of these toxins making them able to accumulate in the plasma membranes of synaptic vesicles and mitochondria. Movement would follow neuron to neuron, synapse to synapse. The search for these neurotoxins may be hampered by their tendency to concentrate along the blood brain barrier damaging tight junctions and creating gaps with increased permeability. There is evidence that the mycotoxins can selectively damage proteins creating the aggregates found at autopsies-amyloid, tau, synuclein and TDP-43. The damage to the olfactory receptors in the upper sinus cavity may bypass the blood-brain barrier leading to the onset of CNS pathology. These neurotoxins cause significant immune damage with progressive immune deficiency undermining treatments. Fusarium is high on the list of potential culprits releasing neurotoxic and immunotoxic mycotoxins.

Proposed Treatment-

1. Screen for olfactory dysfunction
2. Aggressively search for lipophilic neurotoxins including the use of Intracerebral Microdialysis to screen for poisoning in the CNS.
3. Screen patients for opportunistic fungal infections that secret mycotoxins including the use of paraffin block analysis of tissues from patients with neurodegenerative disorders.
4. Assuming finding evidence of poisonings, reassess the value of plasma exchange.
5. ALS patients have been treated with the antifungal agent, Voraconazole coupled with plasma exchange with temporary improvement. The eventual failure of treatment may be due to the progressive immune suppression. Couple antifungal agents and plasma exchange with immunotherapy, in particular, gamma globulin and antibodies against check point inhibitors, such as Nivolumab.
6. Aggressively screen for immune deficits including T-Regulatory cells, PD-1 and CTLA-4, subclass IgG deficiencies.
7. Trials of Gamma Globulin, Interferon, GM-CSF, Interleukin 7 and 15 in ALS patients.
8. Establish the balance between Inflammatory and Anti-inflammatory markers.
9. Screen Parkinson Disease and Alzheimer's Dementia patients for autoimmune levels.

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