Analysis of specific toxic agents as possible triggers of multiple sclerosis

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I. Overview

Multiple sclerosis (MS) is believed to be a multifactorial disorder, requiring the presence of both genetic and environmental factors to initiate the mechanisms that lead to demyelination and neural damage. Toxic agents may be included in the set of factors that can contribute to the development of MS in genetically susceptible individuals. Unlike other types of factors such as genetic variants and pathogens, toxic agents have not yet been extensively studied as possible causes or triggers of MS. Only six toxic agents have been evaluated to more than a minimal extent for a possible connection with MS. Furthermore, the research that has been published regarding the involvement of these agents is often contradictory, with the result that no one toxic agent has been consistently demonstrated to be a risk factor for the disease.

This document surveys six toxic agents that have been evaluated for a role in MS, summarizing the results of the studies that have been conducted to identify possible associations. The research on each of these agents is organized into an analysis table which addresses the following questions:

- Is there a strong correlation between exposure to the toxic agent and risk of MS?
- Is the presence of the toxic agent increased in MS subjects compared with non-MS subjects?
- Does length or intensity of exposure to the toxic agent affect the risk of MS?
- Does the age of exposure to the toxic agent differ among MS and non-MS subjects?
- Does the toxic agent have known effects on the central nervous system (CNS) or immune system that may relate to the pathogenesis of MS?

A description of the toxic agent and brief opinion about the strength of the data is also provided.

In locating and analyzing these studies, we encountered a number of issues that complicate the task of interpreting and comparing results, and may also contribute to the frequency with which study results contradict one another. These issues include lack of uniformity in study methods, missing information about study design and methodology, differences in study locations, small sample sizes, and different criteria for toxic agent exposure. In addition, the methods and criteria used for diagnosing MS have evolved over the past decades, making it somewhat difficult to compare earlier studies with more recent ones.

The purpose of the analysis tables is to provide an overview of the research done and clarify what areas need to be addressed further; they do not represent a complete review of all the studies published to date. Although at this time no toxic agent can be unequivocally implicated as a risk factor for multiple sclerosis, we hope this document will still be helpful in guiding future research into the role toxic agents play in the pathogenesis of multiple sclerosis.

Note: A list of published studies evaluating the role of toxic agents in MS can be found on the Accelerated Cure Project website at:
http://www.acceleratedcure.org/downloads/phase2-toxicagents-studies.pdf or
http://www.acceleratedcure.org/downloads/phase2-toxicagents-studies.xls.

II. Analysis tables

Toxic agent: Cigarette smoke
Description:
Cigarette smoke contains over 4,000 chemical components, many of which are known to have toxic effects in humans. Smoking has been implicated as a major risk factor for numerous diseases including lung cancer, heart disease, bronchitis, emphysema, and stroke (American Cancer Society). Cyanide, lead, nicotine, nitric oxide, and tobacco glycoprotein are several components of cigarette smoke that may damage the central nervous system or alter immune system functionality. • Cyanide - a highly toxic substance whose chemical formula is CN. Its molecular weight is 26.03 kDA and it is a relatively hydrophobic molecule, allowing it to pass through cell membranes readily. • Lead - has been assessed individually – see separate table. • Nicotine - an addictive substance in cigarette smoke that is present in all forms of tobacco. Nicotine is a hydrophilic molecule which readily absorbs water. Its molecular weight is 162.23 kDa. • Nitric oxide - a free radical chemical (molecular formula= NO) that is also produced endogenously in the body and functions as a signaling molecule. NO is slightly soluble in water and has a molecular weight of 30.0 kDa. • Tobacco glycoprotein - a component found in tobacco leaves. It is part of a group of polyphenol-rich compounds, which are present in edible fruits and vegetables.
Is there a strong correlation between exposure to the toxic agent and risk of MS?
A few epidemiological studies have explored the possibility of a connection between cigarette smoke and the risk of developing MS. Results have been mixed. Some, including a few prospective studies, have detected an increased risk of MS in cigarette smokers[1-5]. Others report no significant positive correlation between cigarette smoking and the risk of MS, although some of these studies were case-control studies examining a number of different etiological factors as opposed to focusing on smoking specifically[6-8]. A meta-analysis of six case-control studies concluded that "smoking is a weak but significant risk factor for subsequent development of MS[9]." One study found a hazard ratio of 3.6 for the development of secondary progressive MS in smokers versus non smokers[2]. This indicates that cigarette smoke may also play a role in the progression and/or exacerbation of MS. Finally, smoking was found to be associated with elevated Epstein-Barr virus antibody levels in people with MS, especially females, although whether this finding is related to a causal mechanism is unknown.
Is there evidence of increased toxic agent present in MS subjects compared with non-MS subjects?
Not assessed.
Does length or intensity of exposure to the toxic agent affect the risk of MS?
Only a few studies have addressed the intensity and length of cigarette smoking. In most cases, subjects are divided into the categories of "ever" or "never" smokers. However, one study found a positive association between intensity of exposure to cigarette smoke and risk of MS. The intensity of exposure was defined by the number of packs smoked per day multiplied by the number of years over which that amount was smoked (termed "pack-years"). The study found that smokers in the highest exposure category (>25 pack-years) had the highest increased risk for multiple sclerosis[1]. Similar findings were reported by a case-control study in which the risk of MS was associated with number of cigarettes and number of years having smoked[5].

Another study reported a "borderline significant" correlation between the risk of multiple sclerosis and the number of cigarettes smoked[4].

Does the age of MS subjects when exposed to the toxic agent differ from non-MS subjects?

Not assessed.

What effects does the toxic agent have on the CNS or immune system?

The means by which cigarette smoke could affect the risk of onset or progression of MS is unclear. Several of its components have been demonstrated to affect the CNS or immune system, but not directly in relation to multiple sclerosis.

- Nicotine has been linked to several adverse effects which may have possible implications for multiple sclerosis. Scientists have reported increased microvascular blood flow and flow velocity to certain areas of the brains of rats 1.5 to 2 minutes after an injection of nicotine. This data suggests that nicotine can cause an increased influx of substances across the blood-brain barrier[10]. There is also evidence that nicotine stimulates endogenous production of nitric oxide[11-15], a signaling molecule which is hypothesized to play a large role in the pathogenesis of MS[16]. (One research group, however, found a significant decrease of mean plasma NO levels in smokers compared with nonsmokers.[17]) Scientists have also demonstrated that nicotine may suppress the immune system, for example by inhibiting T lymphocyte responses[18].
- Other components of cigarette smoke have not been studied as extensively as nicotine.
    - Nitric oxide is also a component of cigarette smoke, but the extent to which inhaled nitric oxide contributes to concentrations of nitric oxide in the body is unclear.
    - An in vitro study of human blood cells showed that tobacco glycoprotein may cause over-stimulation of the immune system by activating the proliferation of T cells and differentiation of B cells[19].
    - Demyelination of spinal cord and brain tissue of rats fed cyanide has been documented[20, 21].
    - Lead, which is present in cigarette smoke, is discussed in a separate table.
- Cigarette smoking may contribute to MS by increasing the risk of infections that lead to the development of MS. For instance, one study showed that smoking increases the risk of infection with *Chlamydia pneumoniae*, which is regarded by some to be a potential MS risk factor.[22]
- Finally, a review article outlines various ways in which cigarette smoke may increase the risk of autoimmune diseases including MS, such as by elevating peripheral blood leukocyte counts, upregulating the production of certain inflammatory proteins, and promoting the release of matrix metalloproteinases[23].

Conclusions:

Evidence suggests that cigarette smoke may be a risk factor for MS. However, the mechanisms by which cigarette smoke may contribute to the development of MS are still unknown, and only a few of the 4,000 components in cigarette smoke have been specifically analyzed in conjunction with MS. Further studies are needed to define the involvement of cigarette smoke in increasing the risk of MS.

Toxic agent: Ionizing radiation
Description:
Ionizing radiation, which includes x-rays, alpha particles and gamma rays, can induce mutations in DNA and is the cause of several types of skin cancer. Exposure to this form of radiation as a risk factor for MS has mainly been explored by asking about radiological work done for health purposes, such as x-rays and medical treatments.
Is there a strong correlation between exposure to the toxic agent and risk of MS?
Only two case-control studies have found a positive correlation between ionizing radiation and risk of MS[7, 8]. A pooled analysis of these two studies calculated an odds ratio for developing MS of 4.4 (95% CI 1.6-11.6) for radiological work, and 1.8 (95% CI 1.2-2.6) for X-ray examinations[24].
Is there evidence of increased toxic agent present in MS subjects compared with non-MS subjects?
Not applicable.
Does length or intensity of exposure to the toxic agent affect the risk of MS?
Not assessed.
Does the age of MS subjects when exposed to the toxic agent differ from non-MS subjects?
Not assessed.
What effects does the toxic agent have on the CNS or immune system?
One study by Peterson, et al., suggests that ionizing radiation may increase the rate of demyelination in existing demyelinating conditions. The study presented five separate cases of patients with demyelinating lesions who received ionizing radiation treatment based on a mistaken initial diagnosis of neoplasm. Of the four patients who received high therapeutic doses of radiation, all subsequently experienced a severe decline in clinical status. However, the one patient whose course of radiation was terminated early did not suffer any significant short-term effects[25]. Another case study describes a patient with diagnosed MS who experienced an acute relapse and new hyperintense MRI lesions following radiotherapy for a parotid gland tumor; the new lesions were found to be within the 50% isodose radiation field[26]. More recently, a Mayo Clinic study of 15 MS patients who had received external beam radiotherapy (either before or after MS diagnosis) identified six who had subsequent life-threatening events or death due to neurotoxicity[27]. The mechanisms through which ionizing radiation could cause or accelerate demyelination remain to be determined.
Conclusions:
Only two case-control studies have evaluated exposure to ionizing radiation as a risk factor of multiple sclerosis. Also, researchers have acknowledged that the increased use of radiation treatments in MS subjects may have resulted from their symptoms and therefore may not have played a role in development of MS. Therefore, although case evidence indicates that ionizing radiation can exacerbate demyelination, there is insufficient evidence to support the hypothesis that it may be a risk factor for MS and therefore further research is necessary.

Toxic agent: Lead
Description:
Lead is a heavy metal element (atomic number 82) that has no known physiological role in the human body although it is present throughout the earth. It is characterized by its malleability and resistance to corrosion, is solid at room temperature, and has a bluish-gray color. Lead is not soluble in either water or organic solvents and has a molecular weight of 207.2 kDa. Lead has severe neurotoxic effects, especially in children, and can result in encephalopathy, peripheral neuropathy, convulsions, and mental retardation. One major source of exposure to lead comes from its use as an anti-knock agent in gasoline (this use is now banned in the US for on-road vehicles but still allowed in other parts of the world). People are also exposed to lead through paint, factory emissions, battery production and mining.
Is there a strong correlation between exposure to the toxic agent and risk of MS?
A few authors have reported or discussed possible correlations between the frequency of multiple sclerosis and the lead content in soil in various regions[28-31], but a causal relationship between lead content and MS has not been proven. Furthermore, the studies of soil content did not describe whether or how increased lead content resulted in increased human exposure to lead – e.g., it is unclear whether or not lead levels in the air, food or drinking water were also increased.
Is there evidence of increased toxic agent present in MS subjects compared with non-MS subjects?
There is no strong evidence that MS patients have higher levels of lead in their bodies as compared to non-MS subjects. One analysis of blood lead levels reported no evidence of lead poisoning in multiple sclerosis subjects[32], and another study involving urinary analysis also detected no significant increase of lead levels[33]. In fact, other studies have reported significantly lower levels of lead in blood samples from MS subjects compared with controls[34], as well as similar levels in samples from people with a first demyelinating event compared with controls[35].
Does length or intensity of exposure to the toxic agent affect the risk of MS?
Not assessed.
Does the age of MS subjects when exposed to the toxic agent differ from non-MS subjects?
Not assessed.
What effects does the toxic agent have on the CNS or immune system?
Lead has a multitude of effects on these systems although the physiological consequences of lead poisoning have not been fully defined. One study reported demyelination in the brains of primates exposed to lead[36] but another found that lead implanted in the brains of rats did not cause any significant necrosis[37]. It has also been reported that suckling rats exposed to lead suffered from blood-brain barrier damage which was much more severe than in adult rats[38].
Conclusions:
Lead is the most extensively studied metal in relation to multiple sclerosis, but so far no definitive evidence has implicated it as a risk factor. It is also unclear through what mechanisms lead could contribute to the development of MS.

Toxic agent: Mercury
Description:
Mercury is one of two metal elements that are liquid at room temperature. Like lead, it has no known physiological role and is a neurotoxic chemical. The relationship between mercury and multiple sclerosis has mainly been discussed in the context of dental amalgam for fillings, which contain 50% mercury. Dental amalgam, which was first used in the early 1800s, is used in 80% of all dental caries around the world. Studies have shown that mercury vapors escape from the amalgam and can enter the body.
Is there a strong correlation between exposure to the toxic agent and risk of MS?
There is no strong evidence that dental amalgams are a risk factor for multiple sclerosis. Although one epidemiological study performed in 1978 reported a positive correlation between dental caries and multiple sclerosis[39], four more recent studies that analyzed the use of dental amalgams found either an insignificant positive correlation or no correlation with MS[40-43]. A meta-analysis performed on this topic similarly found only a nonstatistically significant association between amalgam use and MS[44].
Is there evidence of increased toxic agent present in MS subjects compared with non-MS subjects?
A study of dental amalgam removal reported that MS subjects had significantly higher levels of mercury in their hair compared with non-MS controls, but no information was given about the amalgam status of the controls[45]. Another study of dental fillings in MS found no difference between cases and controls in body mercury levels (number of amalgams was similar between the two groups). One post-mortem study of brain tissue found no significant increase of mercury levels in the brains of MS subjects compared with non-MS subjects[46]. Finally, two studies of MS subjects and subjects with a first demyelinating episode found their blood mercury levels to be lower or comparable to those of controls[34, 35].
Does length or intensity of exposure to the toxic agent affect the risk of MS?
This has been addressed by one study, which found only a suggestive increased risk of multiple sclerosis with longer exposure or greater number of amalgams[40].
Does the age of MS subjects when exposed to the toxic agent differ from non-MS subjects?
Not addressed.
What effects does the toxic agent have on the CNS or immune system?
The neurotoxic effects of chronic exposure to mercury include tremors, increased excitability, chronic spasm, memory loss, depression, and delirium. Mercury has also been shown to inhibit immunologic responses by decreasing activation[47] and possibly numbers[45] of T lymphocytes, although a study of dental amalgams determined that T lymphocyte levels were similar in people with and without implants[48]. Mercury may also affect the permeability of the blood-brain barrier. One study found that rats injected with methyl mercuric chloride displayed blood-brain barrier dysfunction[49]. Researchers have also reported an improvement in the mental or physical health of multiple sclerosis patients due to the removal of dental amalgams[50, 51], but these studies were small and unblinded, and did not explore the possible mechanisms behind this effect.
Conclusions:
Studies based on dental amalgams have generally failed to find any correlation between implants and MS. In addition, the typical amount of exposure to inhaled

mercury from dental amalgams is generally considered to be below toxic levels[47]. Overall, insufficient evidence exists to implicate mercury as a risk factor for MS.

Toxic agent: Organic Solvents

Description:

Organic solvents include a variety of carbon-based compounds that can be used to dissolve one or more other substances. Aliphatic hydrocarbons, ketones, ethers, esters, aromatic hydrocarbons, and amines are several classes of chemicals that can be used as organic solvents. Specific chemicals researched in relation to multiple sclerosis include toluene, n-hexane, trichloroethylene, carbon disulphide, acetone, and xylene.

Organic solvents are comprised of non-polar, hydrophobic molecules. They easily dissolve other non-polar substances and permeate organs and cells more readily than hydrophilic substances. The relatively small size of organic solvents (molecular weights typically fall between 50 and 150 kDa) also contributes to their ease of penetrating cell membranes.

These substances are present in glues, paint sprays, industrial cleaners, and other commercially used products. They are known to produce neurological effects in humans such as failed memory, lack of concentration, and tiredness, and have been implicated as a cause or risk factor of several neurological disorders, including peripheral neuropathy, Hodgkin's disease, and leukemia.

Is there a strong correlation between exposure to the toxic agent and risk of MS?

Since 1982, at least 18 epidemiological studies have been published on organic solvents and multiple sclerosis. Several of these reported a connection between organic solvent exposure and MS risk, but some found no evidence for an association. A meta-analysis of 13 studies that examined exposure to organic solvents and frequency of MS found a Mantel-Haenszel ratio of 2.1 (95% CI: 1.6-2.7)[52]. It should be kept in mind that because the majority of studies have been based on occupations typically involving organic solvent exposure, most of these population studies assess likelihood of exposure rather than actual exposure.

Is there evidence of increased toxic agent present in MS subjects compared with non-MS subjects?

Not assessed.

Does length or intensity of exposure to the toxic agent affect the risk of MS?

Not assessed.

Does the age of MS subjects when exposed to the toxic agent differ from non-MS subjects?

Not assessed.

What effects does the toxic agent have on the CNS or immune system?

Increased protein concentrations have been found in the cerebral spinal fluid (CSF) of subjects exposed to organic solvents, indicating a possible increase in permeability of the blood-brain barrier resulting from the exposure. Juntenen, et al. found evidence for slight blood-brain barrier damage (increased levels of albumin in the CSF) in 23% of patients with suspected occupational disease due to solvent exposure[53]. Another study analyzed patients with a diagnosed psycho-organic condition who had experienced occupational exposure to solvents. It reported that three out of 17 subjects had increased albumin ratio in the CSF[54]; each of the three had been heavily exposed to organic solvents. Moen, et al. also found a correlation between albumin in the CSF and solvent exposure, although the extent of the exposure did not seem to correlate with albumin concentrations[55].

Other researchers propose that organic solvents can induce changes in the myelin sheath of the white matter in the brain. MRI scans of toluene abusers have shown

changes in the white matter of their brains[56]. Organic solvents may also affect the immune system, as suggested by a study that correlated exposure to organic solvents with a stimulation of T lymphocytes in the CSF of subjects occupationally exposed to solvents[53].
Conclusions:
Evidence suggests that organic solvents may be a risk factor for MS, but this is based on retrospective studies that did not directly measure the level of exposure in study subjects nor the actual compounds to which subjects were exposed. Organic solvents may initiate the onset of MS by breaking down the blood-brain barrier, but more research is needed to support this hypothesis.

Toxic agent: Radon
Description:
Radon is a gaseous radioactive element (atomic number 86) that is produced by the breakdown of radium in the earth. Radon is an emitter of alpha particles which constitute the most significant source of radiation for most people. Radon is ubiquitous throughout the earth and can build up in large amounts indoors where many people spend a majority of their time, especially in more developed areas. The most significant health effect of radon documented to date is an increased risk of lung cancer.
Is there a strong correlation between exposure to the toxic agent and risk of MS?
Only two epidemiological studies have looked for a connection between radon and the risk of multiple sclerosis. A small pilot study that was conducted in northwestern Ireland compared MS prevalence with surveys by the Radiological Protection Institute of Ireland. Researchers found that the pattern of MS frequency in the area roughly correlated with the varying degrees of radon levels[57]. Bolviken, et al, compared radon levels of 7500 randomly-chosen houses in Norway to the prevalence of multiple sclerosis. They also found a positive significant correlation between radon and MS[58].
Is there evidence of increased toxic agent present in MS subjects compared with non-MS subjects?
Not applicable.
Does length or intensity of exposure to the toxic agent affect the risk of MS?
Not assessed.
Does the age of MS subjects when exposed to the toxic agent differ from non-MS subjects?
Not assessed.
What effects does the toxic agent have on the CNS or immune system?
This has not been assessed. The DNA damage known to be caused by radiation may play a role in MS. Also, one paper (Bolviken, et al) speculated that radon may activate latent viruses which proceed to trigger MS.
Conclusions:
Only two published studies have explored indoor radon levels as a risk factor for multiple sclerosis; although both found a positive correlation, two studies are not enough to implicate radon as a risk factor for the disease. In addition, no thorough explanation has been set forth concerning how radon could cause MS. Therefore, more research is needed before radon can be established as a risk factor for MS.

III. Other toxic agents investigated in MS

Several other toxic agents have been mentioned as possible risk factors for multiple sclerosis, but have not been studied extensively or individually and thus have not been individually described in this document. (Their exclusion from this document by no means indicates that they are not worth investigating as risk factors of MS.) They include:

- Herbicides
- Illegal drugs
- Kerosene
- Pesticides
- Vaccine components

IV. Conclusion

The information provided in these tables highlights the lack of research in the area of toxic agents and multiple sclerosis, as well as the inconclusive nature of the data collected to date. Currently, no toxic agent can be strongly implicated as a risk factor or cause of multiple sclerosis. If it is true that multiple sclerosis is a collection of diseases with varying causes and triggers, it may be difficult to identify (and even more difficult to rule out) any one toxic agent as an unequivocal cause or risk factor of MS. However, the potential benefit in terms of treatment and prevention from linking a toxic agent with the development of MS warrants the performance of additional studies on this topic.

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