Motorneurone disease (MND) – possible etiological factors

What is known about the causes of motor neuron disease (MND)?

This condition disease arises from progressive injury and cell death of motor neurons in the spinal cord and brain stem, and usually also the upper motor neurons in the motor cortex. About 10% have been identified as familial in origin and of a suspected genetic basis, usually with an autosomal dominant mechanism of inheritance.[1] The large majority (90%) appear sporadic, with an incidence (eg in the UK) of 1-2 per 100,000. Clinical variants may affect either just the lower MN's (progressive muscular atrophy) or the upper MN's (primary lateral sclerosis). It is apparent that the pathology in the nervous system is not confined to the motor neurons, but they are the neurons which are the earliest and most severely affected.

The genetically-based cases themselves can arise from a range of different defects, in that over 60 different mutations have been identified.[2] The most common and well studied appears to be mutations on chromosome 21 encoding the enzyme Cu/Zn superoxide dismutase (SOD). This mutation is present in about 20% of familial cases (presumed of genetic origin), and 2% of all cases.[3]

It has been hypothesised (with some supportive experimental evidence) that the defect leads to increased activity of this enzyme, whose normal function is to catalyse the conversion of the toxic superoxide anion to hydrogen peroxide and oxygen. Such a "toxic gain of function" can however result in increased production of damaging free hydroxyl radicals, one initiator of the deleterious process of lipid peroxidation. However intracellular proteins may also be affected, due to formation of nitrotyrosine residues.[4]

Various precipitating factors for motor neuron damage have been proposed, which may have implications for identifying plausible non-genetic causes, such as exogenous chemicals. These include oxidative stress and neuronal "excitotoxicity".

It is thought that even in apparent health, the effects of "normal" oxidative stress, eg due to production of free radical species, can be cumulative, with an eventual toll on non-replicating cells including neurons. This could explain why this disease affects mainly middle aged and older people, with a mean age of onset of 55years, though younger people are occasionally affected. It also could explain why increased production of such free radical species could be an initiating factor. It has been noted from post-mortem examination of tissues, that individuals with motor neuron disease often have more pronounced biochemical changes in DNA/proteins of a type reflecting free radical damage.[5] A further line of evidence comes from animal studies trialling potential treatments of the disease. The antioxidant, VitaminE, apparently delayed the expected time of onset of MND in transgenic mice, which were made particularly vulnerable to it from the gene mutation described above which causes increased SOD enzyme activity, and hence increased free radical production. Another antioxidant, glutathione, extended survival in a further study. This line of argument suggests that sufficient exposure to

chemicals with a <u>significant</u> potential to produce <u>oxidative free radicals</u> could be a factor in the development or progression of the disease.

It has become evident that glutamate is an important excitatory neurotransmitter at certain sites in the nervous system, including the motor neurons. Excess glutamate levels in the synaptic cleft can over-stimulate the post-synaptic glutamate receptors. Normally glutamate is successfully removed from the synapse by transporter proteins. However these proteins are largely located on perisynaptic glial cells, and these cells can be damaged or killed by excessive stimulation of glutamate receptors (partly due to changes in calcium homeostasis). Thus increasing glutamate concentrations can indirectly decrease the levels of the transporter proteins which are designed to keep these concentrations under control. This vicious circle type process has been termed glutamate-mediated "excitotoxicity', and circumstantial evidence suggests that this is a contributory factor in motor neuron disease. This relates to the fact that the function (and expression) of one such transporter protein (EAAT2) is impaired in MND, and that levels of glutamate may be abnormal/high in at least some patients.[6] While this may well have a primarily genetic origin (of uncertain basis), it <u>raises the question as to whether exogenous causes of excess synaptic glutamate secretion</u> could precipitate or aggravate the condition.

It is thought that motor neurons may be especially vulnerable to both the above processes. It has been postulated that oxidative stress may contribute to the early stages of motor neuron injury, with glutamate toxicity playing a later part in cell death.[7] However, neurofilament accumulation and autoimmunity mechanisms have also been proposed, though many trials of immune-modulating therapies have been negative, casting doubt on the latter.[8] Multiple related factors may be involved.
There is increasing evidence that free-radical toxicity and in particular, excitotoxicity , contribute to the process. The neurotoxicity events may be primary or secondary, the latter particularly triggered by mitochondrial damage.[9]

Clinical observations of ALS related syndromes or variants have noted <u>associations with</u> toxins (<u>heavy metals</u>, organic pesticides), endocrine disorders (eg hyperthyroidism), immune or lymphoproliferative disorders, and infections, such as polio and retroviruses.[10] There have been comparatively few animal experiments or human epidemiological studies to investigate the role in practice of specific chemical compounds. However in the UK, descriptive epidemiological studies have suggested an increased risk in the leather industry.[11] Even if real, the <u>causative agent(s) have not been identified.</u> It has been postulated that contact with carcasses or hides (possibly involving transmission of <u>an infectious agent</u>) could be involved, (and such a link has been suggested for the high incidence of a closely related disease, ALS, in the western Pacific region of Guam). However arguments against such a cause include the fact that farmers and rural dweller are not considered by some to be at increased risk of MND.[12] In fact the possible role of a persisting virus infection has been suggested for some time. However, in the context of the leather industry, the role of solvents has also been questioned.[13] A small case-control study noted that the highest odds ratio related to a combination of hereditary (family history of neurodegenerative disease), male gender, and exposure to solvents.[14] They

suggested that individual susceptibility could be partly based on deficient metabolising or detoxifying capacity. A later cluster study suggested however that agricultural work was significantly more common in the cases.[15]

The MND variant, amyotrophic lateral sclerosis (ALS), occurs with an increased incidence in the western Pacific (WP) region. It has been suggested that this arises from nutritional deficiencies of calcium and magnesium (due to low levels in soil and drinking water), but also relatively high aluminium levels,[16] resulting in secondary hyperparathyroidism, which causes deposition of calcium and the aluminium in neurons. Thus in primates subjected to a similar diet, mild calcium and aluminium deposition in neurons were noted,[17] along with degenerative changes. (Furthermore two human cases of MND did apparently show high CNS aluminium and calcium and low magnesium levels.[18] This might be thought to imply that increased aluminium intakes per se from any source could be a risk factor. However it has been suggested that it is only the combination of aluminium with a deficiency of magnesium/calcium that gives rise to the condition.[19] This seems to be supported by Mitani who found that the Al content in CNS tissue was not higher in animals given increased aluminium but with normal dietary Mg/Ca.[20]

Furthermore, not all studies of ALS-WP have demonstrated increased aluminium levels,[21] and this has also not always been found in sporadic ALS elsewhere. Thus Kasarkis[22] found no increased aluminium levels in samples of cervical spinal cord in five such patients. They did however find increased iron (and calcium) levels in spinal neurons (ie 1.5 to 2-fold increases). They speculated that the iron could be a significant causal factor, on the basis of its ability to catalyse the generation of oxygen free radicals. Others have even suggested that the effects of iron and aluminium could interact.[23] Thus in an (in vitro) study, the level of reactive oxygen species produced by iron was enhanced by concomitant aluminium. However the relevance of such an isolated *in vitro* study is unclear, particularly as it sheds no light on the external dose likely to be required to cause such an effect, if real.

Are the chemicals in question a plausible cause (or aggravator) of MND?

This would be the case if they were known to have significant potential to either cause or exacerbate reactions generating free (peroxy or hydroxy) radicals, or having glutamate like effects.

(i) It has been shown that with some diseases, including some degenerative neurological conditions such as Parkinson's disease, oxidative processes cause iron to be mobilised from cellular ferritin stores and released as Fe(II), ie in the reduced (redox) form. This iron reacts in such a way as to exacerbate the production of superoxide and other (eg hydroxyl) free radicals, thus enhancing lipid peroxidation. Post-mortem investigations in patients with Parkinson's disease have shown increased iron (and decreased reduced glutathione) levels, and increased lipid peroxidation and DNA damage in the substantia nigra, the area of the brain most affected in the disease. All of these findings are consistent with the hypothesis that alterations in iron handling can play a significant part

in this disease. It may not be the primary cause of the initial "oxidative stress", but it can be involved in the response, and contribute to the end result of lipid peroxidation and other macromolecular damage. Thus iron can exacerbate the production of free radicals in catecholinergic neurons.[24]

A similar mechanism has been postulated for MND, however there is less evidence to support this. A relevant question is whether increased iron "intakes" per se (whether proven or merely possible) would make this process more likely or more severe. There is only limited evidence in this respect,[25, 26] which supports the plausibility of this. However the former report refers to "pathological iron overload", and in the latter, the iron was injected directly into the affected tissue. Furthermore, the evidence for this mechanism is substantially greater for Parkinson's disease than MND.

(ii) Glutamate excitotoxicity – precipitation or aggravation.

Chemicals featuring in discussions of this topic include ethambutol, "MPTP", mercury (Hg2+),[27] and natural or endogenous compounds including cysteine. The food additive, MSG (monosodium glutamate) is a glutamate agonist, with potentially similar properties, at least at some sites in the nervous system. In terms of dietary factors, kainate, present in some seaweeds is a glutamate analog of much higher toxicity than glutamate itself, and the seeds of Cycas species contain a different excitatory amino acid (BMAA) from glutamate. These plants are part of the diet in Guam and have been proposed as an alternative cause (to metal toxicity) of ALS in this population.

Is the exposure of sufficient intensity and/or duration to be the explanation

Aluminium has been suggested as an important factor in one clinical variant of MND, though increased aluminium intakes on their own (ie without Ca/Mg deficiency) do not appear sufficient to cause this condition. Increased release of iron from nerve tissue stores has been linked with Parkinson's disease, and also been postulated to be a factor in MND. There is however, not much information on the likely hazardous doses, or dose-response relationships for the effects, particularly regarding the iron intakes. Therefore one is restricted in this line of reasoning. The GIT absorption of aluminium is low, generally less than 1%, though this depends on the media or matrix. The absorption of iron is higher (~2-15%), but it is regulated by homeostatic processes. In cases of excess intake, most of the extra iron is present as haemosiderin rather than ferritin, the usual storage protein.

Are there other (more) likely or plausible causes?

While the cause of only about 10% of cases is known, and those to be of genetic origin, it may well be that this is an important factor in a higher percentage of cases. It may or may not be possible to undertake genetic testing.* It also seems that dietary factors could be important, given that foodstuffs contribute in a major way to the production of free radicals[28], and that exposure to such factors occurs regularly and "efficiently" (in terms of reliable gut absorption), starting at birth. MND is a disease of slow onset, thought to only

become clinically apparent after years of cumulative neuronal damage, from whatever the major causes are (apart from genetic). It may be that a larger proportion have a genetic basis than the 10% or so where this is currently demonstrable.

Summary

In reviewing the various chemicals on the list, the only ones where a link has been specifically suggested or speculated upon are aluminium and iron. Thus as outlined above, aluminium has been suggested as an important factor in one clinical variant of MND, though increased aluminium intakes on their own (ie without Ca/Mg deficiency) do not appear sufficient to cause this condition. Increased release of iron from nerve tissue stores has been linked with Parkinson's disease, and also been postulated to be a factor in MND.

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