# Does iron cause disability in multiple sclerosis patients?

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## What is the problem?

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) (the brain and spinal cord) which causes a decline in sensory, motor and cognitive function. In MS, the protective layer of myelin, which wraps around and protects axons (the long thin projections of neurons (nerve cells) which transmit nerve impulses), is degraded. This disrupts neuronal signalling and leaves the axon exposed to damage and eventual degeneration. The cause of multiple sclerosis remains unclear, limiting treatment options for this disease.

## What are we interested in?

A protein called fibroblast growth factor 9 (FGF9) is known to be present in excess in regions of CNS damage in MS patients. FGF9 has been shown to compromise neuronal function/survival by exacerbating damage. We thought that this may be a result of type of cell called microglia, which usually monitor and maintain the appropriate level of iron in the CNS, taking up excess iron (iron levels are reportedly higher than usual in the microglia of MS patients). Haemoglobin breakdown products, particularly hemin, are thought to be a major source of iron in MS. Thus, we decided to investigate the effect of hemin on CNS cells and whether its effect was dependent on microglia.

## What did we do?

We grew rat cells from the CNS in the lab in a system similar to the concept of a "brain in a dish". We took cells from the brain and spinal cord of embryonic rats, which contain all the cells present in the CNS, and grew (cultured) them in the lab. Our cultures develop like in real rats and we can see myelin coating the nerves (see Figure 1). We treated our cultures with different concentrations of hemin and FeCl3 (a control which showed us iron-mediated tissue damage) for 24-48 hrs. Some cells were also treated with a microglia inhibitor, called PLX3397, to determine whether the effect of hemin was dependent on the microglia.

We stained our cultures to identify and count the different cells, viewing them using a fluorescent microscope. We also analysed the amount of lactate dehydrogenase (LDH), an indicator of cell death, in the liquid in which we grew our cells in order to quantify tissue damage.

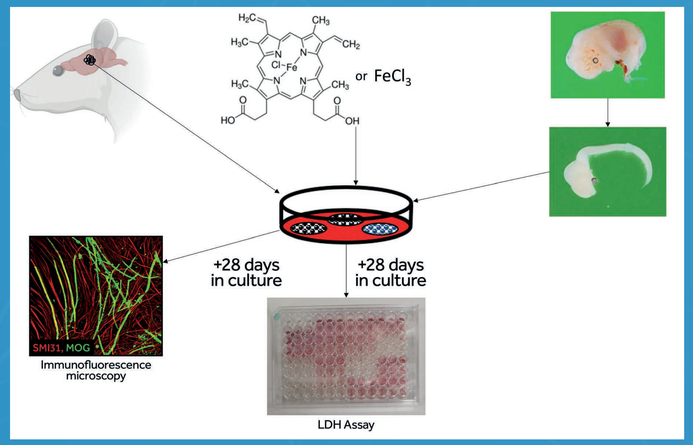


**Figure 1** A "brain in a dish" – brain and spinal cord cells from rat embryos were grown (cultured) in the lab and were treated with hemin and FeCl3, with and without PLX3397 (a microglial inhibitor) and compared to cells grown under standard culture conditions.

## What did we find?

We found that hemin is highly toxic (cytotoxic) to neurons, particularly axons. At high concentrations (10 μM), hemin is extremely toxic to axons and myelin. However, at low concentrations (3.3 μM) it preferentially causes demyelination – slowing, or stopping, nerve impulses being sent (see Figure 2). We also found that the microglia inhibitor, PLX3397, reduced hemin-induced neuronal damage at a low concentration (see Figure 3).

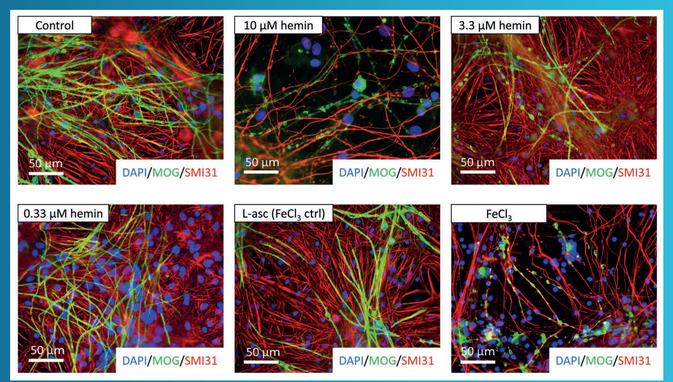


**Figure 2** Images showing the effects of hemin and FeCl3 on axons (red) and myelin (green) compared to controls ('Control' and 'L-asc (FeCl3 ctrl)' had DMSO (used to dissolve hemin) or L-asc (used to dissolve FeCl3) added instead of hemin and FeCl3, respectively). The nuclei (where DNA is stored) of the cells can be seen in blue.

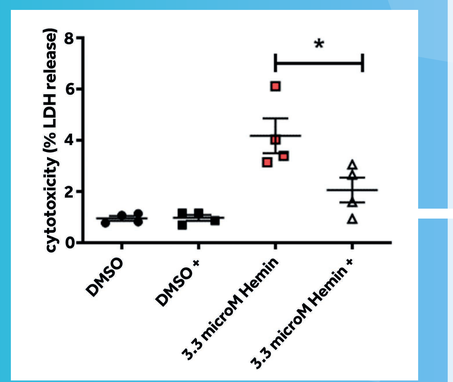


**Figure 3** Hemin-induced cytotoxicity is partly microglia dependent. % LDH release measured following exposure to 3.3μM hemin +/- PLX3397 compared to the control treatment, DMSO +/- PLX3397. Data analysed by one-way ANOVA with Tukey's post hoc test. Significant difference denoted as *p<0.05.

## What does this mean?

Our findings indicate that hemin can cause neuronal cell death. Specifically, it causes axonal degeneration and demyelination. We also found that the effects may be partly microglia-dependent. This is an exciting development in our understanding of MS and, with further study, could lead to novel treatments being developed for MS.

## Who am I?

I am a third year Microbiology student at the University of Glasgow. After my degree I hope to complete a PhD and pursue a research career in Infection Biology, or pursue a career as a clinical scientist within the NHS.

Gillian was supervised during her Vacation Scholarship project, *"Induction of neuronal dysfunction by fibroblast growth factor 9: a therapeutic target to limit accumulation of disability in multiple sclerosis?"*, by Professor Christopher Linington and Dr Katja Muecklisch.