

Abnormal metal homeostasis as a common drug target to combat neurodegenerative diseases

Dagmar Pretsch*

As life expectancy increases, the prevalence of age-related diseases will also increase. There has been an estimation that 50 million individuals are living with dementia in 2019. This number will increase to 75 million in 2030 and 131.5 million by 2050 (McGill-Carter, 2020). Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington disease (HD) disproportionately affect older adult populations, inflicting a considerable physical, emotional, and economic burden.

Current treatments target only the symptoms, and not the causes, because for those disorders no target has yet been identified. In AD, several investigational drugs that target amyloid- β (A β) have failed to show any correlation between a reduction in amyloid burden and an improvement of cognitive functions in large-scale clinical trials. Elevated cortical A β concentrations were not solely responsible for the deposition of A β . Otherwise it would be difficult to explain why A β deposits are focal (related to synapses and the cerebrovascular lamina media) and not uniform in their distribution, especially because the amyloid precursor protein and amyloid- β are ubiquitously expressed. Moreover, to attribute A β accumulation to the presence of A β alone is problematic because the peptide is a normal component of healthy cerebrospinal fluid. Finally, whereas A β deposition is an age-dependent phenomenon, A β production does not appear to increase with age. So it is clear that for the search of new drugs against neurodegenerative disorders, other considerations have to be made.

We are used to think that neurodegenerative disorders are different clinical entities that target different brain regions and have separate pathology and symptoms. But when having a look towards the genetic or the molecular and cellular mechanisms, we should realize that several players and patterns appear consistently. All neurodegenerative disorders show an early vascular dysfunction, an accumulation of misfolded proteins, a selective sensitivity of specific neurons, and an activation of immune responses, to name but a few (Editorial, 2018). Accumulation of

misfolded proteins in the brain is one of the most prominent pathological sign: extracellular oligomers of the peptide A β form the notorious plaques found in AD. The protein α -synuclein accumulates within dopaminergic neurons in PD and the misfolded huntingtin protein oligomerizes in HD.

Another commonality is that each of these proteins interacts with several kinds of metal ions *in vitro*, for example with copper or iron (Xiao et al., 2013). Under physiological requirements Cu and Fe control important mechanisms of the central nervous system like neurotransmitter synthesis, myelin production, oxygen transportation and synaptic signalling to name but a few. To achieve this, enzymes, receptors and neurotransmitters have to be regulated structurally and catalytically. Cu and Fe provide redox properties for most of these cellular functions, but on the other hand they are also a source for the formation of reactive oxygen species too. Consequently, reactive oxygen species promotes neurodegeneration through the oxidation, misfolding, and aggregation of particular proteins. Another fact is that ageing promotes accumulation of metals in brains of humans and mice (Acevedo et al., 2019).

Bush and Tanzi (2008) stated in the "Metal Hypothesis of Neurodegenerative Disease", that an abnormal metal homeostasis during ageing promotes deleterious metallic reactions in the brain and this may provide a more tractable therapeutic target to inhibit disease progression. Many preclinical and clinical studies showed that metal modifying complexes and chelators have therapeutic potential by reducing neurodegeneration and improving clinical symptoms (Acevedo et al., 2019). Copper chelation has been examined as a means to fight free-radical damage previously, with clioquinol shown to reduce aggregation of A β and α -synuclein in transgenic mice (Adlard and Bush, 2018). However there must be further mode of actions than merely chelation of clioquinol because the use of the relatively specific copper chelator D-penicillamine did not show any protection in a mouse model of PD, and in the presence of diethyldithiocarbamate,

another effective copper chelator, enhanced neurotoxicity was observed in the same experimental model (Zhang et al., 2013). Clioquinol is the prototype of the novel drug PBT2. Both compounds translocate Cu²⁺ and Zn²⁺ into the cell, thereby initiating neuroprotective signaling cascades and consequently preventing the effects of breakdown of metal homeostasis (Adlard and Bush, 2018).

Metallothioneins (MTs) provide another possibility for the chelation of metal ions. MTs consist of a diverse superfamily of endogenous multipurpose proteins and their function is the transport, homeostasis, and detoxification of heavy metals. There has been shown that MT-III was markedly reduced in brains in AD, PD, amyotrophic lateral sclerosis, prion disease, brain trauma, brain ischemia, and psychiatric diseases (Zhang et al., 2013). Contrary, other work demonstrated that MTs were more highly expressed in Parkinsonian astrocytes (Michael et al., 2011). Miyazaki and colleagues unravelled the mystery by showing that the expression of MT-III and its mRNA was up-regulated in the healthy aged rat brain. After treatment with lipopolysaccharides, expression of MT-III and its mRNA has been increased only in young but not in aged rat brain regions. Therefore the scientists assumed that the reduced induction of brain MT-III against oxidative stress with aging is related to vulnerability and neurodegeneration of aged brain tissue (Miyazaki et al., 2000).

Therefore we hypothesized that drugs which are able to induce MT in aged model organisms of AD, PD or other neurodegenerative diseases could provide a novel promising therapy. But how can we easily evaluate the impact of compounds on MT induction in a high throughput screening assay?

In the search for new drugs, many variables must be considered if we want to conduct a single definitive screening assay which is based on the metal hypothesis: these include the complex interplay between metals, the magnitude of metal:protein interactions and the non-linear change in metals during ageing and during the course of disease (Barnham and Bush, 2014). These factors are best explored by using a whole animal screening model. The murine model *Mus musculus* is limited by its costs in large scale therapeutic screenings. By contrast, the model organism *C. elegans* is now gaining momentum as a host for screening tools. These nematodes combine genetic amenability, low cost and culture conditions

that are compatible with large-scale screens. Human diseases can be artificially engineered by expressing the human disease gene in *C. elegans*. By the use of such disease models, compounds which are able to suppress the disease phenotype can be identified after treatments. Hits can be further investigated for target identification by easily knocking down single genes using RNAi. In this way, new molecular mechanisms and targets could be found. With *C. elegans* we can bridge the gap between hit identification in cell based assays and the validation in mammalian models. *C. elegans* is widely used in studies of metal homeostasis, aging and neurodegenerative diseases. Results from age-related analyses of the metallome indicated that aging of *C. elegans* is further associated with the accumulation of iron, copper and manganese (Klang et al., 2014). There already exist many transgenic *C. elegans* models of neurodegenerative diseases which can be easily ordered from the Caenorhabditis Genetic Stock Center at the University of Minnesota. In our company, we use different transgenic *C. elegans* models for the initial screening and the mechanistic evaluation of potential new drugs for aging and neurodegenerative diseases. With the strain CL2659, we are looking for compounds which are able to reduce A β ₄₂ toxicity because the expression of A β ₄₂ in the muscle cells leads to a phenotypic paralysis after 48 hours. It has been shown that compounds that encounter AD prolong the time until paralysis (Pretsch et al., 2020). For the PD assay, we use the *C. elegans* strain NL5901, where α -synuclein is GFP-tagged and a reduction in α -synuclein burden can be monitored by a fluorescent reader. After screening several compounds in those models of neurodegenerative diseases, we found out that most hits in the AD screen were active in the PD assay too. To further examine these compound's influence on metalhomeostasis, we used the transgenic strain CL2120 where A β ₄₂ is expressed and MT is GFP tagged so that MT induction through disease progression can be monitored.

Like in the above mentioned study of Miyazaki, where murine models have been used, we were able to show that in the healthy *C. elegans* control strain CL2122, MT slightly increases with age, whereas in the A β ₄₂ expressing strain CL2120, we observed an induction in the young adults until the 6th day of their lifespan whereas after 9 days a breakdown followed (Pretsch et al., 2020). *C. elegans* has a lifespan of 14 to 20 days. Compounds that were active in both, in the

AD and PD assays, like emodin and clioquinol, were further able to prolong the time of MT induction in strain CL2120. When knocking down MT-2 by RNAi, active compounds lost their ability to reduce proteotoxicity in the AD and PD assays. Targeting the failed MT induction in aged nematodes with an AD or PD phenotype reduced the proteotoxicity by presumably restoring the metal homeostasis (Pretsch et al., 2020).

In my opinion, it is worth to have this target in mind when searching for new therapeutics against neurodegenerative diseases, and here I am not only referring to AD and PD. MT promotion should be further taken into account for HD, amyotrophic lateral sclerosis, prion disease, brain trauma, brain ischemia, and psychiatric diseases, as it has been shown that MT is diminished in those disorders too (Michael et al., 2011). Therefore our newly established MT assay could provide a first stage selection of compounds which should be further investigated in the particular disease model.

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Dagmar Pretsch*

Oxford Antibiotic Group GmbH, Konrad-Lorenz-Straße, Tulln, Austria

Department of Pharmacognosy, University of Vienna, Althanstrasse, Vienna, Austria

*Correspondence to: Dagmar Pretsch, MSc, dagmar.pretsch@univie.ac.at or d.pretsch@oxfordantibioticgroup.com. <https://orcid.org/0000-0002-8984-9099> (Dagmar Pretsch)

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