

**Size characterized manganese - and iron species in sprague-dawley rats exposed to  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$  (i.v.)**

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Continuous manganese (Mn) exposure leads to damage of the central nervous system and finally to a *Mn* dependent Parkinsonism, also called “manganism”. However, the exact mechanism, how *Mn* can enter the brain without using the transferrin-receptor mediated transport, is still unknown. Excessive *Mn* load can cause an overflow of the liver and an overload of the original *Mn* carriers (e.g. transferrin or serum albumin). In that case, low molecular mass (*LMM*) compounds can act as ligands and form stable *Mn*-complexes. These *LMM Mn* species (above all *Mn*-citrate) probably cross the blood-brain barrier and accumulate in the brain (*basal ganglia*). Iron (*Fe*) is discussed as a co-factor of the manganese dependent neurodegeneration specifically because it represents the main competitor in the formation of *Mn* species.

The rat is a well established experimental animal for exposure experiments and the adequate analogy of the blood-brain barrier is quite sufficient to investigate its characteristics regarding the permeability of *Mn*- and *Fe* species. The current research therefore investigated the size distribution of the *Mn*- and *Fe* species of the original *Mn*- and *Fe* carrier of unexposed rats fed with a high manganese and iron standard diet as the first characterization step. Subsequently *Mn*- and *Fe* species analysis of exposed sprague-dawley rats (one-time i.v. injection of up to 1 mg Mn per kg body weight and four days incubation) fed with a low *Mn*- and *Fe* special diet (2 weeks pre-feeding) are supposed to improve the understanding of the homeostasis of allegedly harmful *LMM Mn* species. The special diet provides comparable low *Mn*- and *Fe* body levels and accordingly an enhanced Mn absorption in all rats. Unexposed animals (controls) were analyzed after injection of the same infusion solution without Mn. In all experiments the animals were anesthetized and killed painless. The organs and body fluids were frozen in liquid nitrogen for further investigations. After homogenizing under liquid nitrogen and cleaning up the samples under inert gas (Ar), the different rat tissue extracts and sera were size-characterized (eluent pH=7.4). These methodical parameters were found to be the optimum conditions with regard to species stability and quality assurance. The size exclusion chromatography coupled on-line to an inductively coupled plasma – dynamic reaction cell - mass spectrometer (*SEC-ICP-DRC-MS*) offers a valuable tool for speciation analysis of labile *Mn*- and *Fe* species. The recovery was determined by *ICP*-optical emission spectroscopy (*ICP-OES*).

The investigation of different tissues (e.g. brain, kidney, liver and lung) and sera could assign the organic *Mn*- and *Fe* species to a molecular mass between 80-160 kDa. However, nearly each sample showed a minor signal at the retention time according to probably harmful *LMM-Mn*-species (<1000 Da) and inorganic *Mn* and *Fe* respectively. This finding may be discussed either due to a chemical equilibrium between high- and low molecular mass species in the sera and accordingly tissue extracts, or due to a degradation of native *Mn* species. However, polyatomic interferences (e. g. Ar-N-H, Ar-O, Ar-O-H according to the mass <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>57</sup>Fe) which are removed to a large extent by DRC cannot be completely excluded.