7 SUMMARY

This study focused on the regulation of microglial activation under oxidative stress conditions by the trace element selenium.

In response to the cellular damage induced by oxidative stress, the microglial cells become activated and produce large amounts of free radicals of oxygen, nitrogen oxides and toxic cytokines, which contribute to the secondary neuronal damage and account for most of the losses of the brain functions.

The findings of this study demonstrate that hydrogen peroxide-induced microglial activation and deleterious correlated events can be attenuated by selenium through the downstream regulation of intracellular mechanisms, such as the production of ROS, the activation of caspase-3, the nuclear translocation of NF-kB and the activation of iNOS. The activation and migration of microglial cells were also shown to be regulated by the incubation with physiological concentrations of selenium: the FACS analysis showed a decreased expression of the $ß_2$-integrin CD11a after pre-incubation with selenium.

By means of ^{75}Se-labelling and electrophoresis, the selenoproteome of BV2 cells was analysed. After two-dimensional electrophoresis more than 25 labelled spots were detected in the microglial cells. Some selenoproteins could be identified by comparison with the results of previous studies.

All the observed events were related to the capability of selenium to stimulate the expression of the microglial selenoproteins. In particular, selenium administration increased the Gpx1 activity in BV2 cells, as evidenced by the tracer experiments with ^{75}Se and by enzyme activity measurements. Thus, it was likely that selenium could act by scavenging free radicals through an increase in the Gpx1 activity (resulting in the inhibition of the microglial activation induced by hydrogen peroxide). Novel plasmids were constructed for the down-regulation and over-expression of the Gpx1 protein. The over-expression was achieved by cloning a Gpx1-EGFP-C1 vector, which was successfully transfected in BV2 cells and was able to produce recombinant Gpx1-EGFP protein. This also allowed the analysis of the subcellular localization of the protein by confocal microscopy in COS-7 cells. The silencing of the Gpx1 activity was achieved by using the siRNA technology. Target sequences were accurately

selected and at the end of the cloning process in the pSUPER vector, one functional plasmid was obtained. The Gpx1-pSUPER-142 vector was able to reduce efficiently and exclusively the Gpx1 protein expression in microglial cells, shown by the selenoproteome analysis of the transfected cells.

The silencing of the Gpx1 confirmed that this protein is one of the fundamental proteins by which selenium acts in the protection of microglial cells. The essential role of the selenium status was also confirmed in experiments with primary microglial cells isolated from rats fed a selenium-sufficient or a selenium-deficient diet.

Thus, this work helped to elucidate some of the intracellular mechanisms by which selenium produces protective responses via the modulation of toxic events in microglial cells. The results support the use of selenium as a therapeutic agent against the microglial over-activation, which occurs in many neurodegenerative diseases and contributes to the secondary neuronal cell death.