Abstract and keywords

Although reactive oxygen and nitrogen species have a fundamental role in physiological processes occurring in living organisms, their overproduction induced by endogenous and/or exogenous sources may lead to serious imbalance in redox homeostasis, damage to intracellular components and thus, dramatically alter their function or even trigger cell death. Oxidative stress is believed to be very important mechanism of toxicity of xenobiotics, including drugs, and may be responsible for the development of their unwanted side effects. Considering a very low number of studies evaluating oxidative stress after the treatment with oxime reactivators of acetylcholinesterase (AChE) *in vivo* and *in vitro*, the relationship between their toxicity and generation of specific biomarkers of oxidative damage is not still fully understood. In order to monitor antioxidant/prooxidant properties of drugs, high performance liquid chromatography method coupled with tandem mass spectrometry (LC-MS/MS) for simultaneous determination of two biomarkers of oxidative stress, malondialdehyde (MDA) and 3-nitrotyrosine (3-NT), in biological matrices was developed. Validation of this LC-MS/MS method demonstrated the acceptable appreciable selectivity, accuracy, intra- and interday precission, and recovery of sample processing (deviations < 15%). The method is applicable for determination of MDA and 3-NT in biological samples in ranges from 0.025 to 4.00 nmol/mg and 0.0125 to 2.00 nmol/mg, respectively. Additionally, chromatographic method with spectrophotometric detection (HPLC-UV/VIS) was established and optimized for the examination of thiol redox state represented by non-protein thiols (NP-SH) and disulfides (NP-SS-NP). Chromatographic methods were subsequently applied on samples obtained from *in vitro* experiment with HepG2 cells exposed to five clinically most relevant AChE reactivators: obidoxime (LüH-6), asoxime (HI-6), pralidoxime (2-PAM), trimedoxime (TMB-4), and metoxime (MMB-4) for 1, 4 and 24 hours. Based on the results, the ability of selected oximes to induce oxidative/nitrosative stress *in vitro* was in the following order: LüH-6 > TMB-4 > MMB-4 > 2-PAM > HI-6. Regarding the chemical structure of these reactivators, bisquarternary oximes bearing two oxime groups at position 4 of pyridinium rings (LüH-6, TMB-4 and MMB-4) appeared to induce more intensive oxidative/nitrosative stress. The LC-MS/MS and HPLC-UV/VIS methods were further used for determination of oxidative stress biomarkers in cerebrospinal fluid collected from patients diagnosed with Alzheimer disease. Oxidative stress biomarkers were evaluated in patients treated with memantine in comparison to control group without this therapy.

Memantine therapy decreased 3-NT and increased NP-SH concentrations in cerebrospinal fluid, whereas levels of the remaining markers were not significantly changed.

Keywords: oxidative stress, nitrosative stress, organophosphates, nerve agents, oximes, reactivators of acetylcholinesterase, malondialdehyde, 3-nitrotyrosine, high performance liquid chromatography, mass spectrometry, Alzheimer disease, memantine.