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Ex vivo method for determination of oxidative stress and diagnosis of ferroptosis-associated diseases

Keywords: iron species, ferritin, selenium species, sulfur species, CE-ICP-DRC-MS, oxidative stress, ferroptosis, cancer, neurodegeneration

INVENTION NOVELTY

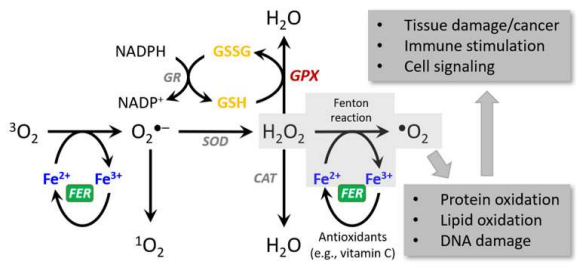
The pathogenesis of several diseases is associated with dysregulation of the cellular redox balance. This type of cell damage is referred to as ferroptosis and mediated by an increase in Fe^{2+} and a decrease in Fe^{3+}. Elevated levels of Fe^{2+} induce the formation reactive oxygen species (ROS), which in turn promote the formation of lipid peroxides. Reduced glutathione (GSH) can donate electrons to ROS under formation of disulfide-bridged glutathione disulfide (GSSG), this way eliminating free radicals. This reaction is catalyzed by the seleno-enzyme GPX4, whose decreased activity (due to a lack of selenocysteine-tRNA) may lead to an accumulation of lipid peroxides and ROS, further promoting oxidative stress and eventually causing e.g., DNA damage. Prior art methods allow to individually determine some of the biological iron, sulfur and selenium species, but not all of them in parallel from one sample. The invention provides an elegant, easy, inexpensive, and quantitative method for determining the composition of iron, sulfur, and selenium species simultaneously, paving the way for using this information for diagnostic and prognostic purposes.

VALUE PROPOSITION

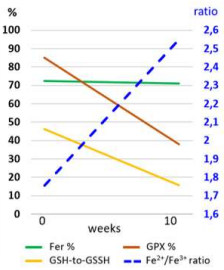
The innovative *ex vivo* method exploits an CE-ICP-DRC-MS-based "*one pot, two shot*" approach to quantitatively determine all biological relevant iron (e.g., Fe^{2+}, Fe^{3+}, ferritin-bound Fe and total Fe), selenium and sulfur (e.g., GSH, GSSG, cysteine, cystine, Se-cystine, Se-methionine, Se^{4+}, Se^{6+} and GPX4-n) species in parallel, in short time (5-15 min) and at low costs. It allows scientists and clinicians to capture redox-biological information, inaccessible with conventional methods, in essentially any biofluid (including cell and tissue lysates, CSF, serum and urine), and to use it as diagnostic and prognostic marker for any disease, related to oxidative stress and ferroptosis. Comprehensive validations on samples derived from patients suffering from e.g., cancer or neurodegenerative diseases demonstrate the method's tremendous clinical value and potential.

TECHNOLOGY DESCRIPTION

The method is based on inductively coupled plasma mass spectrometry (ICP-MS), in which an argon steam is passed through a high-frequency electromagnetic field. In the resulting plasma, the sample aerosols are vaporized and broken down into their atomic components, which can then be analyzed by MS. Due to the special nature of the excitation, the atoms produced are usually singly ionized, making the electropherograms generated very clear and easy to interpret. In addition, the method has a detection limit in the range of a few micrograms per liter, requires minimal sample volumes (nanoliters), and allows quantitative measurements over a wide, linear range. Potential interferences can be suppressed by using a dynamic reaction cell (DRC) along with a suitable reaction gas (e.g., NH_3 or CH_4). The ICP-DRC-MS system allows the quantification of individual elements (e.g., total iron). To distinguish between different species (e.g., Fe^{2+}, Fe^{3+}, Fe-ferritin), a suitable analytical separation (i.e., capillary electrophoresis, CE) must be performed upstream. The coupled ICP-DRC-MS then detects individual elemental peaks (e.g., iron), which can be assigned, to individual species, based on their known CE-elution profile.



ROS generation via Fenton reaction. CAT, catalase; FER, iron transporting ferritin; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; NADPH/$NADP^+$, nicotinamide adenine dinucleotide phosphate (reduced/oxidized); SOD, superoxide dismutase (adopted from: Abe et al., 2022, Molecules 27: 5451).



Change of ferroptose markers during progressive development of prostate cancer cells (Michalke et al., 2022, unpublished).

DEVELOPMENT STATUS

The *ex vivo* method has been extensively and successfully studied *in vitro* and *in vivo*, and its diagnostic and prognostic potential is currently being clinically validated for several diseases associated with oxidative stress and ferroptosis.

COMMERCIAL OPPORTUNITY

The *ex vivo* method is available for in-licensing.

PATENT SITUATION

The method is protected by WO2023/025957A1.

FURTHER READING

Vara-Pérez et al., 2021, EMBO J. 40: e106214; Charlebois et al., 2022, eLife 11: e81332; and Jhelum et al., 2023, A. Neurop. Commun. 11: 121.

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