Technique for quantification of erythrocyte zinc protoporphyrin IX and protoporphyrin IX

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In heme biosynthesis, the terminal step is the insertion of ferrous iron into protoporphyrin IX (PPIX) by the enzyme ferrochelatase. Under physiological conditions, small amounts of zinc protoporphyrin IX (ZnPP) are formed as well. The concentrations of ZnPP and PPIX in erythroid cells are characteristically altered by conditions that affect the availability of ferrous iron, increase the amounts of PPIX, or diminish the enzymatic activity of ferrochelatase. Unfortunately, the standard HPLC-based quantification method is time-consuming and complicated. Alternatives, too, have proven too technically demanding or cumbersome for general adoption. For measurement of ZnPP alone, a portable front-face fluorometer, the hematofluorometer, is commercially available. Yet, its use is limited by a high background fluorescence of other blood constituents making sample washing inevitable.

A research team led by Georg Hennig from the Klinikum der Universität München (Germany) now developed a new method for simultaneous quantification of ZnPP and PPIX in unwashed blood samples. It is based on dual-wavelength excitation to effectively eliminate background fluorescence from other blood constituents. The obtained results were closely correlated with determinations by a reference HPLC assay.

Key to success was the choice of appropriate excitation wavelengths. Both should undergo virtually identical absorption and their spectral separation should be small, so that the penetration depths of the photons

are essentially the same. The excitation efficiency of the fluorophores responsible for autofluorescence should differ by only a small amount between the two excitation wavelengths, so that subtraction of the emission spectra effectively eliminates autofluorescence. In contrast, the analyte fluorophore should exhibit a large difference in excitation efficiency between these two excitation wavelengths, so that the difference between the emission spectra is large and does not eliminate the analyte fluorophore signal.

This is the case for 425 nm (the ZnPP fluorescence excitation maximum, emission maximum at 593 nm) and 407 nm as a corresponding wavelength with identical oxygenized heme absorption, but substantially lower ZnPP excitation efficiency. Moreover, as the excitation wavelength at 407 nm approaches the PPIX excitation maximum at 397 nm, the emission spectrum shows a pronounced PPIX fluorescence emission peak, which is found at 627 nm. As the PPIX excitation efficiency is much lower at 425 nm, the PPIX fluorescence emission peak is not eliminated in the difference spectrum and can be evaluated along with the ZnPP signal.

The new approach could be simply and cost-effectively implemented in a device for use under field conditions. In addition, it might reduce autofluorescence in tissue such as the oral mucosa in vivo, enabling non-invasive measurements of ZnPP and PPIX. (Text contributed by K. Maedefessel-Herrmann)

More information: Georg Hennig, Christian Gruber, Michael Vogeser, Herbert Stepp, Stephan Dittmar, Ronald Sroka, and Gary M. Brittenham, "Dual-wavelength excitation for fluorescence-based quantification of zinc protoporphyrin IX and protoporphyrin IX in whole blood," *J. Biophotonics* 7:7, 514-524 (2014); DOI: 10.1002/jbio.201200228/pdf

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