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Review

Analysis of the kinetics of lipid peroxidation in terms of characteristic time-points

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ABSTRACT

Measuring peroxidation of aggregated lipids in model systems (liposomes, micelles, emulsions or microemulsions) as well as in samples of biological origin ex vivo (isolated lipoproteins, blood sera or plasma) is widely used in medical and biological investigations, to evaluate the oxidative stress, antioxidants' efficiency and lipid oxidizability in different pathophysiological states. To avoid possible artifacts, such investigations must be based on the time course of peroxidation (i.e. on kinetic studies). To be able to compare complex kinetic profiles, it is important to characterize them in terms of mechanistically meaningful and experimentally unequivocal parameters.

In this review, we characterize the typically observed continuous kinetic profiles in terms of a limited number of characteristic time-points (both commonly used and additional time-points and their combinations) that can be derived from experimental time-dependencies. The meaning of each of the experimentally observed characteristic parameters is presented in terms of rate constants and concentrations, derived on the basis of mechanistic considerations. Theoretical expressions for these characteristic parameters are based on a model that includes both the inhibited peroxidation and the uninhibited peroxidation occurring after consumption of the antioxidant(s). Comparison between theoretically predicted dependencies and experimental data support our treatment considered with special emphasis on transition metals-induced peroxidation of lipoproteins.

Contents

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride, organic generator of free radicals; LDL, low density lipoprotein; OS, oxidative stress; TMI, transition metal ions; PUFA, polyunsaturated fatty acid; OD, optical density; LH, lipid, mostly PUFA, (*index zero relates to zero time, i.e. to concentration prior to the beginning of peroxidation, see also footnote to Table 2); AH, antioxidant*; HP = LOOH, lipid hydroperoxide*; Cu_B, copper ions bound to lipoprotein particles; L•, LO•, LO_2•, lipid-derived free radicals: alkyl, alkoxyl and alkylperoxyl radical, respectively; R•, any free radical produced in the considered system (e.g. AAPH-derived or lipid hydroperoxide-derived radicals); R_i, initiation rate, rate of free radical production; v, reaction rates (indices are explained in Sections 3 and 4); k, rate constants (indices are explained in Section 4 and Table 3).

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1. Introduction

Oxidative stress (OS), intuitively defined as excess of reactive oxygen and nitrogen species (RONS) over the antioxidative defense network in cells and biological fluids, is an issue of great importance (Sies, 1997). Lipid peroxidation, induced by RONS, particularly free radicals, is commonly considered responsible for the pathogenesis of many diseases and pathological states, including cardiovascular disease, diabetes mellitus and human immunodeficiency (Halliwell and Gutteridge, 2007). Of special importance is the commonly accepted hypothesis that oxidation products of low density lipoproteins (oxLDL) are responsible for atherogenesis (Steinberg et al., 1989). The evidence for causal relationships between free radical lipid peroxidation of LDL, the major carrier of cholesterol in the circulation, and development of the diseases is indirect (Stocker and Keaney, 2004). However, much effort has been devoted to evaluation of 'oxidative stress' (Costantini and Verhulst, 2009) ex vivo, using many different methods to measure different biomarkers (indices) that can be regarded indicative of OS.

In our previous studies we found that OS can not be defined on the basis of a universal index but that methods based on lipid peroxidation (e.g. malondialdehyde, isoprostanes, lipid hydroperoxides) do correlate with each other (Dotan et al., 2004). Alternative approaches in evaluation of OS are either based on measurements of the concentration of OS biomarkers in the circulation or on the susceptibility of lipid specimen to a flux of free radicals, as monitored upon exposure to free radical 'generators' ex vivo. The response of lipid specimens to radical attack may be monitored either at one predefined time-point or at multiple consecutive time-points. In the latter case, the outcome of the assay is a kinetic profile. The 'ease' of oxidation characterizes the susceptibility of the studied sample to oxidation and is used to evaluate the 'oxidative stress'. The observed kinetics is usually compared with that observed for a control sample under the same experimental conditions (e.g. cardiovascular disease patients vs healthy subjects). The kinetic profile of peroxidation can be based on continuous measurement of the production of oxidation products and/or of the consumption of oxidizable lipids and/or oxygen consumption and/or depletion of antioxidants. Many analytical methods have been applied to monitor different biomarkers, including spectrophotometry (Esterbauer et al., 1989), chromatography (Bowry and Stocker, 1993), fluorescent probes (Hofer et al., 1995), chemiluminescence and electrochemistry (Abuja and Albertini, 2001).

One of the most popular methods used to evaluate the sensitivity of blood lipoproteins to ex vivo peroxidation, is based on of continuous spectrophotometric monitoring of the kinetics of LDL peroxidation induced by transition metal ions (TMI), as proposed by Esterbauer and his group (Esterbauer et al., 1992). This method is based on the absorbtion of UV-light by peroxidation products (shown in Scheme 1), which is monitored continuously at a wavelength of 234 nm after addition of cupric ions. Alternatively, peroxidation might be initiated by other agents that can induce formation of free radicals (e.g. organic generators of free radicals, specific enzymes or radiation).

Regardless of the measured factor, a characteristic kinetic profile of lipid peroxidation is typically sigmoidal (Fig. 1). Specifically, rapid peroxidation (propagation phase) is preceded by a pronounced periods of initial slow peroxidation (usually denoted 'lag phase') and followed by a phase of slow peroxidation after almost all the substrate (oxidizable lipids) became oxidized. The kinetic profiles can be expressed by several parameters, including specific time-points, rates and concentrations of oxidation products at specific time points (Pinchuk and Lichtenberg, 2002). Each of these parameters can differ for different samples. Qualitative interpretation of the observed peroxidation kinetic profiles is commonly based on the length of the lag phase: shorter lag time indicates that the lipids in the studied sample are less resistant to peroxidation or 'more susceptible to oxidation' (Cadenas and Sies, 1998). The latter term is often denoted 'oxidizability', which is somewhat misleading because it is also used for strictly defined combination of peroxidation rate constants.

Analysis of lipid peroxidation induced by organic generators of free radicals is relatively simple. The rate of formation of free radicals is constant and almost independent of the content of preformed hydroperoxides. Hence, the lag is a relatively well-defined function of the concentration of the inducer and lipid, as well as of the concentration(s) and properties of antioxidant(s) present in the system. Quantitative description of this process is well established and, in general, agrees with experimental data (Bowry and Stocker, 1993; Frankel, 2005, 2007; Denisov and Denisova, 2000; Denisov and Afanas'ev, 2005).

By contrast, the kinetics of copper-induced peroxidation depends on the concentration of hydroperoxides (Thomas et al., 1994; Frei and Gaziano, 1993; Pinchuk and Lichtenberg, 1999; O'Leary et al., 1992) and rigorous kinetic description is more complex and much less understood, in spite of the popularity of the assay and the large number of published experimental results (Burkitt, 2001). In other words, adequate characterization of the kinetic profiles in terms of rate constants and concentrations is not available.

Several approaches have been applied to quantitate transition metal-induced peroxidation of lipoproteins:

- *Numerical simulation* of kinetics based on elementary reactions involved in peroxidation, followed by fitting to experimental curves by varying rate constants and concentrations (Abuja and Esterbauer, 1995; Abuja et al., 1997; Herak et al., 2004).
- Fitting of experimental kinetics to appropriate non-linear function without relating to mechanistic interpretation of the obtained parameters, subsequently followed by calculation of characteristic time points based on a *formal mathematical approach* (McPherson et al., 2012).