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EMF and the *in vivo* quantification of oxidative stress by superoxide radical and by DNA fragmentation

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Several mechanisms have been proposed to account for most biological effects after exposure to EMF. Oxidative stress, leading to genotoxicity and disease via oxygen free radicals, is the mechanism best supported by experimental data until today, although not conclusively and only indirectly. And this, because the EMF-oxidative stress relation has been shown (i) by the oxidative damage reactive oxygen species (ROS, such as superoxide radical, hydrogen peroxide, hydroxyl radical, singlet oxygen) can cause in lipids and DNA, (ii) by the non-specific assessment of ROS and the reduction of lipid peroxides and DNA damage by non-specific molecular traps (such as the natural antioxidants melatonin and ascorbic acid, the spin trap N-tert-butyl-a-phenylnitrone, the fluorescent trap rhodamine 123), and (iii) by measuring the specific activity of the main ROS antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. Moreover, EMF-induced genotoxicity via ROS-induced DNA oxidative damage (an indirect indicator of oxidative stress as well) is assessed mainly by qualitative DNA fragmentation assays (such as the problematic in its use Comet assay and the DNA smearing agarose electrophoresis assay), and by measuring 8-oxoguanosine or DNA nicks (which are indicators of transiently damaged DNA because both are cell repairable).

For conclusively showing that EMF induces oxidative stress, my lab proposes its direct assessment by the *in vivo* quantification of superoxide radical in EMF-exposed experimental organisms. The reason being, superoxide radical is central among the ROS components of oxidative stress since it is the precursor of hydrogen peroxide, hydroxyl radical, singlet oxygen. My lab has developed an ultrasensitive assay for the *in vivo* assessment of superoxide radical in various organisms (e.g. rats, mice, rabbits, flies, cell cultures) via the quantification of 2-hydroxyethidium, its specific reaction product with hydroethidine. The sensitivity of assay is <1 pmol 2-hydroxyethidium/mg protein and can be extended to the femto mg protein level when using large size samples [1]. Moreover, for conclusively showing that EMF induce genotoxicity by quantitatively assessing the permanent genome damage, my lab has developed the following complementary and ultrasensitive DNA fragmentation assays: The first assay quantifies fragmented DNA approximately <23 Kb, and, thus, it is restricted to organisms with chromosome size ≥23 Kb; it actually quantifies the fragmented DNA smear formed in a typical agarose gel during DNA electrophoresis. The sensitivity of the assay is 3 ng ml^{-1} (in DNA samples ≥10 ng ml^{-1}) and 5 pg ml^{-1} (in DNA samples ≥15 pg ml^{-1}) for its Hoechst and PicoGreen version, respectively [2]. The second assay quantifies cell necrotic/apoptotic fragmented DNA approximately <1 Kb, with sensitivity 3 ng ml^{-1} and 25 pg ml^{-1} for its Hoechst and PicoGreen version, respectively (in DNA samples ≤2 μg ml^{-1}) [3]. The assay is applicable to any organism regardless of genome size.

The *in vivo* direct evaluation and quantification of EMF-induced oxidative stress and genotoxicity (measured as permanent genomic DNA damage) will help in developing objective criteria for the risk assessment and management of EMF.

References:

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