

METHOD TO ASSESS RNA/DNA OXIDATION FOR RESEARCH IN AGING

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We developed a method for the simultaneous extraction and analysis of total tissue RNA and DNA to quantify the RNA and DNA oxidation products 8-oxo-7,8-dihydroguanosine and 8-oxo-7,8-dihydro-2'-deoxyguanosine using HPLC coupled to electrochemical detection. The method is fast, gives high yields of pure RNA and DNA with low background oxidation levels and also determines the RNA/DNA ratio. In vitro experiments with RNA and DNA exposed to H_2O_2 /ascorbate/ Fe^{3+} (or Cu^{2+}) resulted in significantly greater RNA oxidation. The RNase inhibitor 2-mercaptoethanol commonly used for RNA extraction acted as a pro-oxidant during nucleic acid extraction, an effect attenuated by the inclusion of the metal chelator deferoxamine mesylate. Small but significant increases in liver RNA oxidation was detected from doxorubicin (oxidant generator) administration to Fisher-344 rats. Also, the method could detect significant increases of nucleic acid oxidation in human brains undergoing degenerative diseases including Alzheimer's versus aged controls using only mg amounts of tissue. This novel method could become a useful tool for research in aging.