Free Rad. Res. Comms., Vol. 8, No. 3, pp. 139–141
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FRRC HIGHLIGHT: This note highlights a seminal paper published in Free Rad. Res. Comms. 1, 163–172 (1986)

THE DEVELOPMENT OF A SENSITIVE ANALYSIS FOR 8-HYDROXY-2′-DEOXYGUANOSINE

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(Received September 27, 1989)

We were fortunate to be able to first show that 8-hydroxy-2′-deoxyguanosine (8-OHdG) could be sensitively quantitated (to 20 femto moles) by the use of HPLC with electrochemical detection.[1] This method provides a means to help assess the role of activated oxygen attack on DNA (and RNA) and as such provides data to understand oxidative stress[2] in biological systems.

Tiger in Blake's poem by that title could refer to sagacity, defiance and doubt, but it is equally clear that serendipity, dependence upon the creative and rigorous observations of other researchers and determination fueled by curiosity and dissatisfaction must play an equally important role in exploring new areas and approaches scientifically. Perhaps all three aspects were operating to help make our first observations possible. It is important to acknowledge the following set of circumstances which significantly contributed: (a) Dr. S. Nishimura's generosity in providing us with an authentic standard of 8-OHdG, (b) Dr. P. Wong's collaborative expertise whose training in HPLC-electrochemical detection (HPLC-ED) derives from the pioneering efforts on this analytical technique in biological systems by Drs. L. Blank and R. Adams, (c) our first efforts to use HPLC-ED to detect reduced spin-adducts[3] and hydroxyl free radical reaction products of phenol and salicylate[4] and (d) a persistent fascination with the fundamental chemistry of the toxic aspects of oxygen on biological systems and especially oxygen free radical reactions with nucleic acids.[5]

Since the earlier observations, 8-OHdG has been demonstrated to be formed in the DNA of several biological systems undergoing oxidative stress.[6–10] Nishimura's group has found, using a wide range of systems, a positive correlation between 8-OHdG formation in DNA and conditions enhancing cancer development.[7–9,11–12] We have demonstrated that certain chromium salts and betel nut quid, agents responsible for cancer development, cause formation of 8-OHdG in DNA.[14,15] Of significance is the fact that Kuchino *et al.*[16] demonstrated that 8-OHdG in the DNA template caused miscoding not only of the complementary base but bases on either side of 8-OHdG. It has also been demonstrated that the 8-OHdG content of mitochondrial DNA is higher than that of nuclear DNA. Along this same line, we have noted that the 8-OHdG content of plant chloroplast DNA is higher than nuclear DNA, and that ozone injured plants have a higher content of 8-OHdG in their chloroplast DNA.[10]

In a recent and surprising observation, we discovered that methylene blue plus light causes formation of significant levels of 8-OHdG in DNA apparently by a singlet oxygen mediated mechanism.[18] Progressing from this observation new leads are beginning to emerge regarding the role of 8-hydroxy guanine in RNA. In addition there are areas of research where we are attempting to gain additional knowledge. These include a clear answer to exactly how many 8-hydroxyguanines nature will tolerate in specific populations of DNA and RNA before serious consequences occur; and exactly what are the consequences? Inherent in this question is the need to determine the basal level of these modified bases and the inherent "noise" level that nature will tolerate. That is, if the basal level of 8-OHdG in the nuclear DNA of cultured tumor cells in 1.0 per 10^5 G's, is it possible to consistently determine with confidence if say 1.5 8-OHdG/10^5 G's are produced by a mild oxidative stress. It is possible that 2.0 8-OHdG/10^5 G's is clearly within the limits which are tolerated by the tumor cell line and in fact may be a value that is obtained routinely on certain batches of normal cells. Clearly the analytical methodology of choice must be capable of addressing this question rigorously. We know that meticulous control of DNA/RNA extraction, handling and digestion are necessary to achieve reliable results.

Thus, this brief and incomplete sketch of the developments which have occurred since the first observations[1,19] show that this is a rapidly evolving area of research. Any new analytical development which allows us to probe nature in a different light has inherent limitations. Facts are indeed pesky; but the new vision provided has given a fresh glimpse of the inherent beauty of nature. As it is with most developments the vistas first afforded allows progress to the next limit, beyond which more clearer views are possible.

Acknowledgments

I express appreciation to: B. Chance, B. Commoner, E. Copeland, C. Dye, F. Crane, D. DeVault, C.W.-F, A.F., B. Goldstein, E. Janzen, P. McCay, A. Ohlrogge, H. Sies, B. Singer, D. Tingey, and D. Reed. The financial support of NIH grants CA 42854, ESO4296, NS23307, and EPA R8419801 is acknowledged.

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Accepted by Prof. H. Sies