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The endogenous exposome

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ABSTRACT

The concept of the *Exposome* is a compilation of diseases and one's lifetime exposure to chemicals, whether the exposure comes from environmental, dietary, or occupational exposures; or endogenous chemicals that are formed from normal metabolism, inflammation, oxidative stress, lipid peroxidation, infections, and other natural metabolic processes such as alteration of the gut microbiome. In this review, we have focused on the *endogenous exposome*, the DNA damage that arises from the production of endogenous electrophilic molecules in our cells. It provides quantitative data on endogenous DNA damage and its relationship to mutagenesis, with emphasis on when exogenous chemical exposures that produce identical DNA adducts to those arising from normal metabolism cause significant increases in total identical DNA adducts. We have utilized stable isotope labeled chemical exposures of animals and cells, so that accurate relationships between endogenous and exogenous exposures can be determined. Advances in mass spectrometry have vastly increased both the sensitivity and accuracy of such studies. Furthermore, we have clear evidence of which sources of exposure drive low dose biology that results in mutations and disease. These data provide much needed information to impact quantitative risk assessments, in the hope of moving towards the use of science, rather than default assumptions.

In 2005, Chris Wild brought forward the concept of the "exposome" [1]. He suggested that together with genomics, metabolomics, proteomics, and transcriptomics, we need to also understand relationships between life-time exposures to chemicals and disease. This concept was further explored several years later by Liory and Rappaport [2] and by Wild [3], who pointed out that the assessment of exposures should not be restricted to chemicals entering the body from air, water, food, smoking, etc., but should also include internally generated toxicants produced by the gut flora, inflammation, oxidative stress, lipid peroxidation, infections, and other natural biological processes. In other words, we must focus upon the 'internal chemical environment' arising from all exposures to bioactive chemicals from within and outside the body.

This review will focus on recent advances in our understanding of endogenous DNA damage arising from the internal environment, and how it compares with external exposures. Advances in analytical methods have vastly changed our ability to accurately measure biomarkers such as DNA adducts and protein adducts over the past two decades. When this is coupled with exposure to stable isotope labeled chemicals that cause identical DNA damage, we now can accurately compare the exposures that arise endogenously, with those coming from environmental, occupational and life style chemical exposures. This has important implications for understanding exposure responses, such as causality of mutations, cancer, disease and aging. Likewise, it begins to explain why mutations do not extrapolate to zero when studied at very low exposures. Rather, they often reach thresholds that appear to be driven by the endogenous exposome. The field of low dose mutagenesis, rather than high dose studies for hazard identification, has been grossly understudied, but is of great importance for the advancement of science-based risk assessment.

Research related to endogenous DNA damage and its relationship to a variety of chemical exposures has been a major focus of our laboratory for the past two decades. This review will cover research on abasic sites, oxidative DNA damage and several known human carcinogens that form exogenous adducts identical to endogenous DNA adducts, what the exposure–response relationships are for identical endogenous and exogenous DNA damage, and how this knowledge of the endogenous exposome helps us understand exposure–responses for mutations and disease, as well as informing science-based risk assessment.

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Table 1
The endogenous exposome steady-state amounts of endogenous DNA damage.

Endogenous DNA lesions	Number per cell
AP sites	30,000
OHEtG	3,000
7-(2-Oxoethyl)G	3,000
8-OxodG	2,400
Formaldehyde	1,000–4,000
Acetaldehyde	1,000–5,000
7-Methylguanine	2,300
AcrdG	120
M_1dG	60
N^2,3-Ethenoguanine	36
1N^2-Etheno dG	30
lN^6-EthnodA	12
O^6-Methyl dG	2
Total	40,000+

1. Apurinic/apyrimidinic sites

Apurinic/apyrimidinic (AP) sites are known to be one of the most prevalent types of endogenous DNA lesions (Table 1). Endogenous AP sites in cellular DNA are partly derived from spontaneous depurination and depyrimidination of normal and unstable modified bases (*e.g.*, *N*7-methylguanine (*N*7-meG) and *N*3-methyladenine (*N*3-meA)). In 1973, the rate of spontaneous AP site formation was first estimated to be 10,000 sites/cell/day using the depurination rate of DNA at 70 °C and physical chemistry [4].

Twenty five years later, we directly demonstrated that AP sites are generated at 1.54 AP sites/10^6 nucleotides/day (~9000 AP sites/cell/day) at 37 °C and pH 7.4 using aldehyde reactive probe [5]. In addition to spontaneous hydrolytic base loss, AP sites are also generated by the base excision repair pathway. An AP site serves as an intermediate DNA lesion during the repair of several modified bases [6–8]. Our accumulated results indicate that the steady-state level of AP sites is approximately 30,000 lesions per genome in mammalian cells and tissues [9]. It is important to point out that these endogenous AP sites are likely oxidized deoxyribose [10]. Endogenous hydrogen peroxide, one of the major endogenous reactive oxygen species, generates hydroxyl radicals though the Fenton reaction with ferrous (Fe^{2+}) ions loosely attached to the *N*7 position of guanine and preferentially oxidizing the adjacent deoxyribose to generate various oxidized deoxyriboses, resulting in oxidized AP sites [11–13]. Since deoxyribose lesions are hard to quantitate with high sensitivity and specificity, a steady-state level of oxidized deoxyribose and their biological importance are largely unknown even though they are among the most abundant endogenous DNA lesion in living organisms. The regular AP sites, derived from spontaneous depurination and DNA glycosylase reactions, are cytotoxic through DNA replication blockage. In addition, they are mutagenic through translesion DNA synthesis. Previous articles have extensively reported mutational potential and spectrums of AP sites in cells by transfection of exogenous DNA harboring AP sites.

We utilized an endogenous gene to better understand mutagenicity of AP sites in vertebrate cells under more physiological conditions. DT40 cells (chicken B cells) that naturally express O^6-alkylguanine DNA alkyltransferase (MGMT) were continuously exposed to very low doses of methylmethane sulfonate (MMS). These conditions allowed cells to repair mutagenic O^6-methy-2′-dexoyguanosine (O^6-mdG) before DNA replication and generate AP site-specific mutations [14]. We found a hockey-stick dose response curve with steady-state levels of mutations. Approximately half of the mutations induced by the low concentrations of MMS were transversion mutations at mainly adenine positions and the remaining half were deletions or insertions. These results suggest that *N*3-meA-derived AP sites likely cause transversion mutations and possibly deletion and insertion mutations through *N*-methylpurine-DNA glycosylase function, or via spontaneous depurination. While AP sites in genomic DNA sounds like unwelcome DNA lesions, some of the endogenous AP sites are essential for normal biological function such as cytosine methylation. Activation-induced deaminase (AID) initiates immunoglobulin diversity through deamination of cytosine to uracil [15,16]. The uracil is excised by uracil-DNA glycosylase to generate AP sites, which cause mutations by translesion DNA synthesis and initiate recombination in immunoglobin genes [7]. In contrast to the beneficial property of AP sites, constitutive expression of AID (*e.g.*, under chronic inflammation) causes an increase in global accumulation of uracil, 5-hydroxymethyluracil and possibly AP sites in non-immunogloblin genes, leading to mutations and cancers [7,17,18]. In summary, tightly controlled AP site formation and repair are beneficial for normal biological functions; however, imbalanced repair of AP sites leads to an increase in mutations above the threshold level.

2. Reactive oxygen species

Endogenous DNA adducts have been identified in cellular DNA from cultured cells, tissues of animals and humans. As shown in Table 1, a majority of endogenous DNA damage appears to be derived from oxidative stress [9]. Apurinic/apyrimidinic (AP) sites are the most abundant, spontaneous DNA lesions. One of the characteristics of AP sites suggests that they may be oxidized deoxyribose and exist in cells under normal physiological conditions [9]. DNA single strand breaks (SSBs), which occur adjacent to oxidized AP sites through DNA repair mechanisms, or by spontaneous cleavage, should therefore be among the most profuse endogenous DNA lesions. However, due to technical difficulties in quantitating SSBs with high sensitivity and specificity, accurate steady-state levels are not well characterized to date. The next three most abundant endogenous DNA lesions are *N*7-(2-hydroxyethyl)G, *N*7-(2-oxoethyl)G, and 8-oxodG [9]. All three of these base adducts are caused by oxidative DNA damage due to either lipid peroxidation products or reactive oxygen species (ROS). Among various endogenous ROS, hydrogen peroxide (H_2O_2) is the most diffusible and plentiful, and are mainly produced by mitochondria under physiological conditions. In addition to mitochondria-derived H_2O_2, oxidative demethylation by histone demethylase (*e.g.*, LSD1 and LSD2) produces H_2O_2 and formaldehyde, two major sources of endogenous DNA damage. These two reactive endogenous molecules are produced in close proximity to genomic DNA, suggesting the H_2O_2 resulting from oxidative demethylation of histones could more efficiently damage DNA than mitochondria-derived H_2O_2. In fact, it has been reported that when LSD1-mediated demethylation occurred, oxidative DNA lesions were increased [19,20]. Surprisingly, data have demonstrated that LSD1-mediated local oxidative DNA damage and its repair mechanisms work as a driving force in transcription initiation [19,20]. This suggests that oxidative DNA damage could be beneficial for certain biological functions.

Previously, our lab demonstrated that the formation of single strand breaks, AP sites, and 8-oxo-dG followed a biphasic or polynomial dose response in H_2O_2-treated HeLa cells at concentrations ranging from 60 to 20,000 μM [21,22]. To better understand the association between very low amounts of H_2O_2-induced oxidative base damage (*Biomarkers of Exposure*) and mutation events (*Biomarkers of Effect*), we conducted *thymidine kinase* (*tk*) *gene mutation* assays and 8-oxodG assays in human lymphoblastoid (TK6) cells exposed to H_2O_2 at concentrations ranging from 1 to 56.6 μM. As with MNU [23], H_2O_2 induced a hockey-stick dose response indicative of a threshold (Fig. 1). We also found that H_2O_2

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