

Mechanisms of DNA Oxidation (44449)

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Abstract. Oxidative damage of DNA caused by a variety of chemical and physical agents appears to be linked to cancer. However, it is becoming increasingly clear that endogenous generation of oxidants, such as hydroxyl radical and peroxynitrite, lead to oxidation of DNA, and this may cause cancer in individuals where no obvious exposure to chemical or physical agents known to be carcinogenic has occurred. The mechanisms for generation of these two oxidants in living organisms will be discussed and their reactivities with DNA to produce oxidized products (e.g., 8-oxo-dG) will be presented with special emphasis on the individual characteristics of the generation and reactivity of each oxidant.

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Oxidation of DNA can result in damage to all four bases and the deoxyribose. One of the most abundant and easily measured products of this oxidation is 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG). The relationship between the oxidized bases and the induction of cancer is not entirely clear. However, even though there is no evidence that 8-oxo-dG is directly responsible for cancer, Floyd (1) has noted what he considered to be a significant correlation between elevated levels of 8-oxo-dG in the target organs (2). Treatment of rats with the strong oxidant KBrO_3 causes formation of kidney tumors, containing a high level of 8-oxo-dG in DNA (3). 2-Nitropropane is a liver carcinogen, and its administration caused an increase of 8-oxo-dG in DNA in target tissue, whereas administration of its isomer, 1-nitropropane, did not cause elevation of 8-oxo-dG or liver tumors (4). Administration of iron, chelated with nitrilotriacetate (NTA), caused kidney tumors and yielded a significant increase in 8-oxo-dG (5).

Thus, it appears that treatments with certain chemical or physical agents lead to the formation of 8-oxo-dG, and this may lead to cancer. Is it possible that reactive oxygen species produced under conditions that would not necessarily be recognized as abnormal are involved in DNA damage that leads to cancer? It is becoming increasingly clear that

the answer to this question may be "yes." It is estimated that 2% of the oxygen consumed by humans produces reactive oxygen species (6). This is part of normal metabolism, and the body does not normally produce more than the tissues can catabolize. However, when the production of reactive oxygen species exceeds the ability of the natural defenses of the body to handle them, a variety of diseases can result. For every 10^{12} oxygen molecules entering a cell each day, it is estimated that 1 in 100 damages protein and 1 in 200 damages DNA (6). It is oxidative damage to DNA, whether induced exogenously by radiation or chemical agents or endogenously by free radicals released during respiration, that is generally regarded as a significant contributory cause of cancer.

Current knowledge of endogenous processes that appears to contribute to the oxidation of DNA, evidenced by the production of 8-oxo-dG, suggests that transition metal-catalyzed formation of HO^\cdot or inflammation processes leading to the production of peroxynitrite are the greatest contributors. This review will focus on evidence for the participation of these endogenously generated chemical species in the development of cancer and in the formation of 8-oxo-dG and other oxidized bases. This will include a discussion of the chemistry of generation of these reactive species and differences in their reactivities.

DNA Oxidation and Cancer

Iron. As the most highly utilized metal in biology, iron is essential for life (7). However, excessive iron becomes a risk factor in many human diseases, including cancer. Iron ore miners have been observed to have an increased incidence of lung cancer (8, 9). There is an increased risk of liver cancer among victims of the iron overload diseases, hereditary hemochromatosis (10, 11) and porphyria cutanea tarda (12, 13). There is also a direct cor-

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relation between increased body iron stores and an increased risk of cancer of all organs and tissues in individuals not suffering from iron overload diseases (14–16). Iron has also been shown to be mutagenic in bacteria (17, 18) and in the G12 transgenic strain of Chinese hamster lung V79 cells (19). Because mutations were induced in the G12 strain, but not the parent V79 cells, it appeared that the mutagenicity of iron was mediated by reactive oxygen species (19). Asbestos is a known human carcinogen containing as much as 27% iron by weight. Iron from the asbestos fibers is required for its mutagenicity in the G12 strain of transgenic V79 cells (19) and for its ability to induce 8-oxo-dG in human lung epithelial A549 cells (20). Whether the iron in asbestos and other carcinogenic fibers is responsible for their carcinogenicity is under investigation.

Peroxynitrite. The formation of ONOO⁻ has been linked to tumor formation and/or progression in a number of studies. It has been shown in female Sencar mice that ONOO⁻ was produced by dermal neutrophils during tumor promotion (21). Peroxynitrite production has been identified in human colon adenomas and colon carcinomas, and this was related to iNOS induction (22). It has also been proposed that ONOO⁻ could be involved in breast cancer, formed from the interaction of catechol-estrogen and NO, both of which are known to be formed in the breast and uterus (23). The connection between *Helicobacter pylori* infection and gastric cancer is thought to be immune response-derived ONOO⁻ (24).

The tumor suppressor gene product p52 plays an important role in the cellular response to DNA damage. This protein participates in numerous vital cellular functions including gene transcription, DNA repair, cell cycle control, genomic stability, and apoptosis (25–27). Inactivation of this tumor suppressor gene by deletion or point mutation appears to be a precondition for neoplastic transformation in many types of human cancer (28). Peroxynitrite damage to this gene has been linked to tumor formation (29–31).

Peroxynitrite treatment of the *supF* gene in the pSP189 plasmid induces mutations following replication in bacterial or human cells (32). The majority of mutations are located at G·C base pairs (98.5% in bacteria and 93.4% in human cells), including G·C → T·A transversions (65% of mutations in bacteria and 63% in human cells), G·C → C·G transversions, and deletions (32). Because of the various reactions that ONOO⁻ can undergo, there is also a variety of DNA damage i.e., single-strand breaks (33, 34), nitration (35, 36), and oxidation of bases (34, 36–38).

8-oxo-dG. One of the reasons 8-oxo-dG in DNA is considered a potentially important factor in carcinogenesis is that its presence in the DNA template causes α -polymerase to miscode incorporation of nucleotides in the replicated strands (39). Specifically, 8-oxo-dG is known to cause GC → TA transversions (40–43). It has been speculated that the reason the presence of 8-oxo-dG in DNA causes mutations is related to the perturbations it induces in the macromo-

lecular structure (1), causing mispairing and ultimately producing multiple amino acid substitutions (44).

Measurement of Oxidized DNA Products. Oxidized DNA bases can be identified and quantified by using gas chromatography/mass spectroscopy with selected-ion monitoring (45). This technology requires hydrolysis and derivatization of the samples before analysis. This has led to criticism by some who say that these procedures tend to lead to an artifactual increase in the oxidation products produced during the post-treatment manipulations of the samples. However, this technology has been very useful and has led to the identification of 17 different modified base products (46). One of the major products observed using this technique is 8-oxo-dG.

Floyd *et al.* (47) have developed a more rapid and sensitive means of detecting this oxidized base product by using reversed phase HPLC with electrochemical detection. Because of the availability of HPLC systems and the ease and sensitivity of this assay, most laboratories investigating the production of oxidized base DNA damage are currently using this technique. The disadvantages of this technique are that 1) the samples must be hydrolyzed, usually enzymatically, which can produce oxidized bases if not done carefully, and 2) it does not provide identification of the sample, only quantification.

Recently, another technique, liquid chromatography/electrospray ionization mass spectrometry, has been used to identify and quantify base products (48). This methodology is particularly useful when nucleotides or relatively short oligonucleotides are used to study damage. The advantage over the other GC/MS technique is that samples can be analyzed immediately after reaction without hydrolysis. The disadvantage is that larger oligonucleotides (>20 base pairs) would show only a small mass change associated with the reactions at a single base, which would complicate the identification of the lesions.

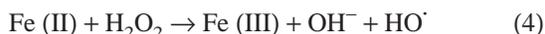
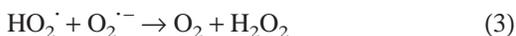
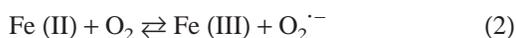
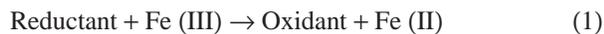
The overall advantage of both of the mass spectrophotometry techniques over the HPLC technique is that mass spectrometry provides the identity of the products, not just quantity of a chemical that is electrochemically active and co-elutes with 8-oxo-dG.

Generation of Oxidizing Agents

Hydroxyl Radical. Reactions of organic molecules with molecular oxygen to generate reactive oxygen radicals do not occur because most organic molecules exist in the singlet spin state whereas O₂ exists in the triplet spin state. Reactions of a triplet with a singlet molecule are formally forbidden and will generally be slower than 10⁻⁵ M⁻¹s⁻¹. However, transition metals like iron can bridge this kinetic restriction by reducing O₂ to form radical species that are capable of reacting with organic molecules (49). Other transition metals with a free coordination site could catalyze the reduction of O₂ or H₂O₂. The exposure to other transition metals is generally low, but they are capable of catalyzing deleterious reactions when introduced into biological sys-

tems. Since iron exists at the highest concentration of any transition metal in most living organisms, it is thought to be responsible for most of the abnormal oxygen radical production observed.

Several mechanisms have been proposed as the potential reactions of iron to generate reactive oxygen species. One of these is shown in the following series of reactions of iron that together lead to the generation of a hydroxyl radical (50).



In addition to generation of HO^{\cdot} , the ferryl iron species, $\text{Fe}^{\text{IV}} = \text{O}$, and $\text{Fe}^{\text{II}} = \text{O}_2 = \text{Fe}^{\text{III}}$ complex have been proposed to be involved in reactions catalyzed by iron. Of all of the reactive species generated by iron, the HO^{\cdot} is the only species that has been studied extensively, probably because methods are more readily available for its detection and for studying its participation in reactions.

Under physiological conditions, virtually all $\text{O}_2^{\cdot-}$ will exist as the unprotonated form (51). This species has several reactivities. In an aqueous environment, it will dismutate spontaneously to yield H_2O_2 and O_2 , at a reaction rate constant of $5 \times 10^5 \text{ M}^{-1}\cdot\text{s}^{-1}$:



Because of this, the formation of $\text{O}_2^{\cdot-}$ is always accompanied by the formation of significant amounts of H_2O_2 (52). The enzymatic dismutation of $\text{O}_2^{\cdot-}$ catalytically by superoxide dismutase (SOD) has a rate constant of $2 \times 10^9 \text{ M}^{-1}\cdot\text{s}^{-1}$, a rate that is three to four orders of magnitude faster than spontaneous dismutation at physiological pH (51). This insures that the steady state levels of $\text{O}_2^{\cdot-}$ *in vivo* approach zero (52). However, this creates another problem, as H_2O_2 is produced as a byproduct of the reaction. Technically, H_2O_2 is not a free radical as it has no unpaired electrons. It is, however, a relatively stable oxidant even though it does not react readily with biomolecules. It becomes biologically significant when it is considered that its interaction with redox-active transition metals results in the formation of HO^{\cdot} .

The reactivity of iron is highly dependent upon its electronic environment. For example, iron bound to low-molecular-weight chelators like citrate or ADP is redox active (49). Graf *et al.* (53) have shown that coordination of iron by these chelators allows water, or other small molecules like O_2 , to coordinate the metal. This allows the Fe(II) chelates to reduce O_2 and generate highly reactive species. The rates at which these redox reactions occur will depend upon the nature of the chelator, thus affecting the rate of formation of these reactive species. When all of the

coordination sites of iron are bound by a chelator, as occurs with desferrioxamine B or ferrozine, which stabilize the Fe(III) or Fe(II) forms, respectively, the redox activity of iron is inhibited. For that reason, these two chelators are often used to determine whether iron is involved in the production of radicals.

Reactivity of Hydroxyl Radical. The hydroxyl radical is an extremely reactive species, reacting immediately with all known biomolecules at diffusion-limited rates of reactions ($\sim 10^7$ – $10^{10} \text{ M}^{-1}\cdot\text{s}^{-1}$). Thus, it is very short-lived and will react at the site where it is formed. Radiation chemists have estimated the diffusion distance of the hydroxyl radical to be only 3 nm, about the average diameter of a typical protein (54). This reactivity dictates that the radical will be injurious to cells only if the metal catalyst is localized immediately adjacent to target biomolecules (52).

Peroxynitrite. Peroxynitrite is a unique oxidant formed from the reaction of the radical species $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$:



This reaction has been determined to be at the near diffusion-controlled rate of $2.0 \times 10^{10} \text{ M}^{-1}\cdot\text{s}^{-1}$ (55), which is faster than the reaction of $\text{O}_2^{\cdot-}$ with superoxide dismutase. The reaction is essentially irreversible because of a 22 kcal/mol decrease in Gibbs energy by forming ONOO^- (56). The $\cdot\text{NO}$ for this reaction is generated enzymatically by the inducible form of nitric oxide synthase (iNOS). $\cdot\text{NO}$ is produced at high levels by macrophages as a cytotoxic agent in the immune or inflammatory response (57, 58). Under these conditions, $\cdot\text{NO}$ is released along with other species, including $\text{O}_2^{\cdot-}$. This can set the stage for high levels of formation of ONOO^- . This may explain the apparent association between inflammation and cancer.

Reactivity of ONOO^- . Peroxynitrite is a strong and relatively long-lived oxidant with a half-life of approximately 1 sec under physiological conditions (59). Peroxynitrite has a pK_a of 6.8, so at physiological temperature and pH, it can be protonated to form peroxynitrous acid (ONOOH), which rapidly decomposes to nitrate:



Peroxynitrous acid has the reactivity of hydroxyl radical and nitrogen dioxide, although it appears to react as a vibrationally activated complex rather than physically separating into two distinct radical species (60, 61). As well as isomerization to nitrate, ONOO^- can undergo reactions that fall into four mechanistic classes: 1) one-electron or 2) two-electron oxidations, 3) oxygen atom transfer, and 4) electrophilic nitrations (62–66).

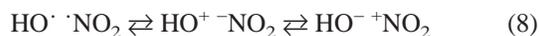
At neutral pH, the oxidative chemistry of ONOO^- is complex and controlled in part by ONOO^- conformation and in part by the type of molecule being oxidized (67). The ONOO^- has two conformations, *cis* and *trans*, based on the bending around the N-O-O bond. The ONOO^- has a long lifetime, which results from its being locked in the stable *cis*

conformation. Estimates of the energy barrier for rotation from the *cis* to the *trans* configuration are as high as 25 kcal/mol (67). It has been suggested that this difference in energy is due to interactions between the terminal peroxide oxygen and the distal oxygen (67). These interactions are weaker in the *trans* anion due to the inability of the terminal oxygens to interact, and this results in the anion being locked into the *cis* conformation.

As well, the negative charge on the terminal oxygen is partially delocalized over the entire molecule while in the *cis* form, and this causes the formation of weak partial bonds between the distal nitrogen and oxygen. These bonds have the strength of a typical hydrogen bond and add to the stabilization (67). This delocalization of charge also inhibits the terminal oxygen from rotating around the OO-NO bond or bending the N-O-O⁻ bond angle to the *trans* configuration (67). This unusual stability as an anion is a major contributory factor to the toxicity of ONOO⁻. While in the *cis* conformation, ONOO⁻ is able to diffuse a considerable distance to critical cellular targets and even cross cellular membranes before becoming activated into the conjugate acid, peroxy-nitrous acid (68). Marla *et al.* (68) have calculated the phospholipid membrane permeability coefficient for ONOO⁻ to be $\sim 8.0 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$, which compares well with water and is approximately 400 times greater than that of O₂⁻ (68). It was suggested that modulation of the pK_a of ONOO⁻ by the hydrophobic vesicular barrier facilitates its transport across the membrane (68). This would suggest that it is actually the acid form and not the anion that crosses membranes.

The diffusion distance of ONOO⁻ has been estimated to be approximately 100 μm in phosphate buffer at 37°C and pH 7.4, based on its half-life (67). However, reactions with bicarbonate and thiols in the cell decrease the diffusion distance to an estimated 9 μm (67). This still gives ONOO⁻ a diffusion distance of approximately ten thousand times further than hydroxyl radical.

The ONOOH can more readily isomerize to the reactive *trans* configuration, a process that occurs at physiological pH. Through quantum mechanical calculations, it has been determined that the *cis* form of ONOOH is lower in energy than the *trans* form by 1–3 kcal/mol (62). This difference is smaller than that of the anion form because the presence of the hydrogen neutralizes the negative charge which limits rotation. The high reactivity of peroxy-nitrous acid is mediated by a vibrationally excited intermediate that is proposed to be able to perform hydroxyl radical-like oxidations (59–61, 69). This excited intermediate, referred to by some as HOONO*, is postulated to have three resonance structures, based on the three distinct oxidative pathways for reactions of ONOO⁻ (62, 67). These three structures are as follows:



The first of these (HO·, ·NO₂) shows the hydroxyl radical- and nitrogen dioxide-like properties; the second (HO⁺, ·NO₂) shows the electrophilic properties that could add to

sulfur atoms to give sulfoxides; the third shows nitronium cation (NO₂⁺) properties indicative of the nitration of aromatic species. These different oxidative pathways are possible due to the ability of the O-O bond of ONOO⁻ to be cleaved either homolytically or heterolytically.

The second of these possibilities (HO⁺, ·NO₂) has been deemed to be too energetically expensive to be considered, as it requires $105 \pm 20 \text{ kcal/mol}$ (67). The reaction of ONOO⁻ with sulfhydryl groups is interesting in that it is one of only two reactions known to occur with ONOO⁻ in the *cis* geometry (67). No rate-limiting isomerization is necessary to take place before ONOO⁻ can oxidize a sulfhydryl group and it attacks directly by a second-order reaction. The third of the possible HOONO* species (HO⁻, ·NO₂) is known to occur and is catalyzed by transition metals (70). The complete separation of peroxy-nitrite into hydroxide ion (HO⁻) and nitronium ion (NO₂⁺) requires only 13 kcal/mol (60). However, to form the charge pair HO^{δ-} ··· δ⁺NO₂ in water initially, an energy barrier must be overcome, and this has been calculated to be as much as 45 kcal/mol (67). Unless metal ions are present, this kinetic barrier favors decomposition to the hydroxyl radical-like pathway. Metal ions circumvent this energy barrier, as shown by Beckman *et al.*, for example, who have found that Fe³⁺EDTA-catalyzed nitration by peroxy-nitrite has an activation energy of 12 kcal/mol (70).

The formation of hydroxyl radical from the excited *trans* configuration of peroxy-nitrite was originally proposed by Beckman *et al.* (59) to occur as a result of spontaneous homolytic cleavage of the O-O bond. This has since been shown to be impossible, as the rate of recombination between hydroxyl radical and nitrogen dioxide is about $10^{14} \text{ M}^{-1} \text{ s}^{-1}$, which is 100–1000 times faster than the diffusion limit (60). Instead of physically separating into free hydroxyl radical and nitrogen dioxide, peroxy-nitrous acid is believed by some to distort into the high-energy intermediate shown in Figure 2, whereby the intermediate oxidizes a target molecule.

In discussing the physicochemical properties of peroxy-nitrite, it is necessary to be aware of how exposure of peroxy-nitrite relates to its concentration. In a physiological system, the concentrations of ONOO⁻ used to inactivate enzymes or kill cells may be as high as 1 mM (71). This seems to be an extreme concentration, but considering that ONOO⁻ decomposition takes place in seconds at pH 7.4, the actual exposure is brief. To understand the reaction of peroxy-nitrite fully, it is important to consider the half-life of the compound as well as the concentration (71). This requires knowledge of the rate of decomposition, which is calculated at any pH in the range of 4–9 by the following equation:

$$k_1 = k_{\text{HA}} * \text{H}^+ / (\text{H}^+ + K_a) \quad (9)$$

At 25°C, k_{HA} is 1.3 s^{-1} and at 37°C, k_{HA} is 4.5 s^{-1} (67). Net exposure to ONOO⁻ is calculated at any pH by combining

and rearranging the above equation as follows:

$$\text{Exposure} = [\text{ONOO}^-]_0 (1 + 10^{(\text{pH}-\text{pK}_a)})/k_{\text{HA}} \quad (10)$$

At acidic pH well below the pK_a of ONOO^- , the rate of decomposition is independent of pH, and the equation reduces to $[\text{ONOO}^-]_0/k_{\text{HA}}$. At the pK_a of ONOO^- , the exposure is twice as great as at acidic pH. At alkaline pH, the exposure becomes much greater because ONOO^- is longer lived.

Reactions with DNA

Hydroxyl Radical. Reactions of HO^\cdot can be classified into three main types: hydrogen abstraction, addition, and electron transfer. These reactions produce other radicals that are less reactive than HO^\cdot . Reaction of HO^\cdot with DNA leads to a wide range of damage to all four bases and to the deoxyribose. The reaction of HO^\cdot with deoxyribose proceeds by hydrogen abstraction forming carbon-centered radicals. Further reactions of the carbon radicals can occur (e.g., reaction with oxygen to give peroxy radicals or the joining of two radicals to produce a nonradical product). Hydrogen can be abstracted by HO^\cdot from all five carbons of deoxyribose (72). Under anaerobic conditions, the C4'-centered radical undergoes β cleavage leading to DNA strand breakage followed by release of an intact base and formation of altered sugars (73, 74). The oxidation of the C1'-centered radical results in sugar lactone formation and release of an intact base (75). Under aerobic conditions, peroxy radicals are formed by addition of molecular oxygen to carbon-centered radicals. This results in cleavage of a carbon-carbon bond giving rise to an alkali-labile site (76). The C5'-centered peroxy radical converts to an oxyl radical leading to β -cleavage and strand breakage followed by release of an altered sugar and an intact base (73), or to strand breakage followed by aldehyde formation at the C5' end (77).

Hydroxyl radical reacts with the heterocyclic bases in DNA by addition. In pyrimidines, HO^\cdot adds to the C5-C6 double bond, giving rise to formation of base radicals. Of the radicals produced, the 5-hydroxy-6-yl radicals have reducing properties whereas the 6-hydroxy-5-yl radicals are oxidizing (78). An additional reaction with thymine is abstraction of a H atom from the methyl group. In purines, HO^\cdot adds to C4, C5, and C8 positions resulting in equal amounts of oxidizing and reducing types of adduct radicals (79). The C4-OH and C5-OH adduct radicals dehydrate and are converted to oxidizing radicals (78, 80, 81). The C8-OH adduct radicals of purines can undergo unimolecular opening of the imidazole ring.

Peroxynitrite. Peroxynitrite preferentially reacts with guanine resulting in the formation of 8-oxo-dG (34, 37), 8-nitroguanine (35, 36), 2,2-diamino-4-[(2'-deoxy- β -D-erythro-pentafuano-syl)amino]-5-(2H)-oxazolone (oxazolone) (37), 4-hydroxy-8-oxo-4,8-dihydro-2'-deoxyguanosine (37), and 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2'-deoxyguanosine (38) when deoxyguanosine is treated with ONOO^- . Treatment of calf thymus DNA

with ONOO^- results in the formation of 8-oxo-dG, 8-nitro-G, and oxazolone (34, 36, 37).

Studies have shown that 8-oxo-dG is highly susceptible to secondary oxidation, making it a target for further oxidation by ONOO^- . The formation of 8-oxo-dG by ONOO^- has been controversial. Uppu *et al.* (82) observed that 8-oxo-dG reacts very efficiently with ONOO^- , becoming a better substrate for ONOO^- than dG itself. As a nucleoside, 8-oxo-dG is approximately 1000-fold more reactive with ONOO^- than dG (82). Thus, the amount of 8-oxo-dG recovered after ONOO^- treatment depends upon the ratio of $\text{ONOO}^-/8\text{-oxo-dG}$. One of the major products of oxidation of 8-oxo-dG by ONOO^- is oxaluric acid (48, 83). Therefore, if further oxidation is suspected, one can monitor the production of oxaluric acid.

Summary

Living organisms in an aerobic environment are constantly exposed to reactive oxygen species as a consequence of normal and, in some cases, abnormal metabolism. This can occur as the result of the generation of HO^\cdot by transition metal-catalyzed (likely iron) reactions with molecular oxygen or through the generation of ONOO^- after activation of macrophages during inflammation. Although both species have the potential to be strong oxidants, the rate of reaction of HO^\cdot is significantly higher than that of ONOO^- such that HO^\cdot will damage biomolecules only within 3 nm of the site of its formation whereas ONOO^- can travel up to 9 μm with a capability of passing through membranes. This greatly increases the biomolecules, which could be targets for reaction with ONOO^- . For HO^\cdot to damage DNA, the transition metal generating the oxidant, would have to be in the nucleus immediately adjacent to the DNA. The damage that HO^\cdot inflicts on DNA appears to be much more varied than that observed for ONOO^- , since all four bases and deoxyribose are targets for HO^\cdot , whereas guanine and to some extent deoxyribose are the preferred targets for ONOO^- . In addition, ONOO^- appears to react preferentially with one of the primary products of its own oxidation with guanine, 8-oxo-dG, raising the question as to whether 8-oxo-dG is the best indicator for the presence of ONOO^- as a DNA damaging agent. Another product, 8-nitro-dG may be the best indicator, although this adducted base is more difficult to detect and also undergoes further reactions. Increasing understanding of the conditions under which each of these oxidants is produced and of methods of inhibiting their formation will likely prove very important in preventing cancer and other diseases of previously unknown origin.

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