Free Rad. Res. Comms., Vol. 6, No. 2-3, pp. 155-156
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OXIDATIVE DNA SUGAR DAMAGE BY TARGETED ANTIBIOTIC FREE RADICALS

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Neocarzinostatin (NCS) is a member of a group of antitumor antibiotics possessing ene-diyne structures that bind to DNA in a specific manner and are converted to a diradical species that directly attacks DNA sugars. The biologically active component is a labile non-protein chromophore (NCS-Chrom) composed of three discrete structural components: 1) a substituted naphthoic acid moiety, 2) an amino sugar, and 3) a novel, highly strained bicyclo [7.3.0] dodecadiendiyne epoxide with two acetylenic bonds. NCS-Chrom binds to DNA by intercalating of its naphthoate moiety and electrostatic interaction of its aminosugar in the minor groove of duplex DNA. The drug prefers 5'-d(GNT)-3' sequences, intercalating between G and N and cleaving mainly at T residues. DNA-bound NCS-Chrom is activated by thiol (or sodium borohydride) by adduction at C-12 of the chromophore bicyclic core, opeining of the epoxide, and rearrangement of the diyne-ene system to form a C-2, C-6 diradical species.

DNA damage produced by NCS-Chrom consists mainly of 1) direct single strand breaks, 2) base release, 3) alkali-labile breaks, and 4) covalent adducts between the drug and the DNA sugar. The free radical form of NCS-Chrom abstracts a hydrogen atom from C-5' of deoxyribose of mainly T residues in DNA to form a carbon-centered radical at C-5'. Dioxygen adds to the radical to form a peroxyl radical intermediate that mainly ($> 80\%$) is converted to a strand break with a 3' phosphate and a 5' nucleoside 5'-aldehyde; less than 20% of the breaks have phosphate at both ends due to the cleavage between C-4' and C-5' of an oxyradical species (at C-5') to form 3'-formyl phosphate-ended DNA, an energy-rich DNA damage intermediate and formyl donor. In the absence of dioxygen, instead of strand-breaks, NCS-Chrom itself forms a stable covalent adduct at C-5' of the deoxyribose. When the radiation sensitizer misonidazole substitutes for dioxygen, strand breaks have phosphates at both ends and formyl $\sim$ P-ended DNA is the main sugar damage intermediate. A mechanism involving a nitroxide radical adduct intermediate and oxyradical formation at C-5' has been proposed in the generation of this lesion.

NCS-Chrom also induces base release with the formation of alkali-labile, abasic sites in the DNA. Abasic lesions, which can account for up to 25% of the total strand breaks, occur with an especially high frequency at C residues in d(AGC) sequences and have an atypical response to enzymatic or chemical hydrolysis when compared with abasic sites generated by acid-induced depurination. These lesions are mutagenic and when they involve the C residue in d(AGC), are responsible for GC to AT transitions. At such sequences the free radical form of NCS-Chrom abstracts a hydrogen atom from C-1' of the deoxyribose of the C residue. Oxidation of the primary radical at C-1' generates 2-deoxyribonolactone with cytosine release.

All abasic sites at the C residue in d(AGC) are accompanied by a direct strand break at the T residue on the complementary strand two nucleotides to the 3'-side (i.e.,

opposite the A in d(AGC)), but not all direct strand breaks have closely opposed abasic sites. If in the repair of the bi-stranded lesions the first event is an endonucleolytic cleavage at the abasic site, this would generate a double strand break, a possibly lethal event; if the strand break is repaired first by a process involving gap generation and filling, an incorrect nucleotide would likely be placed opposite the abasic site on the complementary strand during gap filling to generate a mutagenic lesion. This novel type of mutagenesis does not involve DNA replication. These results raise the possibility that related bi-stranded lesions may also be caused by ionizing radiation with similar biological consequences.

It is proposed that the bi-stranded lesions are formed when NCS-Chrom diradical is appropriately positioned in the minor groove of the DNA so that it can concertedly attack C-5' in one strand and C-1' of the deoxyribose two nucleotides to the 3'-side in the complementary strand. Support for such a mechanism comes from recent ^{1}H-NMR and mass spectroscopic studies showing that DNA is the probable source of the two hydrogen atoms abstracted by NCS-Chrom into the C-2 and C-6 positions of its bi-cyclic core. Further, it is shown that while deuterium from borodeuteride is incorporated into C-2 and C-6 of NCS-Chrom in the absence of DNA, no deuterium from the thiol sulfhydryl is so incorporated. This result raises the possibility that carbon-bound hydrogen of thiol and not the exchangeable sulfhydryl hydrogen is abstracted by the free radical form of NCS-Chrom in the absence of DNA.

References

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