Photochemical reduction of tirapazamine and related di-*N*-oxides to nitroxide free radicals

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Tirapazamine, 3-amino-1,2,4-benzotriazine 1,4-dioxide, (TPZ) selectively kills hypoxic cells in solid tumors. Recently it has been reported that TPZ and 3-amino-2-quinoxalinecarbonitrile 1,4-di-*N*-oxide (TPZCN) cause light dependent DNA damage in plasmid DNA and oligonucleotides, respectively (Fuchs *et al.,* Chem. Res. Toxicol. 12, 1190, 1999; Daniels *et al*., J. Org. Chem. 63, 10027, 1998). Under anaerobic conditions UVA irradiation of TPZ causes DNA cleavage, which is prevented when oxygen is present (Daniels *et al*., ibid). In this study, the photochemical generation of nitroxide radicals from TPZ, TPZCN, and the related analog quinoxaline-1,4-di-*N*-oxide (quindoxin) was examined with respect to their potential to damage DNA both oxidatively and reductively. We have employed direct electron spin resonance (ESR) and the ESR spin-trapping technique using 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) to identify the free radical intermediates formed by Type I reactions during irradiation of TPZ and direct detection of $^1O_2$ to provide evidence for a Type II reaction. Irradiation (λ > 300 nm) of TPZ or quindoxin at pH 9.5 under anaerobic conditions in the presence of a reducing agent, such as GSH or NADH, generated the corresponding nitroxide radical. This same radical has been detected during the microsomal reduction of TPZ (Lloyd *et al.*, Mol. Pharm. 40, 440, 1991). Irradiation of TPZ or TPZCN and DMPO in phosphate buffer solution (pH 7.4) under aerobic conditions in the presence of GSH gave rise to the corresponding glutathiyl adduct, DMPO/GSh(aN = 15.3 G; aH = 16.3 G). In the presence of oxygen, TPZ generated the superoxide radical, which was trapped using DMPO. In the case of TPZCN, during irradiation with DMPO in phosphate buffer solution (pH 7.4) we observed the DMPO oxidation product 5,5-dimethyl-2-oxopyrrolin-1-oxyl (DMPOX). After the light was turned off, a weak multiline spectrum of the TPZCN nitroxide radical was seen. We found that TPZ, TPZCN and quindoxin photosensitized the generation of $^1O_2$ with quantum yields of 0.007, 0.19 and 0.02 respectively, in acetonitrile. These studies suggest that DNA damage under anaerobic conditions is due to the oxidation of DNA by 1,3[TPZ]* and/or the aforementioned nitroxide radical and that inhibition of DNA damage by oxygen results from reaction of these species with oxygen to form superoxide.