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# INDUCTION OF BREAKS IN DEOXYRIBONUCLEIC ACID BY PHOTOEXCITED PROMAZINE DERIVATIVES

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**Abstract**—Near-u.v. photoexcited promazine and three of its derivatives are shown to induce single-strand breaks in ΦX174-DNA replicative form. The mechanisms of this DNA breakage depend upon the various photochemical properties of the promazine derivatives. Chlorpromazine is shown to act predominantly via the photodechlorination reaction both in aerobic and anaerobic conditions. The three other promazine derivatives (promazine, trifluopromazine and methoxypromazine) display two mechanisms for DNA breakage. One of them occurs through the cation radical, which is formed during near-u.v. irradiation of promazine derivatives. The second mechanism is demonstrated to act via an hydroxyl radical-dependent pathway. Acepromazine is without photoactivated action. EPR-spin-trapping studies of irradiated mixtures, containing the drugs and 5,5-dimethyl-1-pyrroline-*N*-oxide (as spin trap), suggest the production of superoxide radical by photoexcited promazines. When DNA is present in the irradiation mixture, this superoxide radical is converted into hydroxyl radical probably via a Haber Weiss-type reaction, catalysed by DNA–iron complexes.

Phenothiazines are widely used as psychotherapeutic agents, but phototoxic responses have been described in patients receiving these drugs [1, 2]. Several studies on various models have been undertaken in order to elucidate the mechanisms responsible for these undesirable side effects. Phenothiazine derivatives have been reported to cause near-u.v. sensitized lethal effects on mammalian cells [3, 4], bacterial cells [4–6], viruses [7] and bacteriophages [8].

Targets responsible for the cell killing by phenothiazine derivatives were suspected to be cell membranes [9, 10] because PZD§ had a high affinity for membrane components [11]. One of the main lesions in membranes promoted by phenothiazine derivatives photosensitization was membrane protein photoaggregation, mainly spectrin molecules [12]. Nevertheless, the photochemical mechanisms and the photomodification of target molecules are still under investigation to explain the phototoxic effects of these drugs.

In addition to membrane proteins, DNA could also be damaged by PZD photoexcitation. When PZD are taken up by cells, they can induce loss of infectivity [13] and mutations [13–16].

Even though, photoexcited phenothiazine derivatives do not produce any detectable singlet oxygen in aqueous neutral solutions [17], other activated oxygen species could explain photodynamic properties of phenothiazines. This aspect of the photochemistry of PZ and of four PZD was investigated in this work, keeping in view its involvement in the induction of DNA single-strand breaks. Therefore, EPR-spin-trapping experiments and the effects of specific scavengers have been carried out to identify the activated oxygen species produced. The DNA breakage has been investigated by neutral agarose gel electrophoresis, which allows detection of true breaks and not alkali-labile bonds [18].

## MATERIALS AND METHODS

*Chemicals.* PZD were from Specia (Paris, France) except TFPZ which was from M.S. Chemicals (Milano, Italy). They were used without further purification. ΦXRFI was prepared according to Piette *et al.* [19]. Stock solutions (±100 μg/ml in 0.15 M NaCl–5 mM EDTA–10 mM Tris–HCl, pH 8.0) were kept at 4°. DMPO (Aldrich) was purified and stored as described by Finkelstein *et al.* [20]. Aqueous solutions of MNP (Aldrich) were prepared according to Lion *et al.* [21]. Catalase (from beef liver E.C.1.11.1.6) and HRP were from Boehringer. DNA calf thymus (type V) was from Sigma. The phosphate buffer used was 13.5 mM $Na_2HPO_4 \cdot 2H_2O$, 9 mM $KH_2PO_4$, pH 7.0. All other chemicals were analytical grade. All solution concentrations are final concentrations.

*Single-strand breaks induction experiments.* Mixtures containing ΦXRFI (50 μg/ml, 0.121 mM P), the selected PZD (0.08 mM), the phosphate buffer were irradiated at room temperature in quartz cells (i.d. 4 mm) using a Xenon lamp (Osram XB0150) equip-

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§ Abbreviations used: ACPZ, acepromazine; CPZ, chlorpromazine; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; DMSO, dimethyl sulfoxide; HRP, horseradish peroxidase; EPR, electron paramagnetic resonance; MNP, 2-methyl-2-nitrosopropane; MTPZ, methoxypromazine; $O_2^{\cdot}$, superoxide radical; $OH^{\cdot}$, hydroxyl free radical; PZ, promazine; PZD, promazine derivative, $PZD^{\cdot+}$, promazine derivative cation radical; TFPZ, trifupromazine; ΦXRFI, ΦX174-DNA replicative form, closed circular, double stranded.

ped with a Schott filter WG305 ($\lambda > 290$ nm). Aliquots (15 $\mu$l, containing 750 ng of DNA) of the irradiated mixtures were removed after increasing irradiation times and analyzed by agarose gel electrophoresis. The fluence rate at the irradiation position had a mean value of 390 W/m$^2$ (between 290 and 400 nm). This value was obtained by graphical integration after measurement of the light intensity transmitted through an interferential filter (with a thermopile Kipp and Zonen, Holland). The agarose gel electrophoresis was performed according to Piette *et al.* [19], 15 $\mu$l of bromophenol blue in glycerol (0.2 mg/ml) were added to the samples and loaded on agarose gels without any clean up (agarose 1%, sodium acetate 20 mM, Tris 40 mM, EDTA 2 mM, ethidium bromide 1 $\mu$g/ml, pH 8.0 acetic acid). After migration, gels were photographed under u.v. light (u.v. transilluminator, u.v. Product) with a Polaroid camera (MP4) equipped with an u.v. filter (Kodak 1A) and an orange filter (Kodak 21). The negative films were scanned with a SL504 Zeineh soft laser scanning densitometer (Biomed Instruments Inc., U.S.A.) using a scanning device at 633 nm. The peak height was used to estimate the ΦXRFI amount (in arbitrary units).

*EPR-spin-trapping experiments.* When DMPO was used as spin-trap, the selected PZD were (0.1 mM) dissolved in the phosphate buffer, mixed with DMPO (50 mM) and irradiated at room temperature in the EPR cavity using a high pressure mercury lamp (Osram HB0500) equipped with the WG305 Schott filter. The fluence rate at irradiation position had a mean value of 2600 W/m$^2$ (between 290 and 400 nm). This value was obtained as described for the XB0150-WG305 device. The EPR spectra were recorded after irradiation using a Varian E9 spectrometer (at room temperature, microwave power 20 mW, modulation 1 Gauss). When MNP was used as spin trap, the mixtures containing the selected PZD (0.1 mM), MNP (0.5 mg/ml), DMSO (25% vol.) or DL-alanine (1 M final concentration) were irradiated, and the EPR-spectra were recorded as described for DMPO. In order to determine the effects of PZD sensitization on DMPO-OH adduct, further experiments were performed. Hydroxyl radicals were generated in the dark using the Fenton reaction (50 $\mu$M $FeCl_2$, 0.25% $H_2O_2$) in the presence of 50 mM DMPO and 0.1 mM PZD in the phosphate buffer. The mixtures were then irradiated in the EPR cavity using the HB0500-WG305 device as described above and the amount of DMPO-OH were recorded by EPR spectrometry during irradiation.

*PZD cation radical effects on ΦXRFI.* In order to investigate the participation of $PZD^{+\cdot}$ in the DNA breakage, $PZD^{+\cdot}$ were prepared in the presence of ΦXRFI by PZD oxidation induced by enzymatically generated triplet carbonyl according to the technique described by Duran *et al.* [22]. ΦXRFI (50 $\mu$g/ml) was mixed with 8 mM isobutanal, 2 $\mu$M HRP, 0.1% $H_2O_2$ and 0.1 mM PZD. The mixtures were incubated in the dark at room temperature and then analyzed by agarose gel electrophoresis as described above.

## RESULTS

*Induction of single-strand breaks.* Neutral agarose gel electrophoretic analysis allowed to separate superhelical ΦXRFI, relaxed ΦXRFII and linear ΦXRFIII molecules. This method was used in order

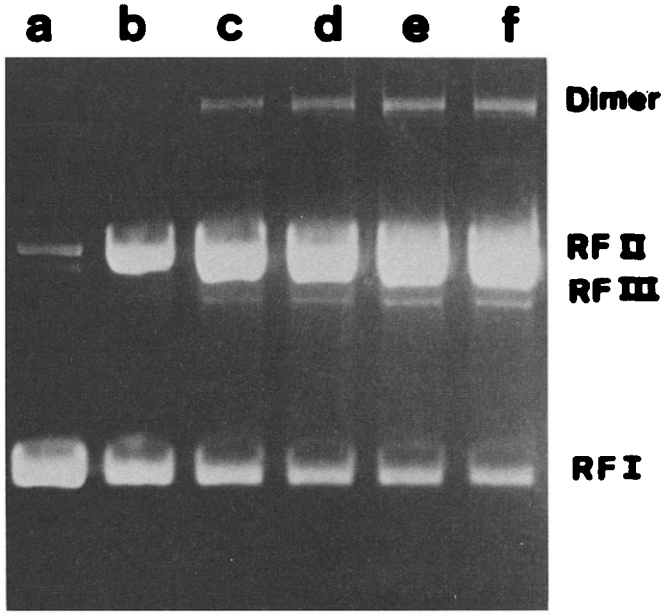


Fig. 1. Photosensitized conversion of ΦXRFI DNA into ΦXRFII DNA mediated by TFPZ. ΦXRFI-DNA (50 $\mu$g/ml), TFPZ (0.08 mM), phosphate buffer (13.5 mM $Na_2HPO_4$, 9 mM $KH_2PO_4$, pH 7.0). Irradiation times 0 min (a), 20 min (b), 40 min (c), 60 min (d), 80 min (e), 100 min (f). Fifteen microliters of bromophenol blue in glycerol (0.2 mg/ml) were added to the samples before electrophoresis. DNA load per gel channel: 750 ng.

to monitor the effect of near-u.v. light plus PZD treatment on ΦXRFI. A single-strand break converted ΦXRFI into ΦXRFII. A double-strand break or close-spaced single-strand breaks changed ΦXRFI into ΦXRFIII. Figure 1 illustrates a typical effect of light induced activity of PZD. Unirradiated ΦXRFI-PZD samples (channel a) showed a major band corresponding to ΦXRFI and weaker bands corresponding to ΦXRFII and ΦXRFIII. Increasing irradiation times (channels b to f) of ΦXRFI sensitized by PZD led to a gradual decrease of ΦXRFI band. In parallel, an increasing ΦXRFII band appeared. This conversion was not observed when irradiation was carried out in the absence of PZD or when preirradiated PZD solutions were mixed in the dark with ΦXRFI. Light induced activity of PZD on ΦXRFI clearly consisted in single-strand breaks induction.

The decrease in ΦXRFI content followed single hit kinetics as a function of irradiation time. The nicking efficiency largely varied according to the PZD investigated. As shown in Fig. 2: CPZ was the most efficient sensitizer ($k = 0.400\ \text{min}^{-1}$) whereas MTPZ, TFPZ and PZ were less active ($k = 0.026$, 0.022 and $0.014\ \text{min}^{-1}$ respectively). ACPZ was without measurable activity. Removing oxygen prior to irradiation by nitrogen flushing over the sample (to avoid mechanical breakage) reduced the nicking efficiencies of PZ, TFPZ and MTPZ with dissimilar quantitative effects (Table 1). When CPZ was used as sensitizer, removing oxygen strongly increased the efficiency of CPZ (Table 1).

In order to investigate the participation of OH˙ in the reaction, irradiations of ΦXRFI sensitized by PZD were carried out in the presence of OH˙ scavengers, such as *t*-butanol, sodium benzoate and sodium formate. Tested at two different concentrations, these three compounds partially inhibited the nicking reaction with efficiencies depending upon the sensitizer used, as observed for oxygen removal (Table 1). The presence of catalase, known to be able to dismutate $H_2O_2$ into $H_2O$ and $O_2$ [23], had no effect on the single-strand breaks kinetics. Since catalase was without effect on the single-strand breaks induction and since superoxide dismutase was inactivated by near-u.v. plus PZD treatment (Decuyper, unpublished result) further

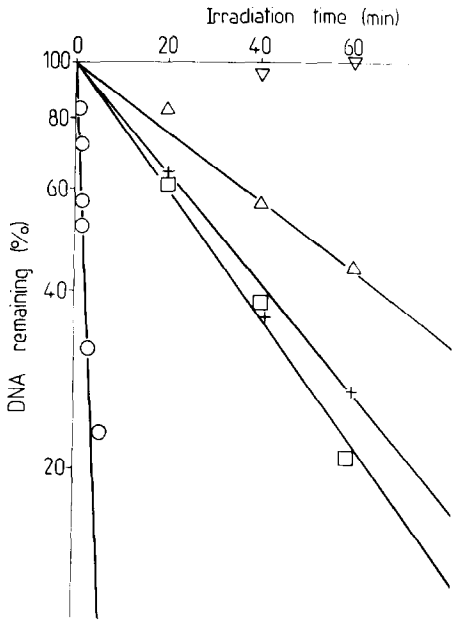


Fig. 2. Nicking activity of the PZD and near-u.v. light treatment on ΦXRFI-DNA. The remaining amounts of intact RFI molecules are reported in log 10 per cent as a function of irradiation time (in min). ●, CPZ; ■, MTPZ; ×, TFPZ; ▲, PZ; ▼, ACPZ.

experiments were needed to elucidate the mechanism of the OH˙ production.

*EPR-spin trapping experiments.* Irradiation of mixtures containing DMPO and the selected PZD led to the generation of an EPR signal as shown in Fig. 3, A for CPZ. In this signal we observed the characteristic 1:2:2:1 quartet ($A_N = A_H = 14.9$ Gauss) of the DMPO-OH adduct, the triplet of triplets [$A_N = 16.7$, $A_H$ (2H) = 22.4 Gauss] of the DMPO-H adduct and an unattributed triplet. The amount of DMPO-OH produced varied according to the following scale PZ > MTPZ ≃ CPZ > TFPZ ≃ ACPZ = O. However, this last scale do not reflect the relative ability of the various PZD to promote the generation of the species trapped by DMPO.

Table 1. Effect of various inhibitors on DNA strand scission

| Added scavenger | PZ | CPZ | TFPZ | MTPZ |
|---|---|---|---|---|
| — | 100 | 100 | 100 | 100 |
| $N_2$ | 31 | 127 | 13 | 71 |
| 0.1 M ter-Butanol | 43 | 99 | 65 | 76 |
| 1 M ter-Butanol | 25 | 81 | 63 | 76 |
| 10 mM sodium benzoate | 45 | 90 | 65 | 94 |
| 100 mM sodium benzoate | 37 | 51 | 0 | 39 |
| 10 mM sodium formate | 34 | 94 | 73 | 84 |
| 100 mM sodium formate | 14 | 64 | 46 | 69 |

Samples containing ΦXRFI DNA (50 μg/ml), the PZD (0.08 mM), phosphate buffer and the selected scavenger were irradiated at a given dose: 3 min for CPZ; 40 min for PZ and TFPZ, and 30 min for MTPZ with the XB0150 WG305 device. Results are expressed in percentage of broken ΦXRFI in the presence of inhibitor vs in the absence of inhibitor.

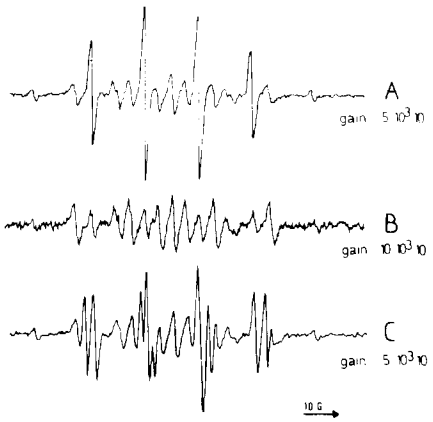


Fig. 3. EPR spectra obtained after 5 min irradiation of the mixtures containing: (A) CPZ (0.1 mM), DMPO (50 mM) and phosphate buffer under air; (B) CPZ (0.1 mM), DMPO (50 mM) and phosphate buffer, under nitrogen; (C) CPZ (0.1 mM), DMPO (50 mM), phosphate buffer and sodium formate (0.1 M), under air.

Indeed, DMPO-OH should have the same stability under the irradiation conditions with all the PZD. As shown in Fig. 4(A) $OH^{\cdot}$ generated in the dark by a Fenton reaction led to the formation of DMPO-OH in the presence of PZD. When this mixture was irradiated we observed that TFPZ could sensitize the decay of DMPO-OH (Fig. 4B). The other PZD did not display this property. Irradiation after nitrogen bubbling through the solutions gave rise to a very little production of DMPO-OH (Fig. 3B for CPZ). The addition of *t*-butanol or sodium benzoate to the mixtures prior to irradiation reduced the DMPO-OH signal (Table 2) and inhibited the DMPO-H production. When sodium formate was used, the DMPO-H generation was not affected and an effect was only observed on DMPO-OH production with CPZ as sensitizer. In this case a DMPO-$CO_2$ adduct ($A_N$ = 15.6, $A_H$ = 18.7 Gauss) was observed (Fig. 3C), this adduct appeared shortly after the beginning of irradiation. The DMPO-$CO_2$ adduct induction was also observed under anaerobic conditions.

Irradiation of MNP-DMSO-PZD or MNP-alanine-PZD mixtures were performed as another $OH^{\cdot}$ detection technique [21]. The experiments led to the detection of MNP-$CH_3$ or MNP-alanine adduct only when CPZ was used as sensitizer [21]. Under anaerobic conditions, these radical formations were strongly increased.

*PZD cation radical effects on ΦXRFI DNA.* The enzymatic generation of a high steady-state level of the cation radicals from CPZ, TFPZ and PZ using the peroxidase-$H_2O_2$-isobutanal system; and a subsequent mixing with ΦXRFI DNA led to DNA breakage. As shown in Fig. 5, the agarose gel electrophoresis analysis led to the detection of some conversion to ΦXRFII DNA, which was between 10 and 15% in the case where CPZ, PZ and TFPZ cation radicals were used. When the mixture was done with the MTPZ cation radical, the ΦXRFI DNA band disappeared completely without a concomitant increase in the intensity of the ΦXRFII DNA band. No ΦXRFI conversion into ΦXRFII was observed in the absence of HRP and PZD. Moreover, EPR-spin trapping experiments demonstrated that all PZD were unable to catalyze a generation of $OH^{\cdot}$ radical by a Fenton-like reaction (0.1 mM PZD, 50 mM DMPO, 0.1% $H_2O_2$, in the phosphate buffer).

## DISCUSSION

In the EPR spin trapping experiments, two kinds of signals were recorded at the same time; one being due to a DMPO-H adduct and the other was due to an oxygen dependent photogeneration of DMPO-OH.

The generation of a DMPO-H signal during near-u.v. irradiation of PZD can be explained by a direct

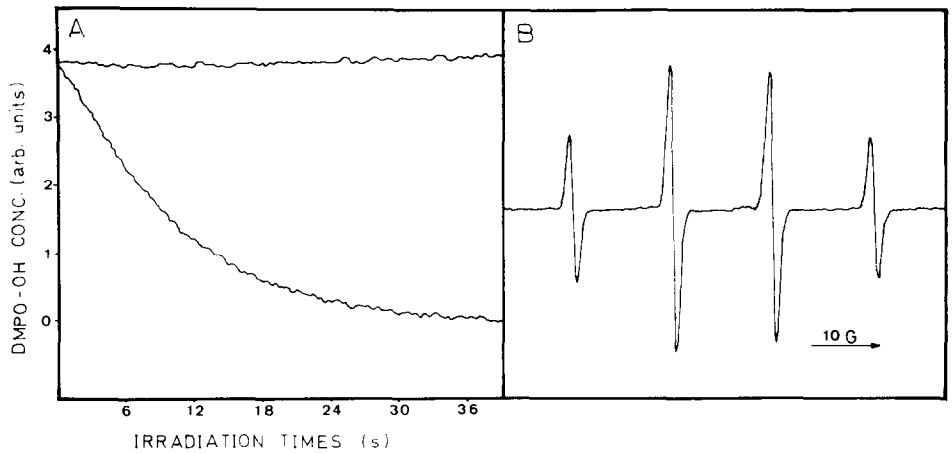


Fig. 4. (A) Decay of DMPO-OH concentration during irradiation of mixtures containing 50 mM DMPO, 0.1 mM PZD, 50 μM $FeCl_2$, 0.25% $H_2O_2$ in the phosphate buffer. Upper curve CPZ, lower curve TFPZ. The magnetic field was set on the maximum amplitude of the low field line of the DMPO-OH EPR signal. (B) EPR signal corresponding to the DMPO-OH adducts generated by a Fenton reaction, in the presence of 0.1 mM TFPZ.

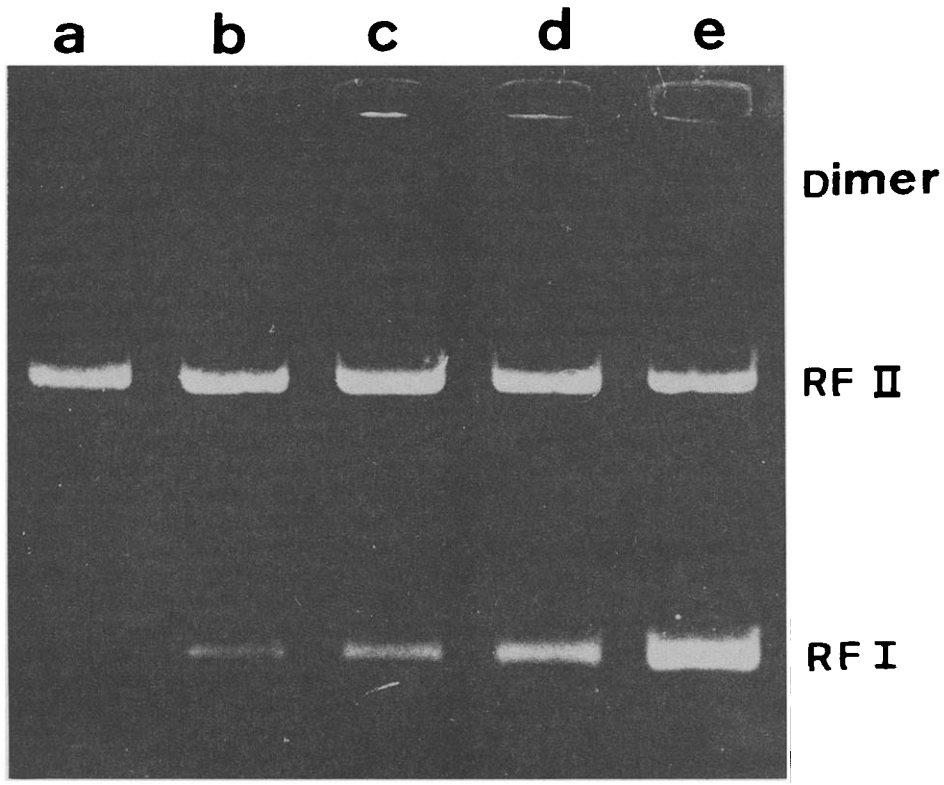


Fig. 5. Nicking activity of $PZD^{+\cdot}$ on ΦXRFI DNA. The $PZD^{+\cdot}$ are generated in the dark by the HRP-$H_2O_2$-isobutanal system with the presence of ΦXRFI DNA. Channels (a) to (d) are: (a) $MTPZ^{+\cdot}$; (b) $TFPZ^{+\cdot}$; (c) $CPZ^{+\cdot}$; and (d) $PZ^{+\cdot}$. Channel (e) is the reaction mixture without PZD.

reaction between DMPO and electrons. As it was shown by Navaratnam *et al.* [24], PZD are photo-ionized upon near-u.v. irradiation; thus, the photo-ejected electrons can reduce the spin trap and generate the DMPO-H adduct [25]. This interpretation is confirmed by the effects of either ter-butanol or sodium benzoate on DMPO-H adduct formation. Indeed, these compounds have significant reactivities with electrons ($[k]^{-}_{aq}$, in $10^8$ $dm^3$/mole/sec are 1 and 35 for *t*-butanol and sodium benzoate respectively, [26]), and contribute to the disappearance of the DMPO-H signal when these compounds are present during irradiation. Sodium formate which does not display this reactivity with electrons ($[k]^{-}_{aq} \leq 1.4\ 10^4$ $dm^3$/mole/sec, [26]) does not affect the DMPO-H adduct generation.

On the other hand, DMPO-OH generation mediated by PZD irradiation could be explained by two different mechanisms: (i) an $OH^{\cdot}$ trapping by DMPO or (ii) a decay of a DMPO-OOH adduct, resulting from an $O_2^{\cdot-}$ trapping by DMPO. This resulting DMPO-OOH adduct is known to undergo rapidly conversion into DMPO-OH [20]. The lack of sodium formate effect on DMPO-OH generation (Table 2; except in the case of CPZ, which will be discussed below) allows to demonstrate that the DMPO-OH signal does not result from an $OH^{\cdot}$ trapping. Indeed, OH radicals should react with formate anions and form $CO_2^{\cdot-}$ which should be subsequently trapped by DMPO leading to DMPO-$CO_2$ adduct formation. Since no DMPO-$CO_2$ adduct is detectable, and since no protective effect of sodium formate is observed, we conclude that OH radicals are not directly produced during the near-u.v. photolysis of PZD. This demonstration is confirmed by EPR spin trapping experiments using either MNP-alanine or MNP-DMSO as traps during PZD (except CPZ) photolysis [21]. Therefore, the observed DMPO-OH adducts can result only from the decay of a DMPO-OOH. Because the $O_2^{\cdot-}$ steady state concentration is, in our irradiation conditions, always very low, the DMPO-$O_2^{\cdot-}$ adduct is not observable.

The spin trapping experiments clearly suggest that $O_2^{\cdot-}$ is generated during the PZD photolysis. The $O_2^{\cdot-}$ formation which is suggested by Iwaoka and Kondo [27] is probably formed by a reaction between molecular oxygen and the photo-ejected electrons [28]. As shown in Table 2, sodium benzoate and *t*-butanol decrease the amount of DMPO-OH formed during the PZD irradiation. These protecting effects should be definitively interpreted as resulting from an electron scavenging by benzoate or *t*-butanol and not by an $OH^{\cdot}$ scavenging by these compounds. Near-u.v. photolysis of CPZ in the presence of DMPO leads to the detection of EPR signals which can be attributed to DMPO-H and DMPO-OH adducts. The interpretation of the origin of the DMPO-H signal is similar to those mentioned for the four other PZD. Under anaerobic conditions, no DMPO-OH signal is recorded after CPZ photolysis. The presence of either *t*-butanol or sodium benzoate during irradiation partially abolishes the DMPO-

Table 2. Effect of various inhibitors on DMPO-OH induction

| Added scavenger | PZ | MTPZ | CPZ | TFPZ |
|---|---|---|---|---|
| — | 100 | 100 | 100 | u.m. |
| Nitrogen | 16 | 20 | 14 | |
| 0.5 M ter-Butanol | 80 | 64 | 83 | |
| 50 mM sodium benzoate | 84 | 70 | 69 | |
| 100 mM sodium formate | 120 | 131 | 82 | |

Samples containing PZD (0.1 mM), DMPO (50 mM), the phosphate buffer and the selected inhibitor were irradiated 5 min in the EPR cavity using the HBO500-WG305 device. Results are expressed in percentage of the DMPO-OH formed in the presence of inhibitor vs in the absence of inhibitor.

u.m., unmeasurable.

OH signal; and when sodium formate is used as scavenger, DMPO-$CO_2$ adducts are rapidly observable in the course of irradiation. Surprisingly, this DMPO-$CO_2$ signal is also detectable under anaerobic conditions. The use of the MNP-DMSO or MNP-alanine systems as traps during CPZ photolysis allows to detect MNP-$CH_3$ and MNP-alanine radical adducts and has been interpreted by Lion *et al.* [21] as an OH radical trapping. However, these adducts can also be observed under anaerobic conditions with a better yield. This effect does not seem to be easily interpretable as an OH radical trapping. As mentioned above, CPZ can rapidly undergo photodechlorination yielding chlorine and promazynyl radicals [29, 30]. These authors mentioned that this dechlorination was really more efficient when oxygen was absent. Thus the MNP-$CH_3$ and MNP-alanine adducts could be explained by a reaction between either the chlorine or the promazinyl radicals with either DMSO or alanine. This interpretation is supported by the detection of identical adducts when either MNP-DMSO or MNP-alanine are used as traps during u.v. photolysis of $CCl_4$ as chlorine radical sources [31, Decuyper, unpublished results]. It thus clearly appears that chlorine radicals can abstract hydrogen. In the same manner, promazinyl radicals can also abstract hydrogen since a promazine formation is detected by mass spectrometry after CPZ photolysis [30]. These two radicals can be responsible for the formation of the DMPO-$CO_2$ adduct under aerobic and anaerobic conditions. In conclusion, the DMPO-OH adducts should be due to a decay of DMPO-OOH adducts as for the four other PZD.

One of the damages detected by agarose gel electrophoresis in the ΦXRFI DNA photoreacted with PZD is the induction of single-strand breaks which promote the conversion of the superhelical DNA molecule into a relaxed form (ΦXRFII DNA) [32]. Except ACPZ, all four other derivatives under investigation promote the nicking reaction with variable efficiencies (see Fig. 2). All scavengers used, i.e. ter-butanol, sodium benzoate and sodium formate, protect ΦXRFI DNA against single-strand breaks induction ($[k]_{OH^\cdot}$, in $10^8$ $dm^3$/mole/sec are 4.2, 55 and 29, respectively; [33]). These results (especially the protecting effect due to sodium formate) contrast with data obtained by EPR-spin-trapping. Therefore, it appears clearly that OH radicals are involved in the strand breakage reaction, thus in the presence of DNA, $O_2^{\cdot-}$ seems to be efficiently converted into OH radicals. Two hypotheses can be proposed to explain the production of $OH^\cdot$ in the presence of DNA. (1) A Fenton reaction which involves $H_2O_2$ conversion into $OH^\cdot$ catalyzed by DNA-Fe(II) complexes [34]. This hypothesis seems to be very unlikely because no ΦXRFI conversion into ΦXRFII is observable after incubation of ΦXRFI in the presence of $H_2O_2$. Thus, iron complexed with DNA molecules is probably in a ferric stage (FeIII). (2) a Haber–Weiss reaction which involves to redox half reactions (i.e. $Fe(III) + O_2^{\cdot-} \rightarrow Fe(II) + O_2$ and $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^\cdot + OH^-$). From the data presented above, this last hypothesis seems to be more realistic because $O_2^{\cdot-}$ has been shown to be produced in our system and is known to reduce Fe(III) complexes [35]. Moreover, $O_2^{\cdot-}$ can be dismutated leading to $H_2O_2$ formation [28]. However the $H_2O_2$ participation in the $OH^\cdot$ generation cannot be unambiguously demonstrated because catalase is found to be almost ineffective at decomposing low concentrations of $H_2O_2$ [23]. Moreover, this enzyme could be inactivated under our irradiation conditions according to Cheng *et al.* [36].

If OH radicals are the only species, the inhibiting effects of $OH^\cdot$ scavengers and $O_2$ removal should have similar quantitative effects on DNA damages initiated by all PZD. Instead, Table 1 shows large variations in the protecting effects emphasizing that other mechanisms than the $OH^\cdot$ pathway are involved in the reaction. In the case of CPZ, removing oxygen strongly increases the nicking activity of this sensitizer as also pointed out by Schothorst *et al.* [37]. This anaeorbic DNA breakage mechanism could be explained by the involvement of the promazinyl and/or the chlorine radicals. Chlorine radicals could be involved in the phenomenon. Krasin and Hutchinson [38], using *N*-bromosuccinimide photolysis as bromine radical source, demonstrated the ability of this radical to induce DNA breakage. Chlorine radicals probably display similar reactivity. However, the formation of the promazinyl radicals does not take place when MTPZ, TFPZ or ACPZ are used as photosensitizers. Indeed experiments similar to those carried out by Dao Viet *et al.* [30] show that these PZD do not lose their substituents (Decuyper, unpublished results). Thus another mechanism which does not involve molecular oxygen should be involved.

Under near-u.v. irradiation, Navaratnam *et al.*

[24] demonstrated that PZD undergo photoinonization leading to the formation of $PZD^{+\cdot}$, except in the case of ACPZ [39]. Experiments using high steady state concentrations of $PZD^{+\cdot}$, generated in the dark by the HRP-$H_2O_2$-isobutanal system, show clearly that $PZD^{+\cdot}$ could promote DNA breakage. This phenomenon is not very important with $PZ^{+\cdot}$, $CPZ^{+\cdot}$ and $TFPZ^{+\cdot}$, however $MTPZ^{+\cdot}$ shows a specially high activity which could be in accordance with peculiar reactivity of this cation radical as shown by Sackett and McCreery [40]. $PZD^{+\cdot}$ decay via several intermediates into stable degradation products [40]. However, these stable degradation products do not seem to act as DNA breakers, because no DNA cleavage can be observed when preirradiated PZD are mixed with DNA. Thus, $PZD^{+\cdot}$ or one of the non stable intermediates can participate in the DNA cleavage reaction.

In conclusion, promazine derivatives show large variations in their photosensitizing activities towards DNA, which result from differences in the efficiencies of mechanisms involving essentially two intermediates: OH radicals and $PZD^{+\cdot}$ or one of its degradation products. CPZ which undergoes an important dechlorination under irradiation should therefore be distinguished from the other promazine derivatives.

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