Radiation-chemical Properties of the Hypoxic Cell Radiosensitizer Doranidazole (PR-350)

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This study was performed to confirm the radiation-chemical properties of the 2-nitroimidazole derivative doranidazole, (±)-(2RS,3SR)-3-[(2-nitroimidazol-1-yl)-methoxy]butane-1,2,4-triol [CAS 137339-64-1], PR-350, which was synthesized as a hypoxic cell radiosensitizer with low toxicity. Radiation-chemical experiments using doranidazole showed that (1) unlike O2, it had high reactivity toward not only hydrated electrons (eaq−), but also hydroxyl radicals (•OH), (2) the reduced intermediates of doranidazole had no ability to induce immediate strand breaks of colE1 plasmid DNA, (3) doranidazole enhanced radiation-induced DNA strand breaks of colE1 plasmid DNA in the aqueous state, whereas it did not enhance the base alteration, such as 8-oxo-deoxyguanosine, (4) it enhanced the radiation-induced formation of strand breaks with 3’-phosphate and 3’-phosphoglycolate termini, and (5) it was bound to DNA after irradiation. These facts revealed that the majority of radiation-chemical properties of doranidazole, except for the high reactivity toward ‘OH, were similar to those of oxygen.

INTRODUCTION

Many nitroimidazole derivatives have been developed as hypoxic cell radiosensitizers with high electron affinity and tested in vitro and in vivo1. Among them, misonidazole ([2-nitroimidazol-1-yl]-3-methoxy-2-propanol) and etanidazole (N-[2-hydroxyethyl]-2-nitroimidazole-1-acetamide) were extensively examined and their clinical evaluation was reported2–5. The clinical results with hypoxic cell radiosensitizers have been disappointing because of their toxicity to neurons and the gastrointestinal tract. Recently, a newly synthesized 2-nitroimidazole derivative, doranidazole, which is less toxic and produces fewer side effects, was examined to determine its
fundamental physicochemical properties and LD_{50} as well as its radiosensitizing ability. Hypoxic cell radiosensitizers have been developed to increase the radiosensitivity of radioresistant hypoxic tumor cells by forcing them to take part in radiation-chemical reactions to increase cell killing. Therefore, it seems significant at this time to characterize the chemical aspects of doranidazole by radiation-chemical experiments. The present study was carried out based on the facts that (1) the concentration-dependence of the radiation-enhancement ratio in Chinese hamster ovary cells on oxygen was very similar to that for radiosensitizers, except for the concentrations necessary to achieve maximum sensitization (for example, maximum enhancement ratios of 2.85 for oxygen and 2.8 for misonidazole and K (oxygen constant, the concentration of oxygen or sensitizer giving rise to the intermediate value between maximum radiosensitization and the radiosensitivity under 100% nitrogen) values of 4 μM for oxygen and 3 mM for misonidazole, which is 750-fold less efficient than oxygen because of the difference in the one-electron reduction potential E between oxygen -0.15 V and misonidazole -0.34 V)\textsuperscript{10,11}, and (2) radiosensitizers cannot sensitize fully aerobic cells though the combination of misonidazole at high concentrations and oxygen at low concentrations of \( \Box 1\% \) yields a small additional effect, suggesting that the selectivity towards hypoxic cells may not strictly be a correct concept\textsuperscript{12}. To compare radiation-chemical properties of doranidazole with those of oxygen, (1) the reaction rate constants of doranidazole with 'OH and e\textsubscript{aq} were determined using a nanosecond pulse radiolysis system, (2) the ability of reduced intermediates of doranidazole to induce immediate strand breaks of DNA was examined by employing an experimental system using the obtained rate constants, (3) the effect of doranidazole on 'OH-induced base damage (7,8-dihydro-8-oxo-2'-deoxyguanosine, 8-oxodG) was examined, (4) the ability of doranidazole to enhance 'OH-induced DNA strand breaks was examined in an aqueous solution of colEl plasmid DNA, (5) the influence of doranidazole on the formation of 3'-termini at strand breaks was studied on oligo(dA)\textsubscript{12}/oligo(dT)\textsubscript{12}, and (6) the adducts between doranidazole and DNA were measured with \(^{14}\text{C}\)-labeled doranidazole.

**MATERIALS AND METHODS**

**Chemicals**

Doranidazole (PR-350), shown in Fig. 1, and \(^{14}\text{C}\)-labeled doranidazole (452 MBq/mmol) were supplied by Pola Chemical Industries, Inc. (Yokohama, Japan). CoIE1 plasmid DNA and salmon sperm DNA were purchased from Nippon Gene Co., LTD. (Tokyo, Japan). Oligonucleotides (dA)\textsubscript{12} and

![Fig. 1. Chemical structure of doranidazole (PR-350). Doranidazole is an equal mixture of enantiomers.](https://academic.oup.com/jrr/article-abstract/43/1/77/910227)
(dT)$_{12}$ were purchased from Sawady Technology Co., Ltd. (Tokyo, Japan). [$\gamma$,$^{32}$P]ATP (>148 TBq/mmol) was obtained from ICN Biomedicals Inc. (Costa Mesa, USA). λDNA and T4 polynucleotide kinase were from Takara Shuzo Co., Ltd. (Otsu, Japan). Oligonucleotides (dA)$_{12}$ and (dT)$_{12}$ were labeled at the 5’-end group by T4 polynucleotide kinase, as described previously

Pulse radiolysis experiments

Pulse radiolysis measurements were performed to determine the rate constants between 'OH and doranidazole or λDNA, and $e_{aq}^\cdot$ and doranidazole or λDNA with 50ns electron pulses from a 45 MeV linear accelerator (Model ML-45L, Mitsubishi) of the Graduate School of Engineering, Hokkaido University. Technical specifications have been published elsewhere

First, a determination of the reaction rate constant of doranidazole with 'OH was carried out according to the following competitive reactions of 'OH between doranidazole and the thiocyanate anion SCN$^-$:

$$\text{SCN}^- + '\text{OH} \xrightarrow{k_{SCN}} \text{SCN}' + \text{OH}^- \xrightarrow{1} (\text{SCN})_2^-,$$

$$\text{doranidazole} + '\text{OH} \xrightarrow{k_{doran}} \text{products},$$

from equation (5), the following decay equation was obtained when $[e_{aq}^\cdot]$ << [doranidazole]:

$$\ln \left( \frac{[e_{aq}^\cdot](t)}{[e_{aq}^\cdot]_0} \right) = -k_{doran} \cdot [\text{doranidazole}] t.$$

where $k_{doran}$ is the reaction rate constant of $e_{aq}^\cdot$ with doranidazole, and [doranidazole] is the concentration of doranidazole. From equation (5), the following decay equation was obtained when $[e_{aq}^\cdot]$ << [doranidazole]:

$$\sqrt{G[\text{OH}]} = \sqrt{G[(\text{SCN})_2^-]} = 1 + \frac{k_{doran}}{k_{SCN}} \cdot [\text{doranidazole}] [\text{SCN}^-].$$

where $k_{SCN}$ and $k_{doran}$ are the reaction rate constants of 'OH with SCN$^-$ and doranidazole, respectively, and [doranidazole] and [SCN$^-$] are the concentrations of doranidazole and SCN$^-$, respectively. The concentrations of 'OH generated were determined by the optical absorbance of (SCN)$_2^-$ at 475 nm ($\varepsilon_{475\text{nm}} = 7,600$). $G[\text{OH}]$ is the yield of 'OH generated by one electron-pulse irradiation and equals $G[(\text{SCN})_2^-]$ at [doranidazole] = 0. $G[(\text{SCN})_2^-]$, corresponding to the concentration of OH obtained when doranidazole coexists at an adequate concentration. A value of 1.1 $\times$ 10$^{10}$ M$^{-1}$s$^{-1}$ was employed for $k_{SCN}$.

Second, determination of the reaction rate constant of doranidazole with $e_{aq}^\cdot$ was carried out by observing the dependence of the decay rate of $e_{aq}^\cdot$ generated by one-pulse irradiation on the concentration of doranidazole. In this system, O$_2$ and 'OH were removed by N$_2$-gas bubbling and by adding 100 mM formate ions (HCO$_2^-$) to the solution, respectively. The decay of $e_{aq}^\cdot$ and the decay rate are expressed as follows:

$$\text{doranidazole} + e_{aq}^\cdot \xrightarrow{k_{doran}} \text{products},$$

$$- \frac{d[e_{aq}^\cdot]}{dt} = k_{doran} [e_{aq}^\cdot] [\text{doranidazole}],$$

Reduction of doranidazole by $e_{aq}^\cdot$ and the ability of its reduced intermediates to induce immediate DNA strand breaks

ColE1 plasmid ccDNA (closed-circular form), doranidazole and sodium formate were dissolved in water at concentrations of 6.1 $\times$ 10$^{-5}$ M (nucleotide concentration), 6.1 $\times$ 10$^{-5}$ M and 0.1 M, respectively. The pH of the solution was 6.7. After saturation with Ar gas, the solutions were exposed to X-rays with a Shimadzu HF-320 (200 KV and 20 mA) at doses of 0~450 Gy corresponding to 0~121.2 µM $e_{aq}^\cdot$ (calcu-
lated by the method described in Fig. 2 of ref. 17). Agarose gel electrophoresis of plasmid DNA on 0.7% gel was then performed to measure the strand breaks of DNA. The ratio of the band intensities of ccDNA before and after irradiation was plotted against the concentration of \( \text{e}_{\text{aq}}^- \) on a semilogarithmic scale, \( \ln(S/S_0) \) vs. \( \text{[e}_{\text{aq}}^-\text{]} \).

Effect of doranidazole on 'OH-induced 8-oxodG

2'-Deoxyguanosine (2'-dG) and doranidazole were dissolved in water at a concentration of 1 mM for each. After the solution was saturated with \( \text{N}_2\text{O} \) gas, it was exposed to X-rays at 0°C with doses ranging from 0 to 120 Gy, corresponding to 0–64.4 \( \mu \)M \('\text{OH}\). Under these conditions, 95.3% of \( \text{e}_{\text{aq}}^- \) was converted to \('\text{OH}\) by a reaction with \( \text{N}_2\text{O} \) (calculated by \( k_{\text{N}_2\text{O} + \text{e}} = 9.1 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \) (ref. 17), \( k_{2'\text{-dG} + \text{e}} = 3.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \) (ref. 17), \( k_{\text{doran} + \text{e}} = 2.3 \times 10^{10} \text{ M}^{-1}\text{s}^{-1} \) obtained by present experiments, see Table 1); 75.9% of \('\text{OH}\) reacted with 2'-dG and 24.1% of \('\text{OH}\) reacted with doranidazole (calculated by \( k_{2'\text{-dG} + \text{e}} = 8.8 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \) (ref. 17) and \( k_{\text{doran} + \text{e}} = 2.8 \times 10^9 \text{ M}^{-1}\text{s}^{-1} \) obtained by present experiments, see Table 1).

Reverse-phase HPLC (TSKgel ODS-80T column equipped with an EC-detector [600 mV]) elution with a 50 mM phosphate buffer containing 10% ethanol was employed to measure 8-oxodG. The yield of 8-oxodG was plotted against the amount of \('\text{OH}\) that reacted with 2'-deoxyguanosine.

Ability of doranidazole to enhance \('\text{OH}\)-induced DNA strand breaks

ColE1 plasmid ccDNA and doranidazole were dissolved in water at concentrations of \( 6.1 \times 10^{-5} \) (nucleotide concentration), \( 6.1 \times 10^{-6} \) M or \( 6.1 \times 10^{-5} \) M, followed by saturation with \( \text{N}_2\text{O} \) gas. X irradiation was performed at 0°C with doses of 0–16 Gy, corresponding to 0–8.8 \( \mu \)M \('\text{OH}\). The measurement of DNA strand breaks was carried out according to the method described above. The band intensity of ccDNA was plotted against the concentration of \('\text{OH}\) that actually reacted with it on a semilogarithmic scale, \( \ln(S/S_0) \) vs. \('\text{OH}\).

![Fig. 2. Effects of short-lived intermediates of doranidazole in its reduction process on the induction of immediate strand breaks of colE1 plasmid DNA. The band intensities of ccDNA before (S0) and after (S) X irradiation in the presence ( ) or absence ( ) of doranidazole were plotted against the concentration of \( \text{e}_{\text{aq}}^- \) that actually reacted with DNA on a semilogarithmic scale. The error bars are the S.D. from three experiments. The insert shows a plot of \( \ln(S/S_0) \) against the X-ray dose (Gy) when irradiated in the presence ( ) or absence ( ) of 0.1 M formate.](https://academic.oup.com/jrr/article-abstract/43/1/77/910227)

| \( '\text{OH} \) | \( \text{e}_{\text{aq}}^- \) |
|-----------------|-----------------|
| Doranidazole    | \( 2.8 \times 10^9 \) | \( 2.3 \times 10^{10} \) |
| Misonidazole    | \( 7.1 \times 10^9 \) | \( 2.5 \times 10^{10} \) |
| DNA             | \( 2.9 \times 10^9 \) | \( \sim 10^8 \) |
| \( \text{O}_2 \) | –               | \( 1.9 \times 10^{10} \) |

\(^a\text{Whillans et al. (1975)}\); \(^b\text{Gattavecchia et al. (1984)}\); \(^c\text{Buxton et al. (1988)}\).
Influence of doranidazole on the formation of 3’-termini at strand breaks

Oligo(dA)$_{12}$ was 5’-32P-end labeled by T4 polynucleotide kinase. A duplex was then prepared by annealing with its complementary strand oligo(dT)$_{12}$. The duplex and doranidazole were dissolved in water. The concentration of the duplex was adjusted to 120 µM (nucleotide equivalent) by adding cold duplex. The concentration of doranidazole was adjusted to 0.01 ~ 10 mM. The solution was then saturated with N$_2$-gas and exposed to 200 Gy of X-rays. The unreacted fragments of oligonucleotides were separated on 20% PAGE gel containing 7 M urea and analyzed with a Fujix BAS-1000 Bio Image Analyzer. Quantitative measurements and experimental details were reported elsewhere.$^{13,18}$ To estimate the total quantity of fragments, the sum of radioactivities of all bands excluding the origin in each lane was measured and compared to each other after subtracting the total radioactivity of all bands excluding the origin of the band of the unirradiated duplex.

The adduct formation between doranidazole and DNA

After salmon sperm DNA was purified once to remove proteins, it was dissolved in water at a concentration of 12 mM (nucleotide concentration) together with 120 µM $^{14}$C-labeled doranidazole. The aqueous solution was then N$_2$O-saturated and exposed to 0~200 Gy of X-rays. The fragments of oligonucleotides were separated on 20% PAGE gel containing 7 M urea and analyzed with a Fujix BAS-1000 Bio Image Analyzer. Quantitative measurements and experimental details were reported elsewhere.$^{13,18}$ To estimate the total quantity of fragments, the sum of radioactivities of all bands excluding the origin in each lane was measured and compared to each other after subtracting the total radioactivity of all bands excluding the origin of the band of the unirradiated duplex.

RESULTS

Reaction rate constants obtained from pulse radiolysis experiments

The reaction rate constants are listed in Table 1. The reaction rate constant of doranidazole with ‘OH was 2.8 × 10^9 M$^{-1}$s$^{-1}$, which was slightly less than that of misonidazole$^{19}$. That of λDNA with ‘OH was 2.9 × 10^9 M$^{-1}$s$^{-1}$. The reaction rate constant of doranidazole with e$_{aq}^{-}$ was 2.3 × 10^10 M$^{-1}$s$^{-1}$, which was quite similar to those of misonidazole$^{20}$ and O$_2$$^{17}$. That of λDNA with e$_{aq}^{-}$ was less than those values by two orders of magnitude.

The ability of the reduced intermediates of doranidazole to induce immediate DNA strand breaks

We examined the induction of immediate strand breaks of DNA by short-lived intermediates of doranidazole formed in its reduction process. For this purpose, an Ar-saturated aqueous solution containing colE1 plasmid ccDNA (6.1 × 10$^{-5}$ M), doranidazole (6.1 × 10$^{-5}$ M) and formate ions (0.1 M) was exposed to X-rays according to the method described above. Under this condition, reactions of ‘OH with DNA and doranidazole were neglected (only 0.055% of ‘OH and 0.053% of ‘OH were calculated to react with DNA and doranidazole, respectively, by applying k$_{HCOO^{-}+‘OH}$ = 3.2 × 10^9 M$^{-1}$s$^{-1}$ [ref. 17], and k$_{DNA+‘OH}$ = 2.9 × 10^9 M$^{-1}$s$^{-1}$ and k$_{doram+‘OH}$ = 2.8 × 10^9 M$^{-1}$s$^{-1}$ in Table 1). Furthermore, the reaction rate constants of e$_{aq}^{-}$ to DNA and HCOO$^{-}$ are two or three orders of magnitude lower than those for doranidazole (Table 1 and ref. 17). Therefore, e$_{aq}^{-}$ react exclusively with doranidazole to produce its reduced intermediates. Figure 2 shows plots of ln(S/So) vs.[e$_{aq}^{-}$] in the presence (□) and absence (□) of doranidazole, where S and So are the amounts of ccDNA with irradiation and without irradiation, respectively, measured by agarose gel electrophoresis. The insert is ln(S/So) vs.[X-ray dose] in the presence (□) and absence (□) of 0.1 M formate ions. It was shown that the induction of DNA strand breaks was largely protected by formate ions, suggesting that the reac-

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The formulae and equations are not properly rendered in the text. The context and meaning are clear, but the exact formatting is missing.
tion of \(\cdot\text{OH}\) with DNA was neglected. The reaction of doranidazole with \(e_{\text{aq}}^-\) induced few DNA strand breaks and no significant difference was observed in either case. These results indicated that short-lived intermediates of doranidazole formed in its reduction process did not induce immediate strand breaks of DNA.

**Effect of doranidazole on \(\cdot\text{OH}\)-induced base alteration (8-oxodG)**

To examine the effect of doranidazole on \(\cdot\text{OH}\)-induced base alteration in DNA, we chose 8-oxodG as a typical base alteration and measured its formation after the aqueous solution of 2'-dG was exposed to X-rays in the presence or absence of doranidazole. Figure 3 shows plots of the amounts of 8-oxodG vs. the concentration of \(\cdot\text{OH}\) that actually reacted with 2'-dG. The tendency for the formation of 8-oxodG to be decreased by doranidazole (●) compared to the control ( □) rather than enhanced means that the hypoxic cell radiosensitizing ability of doranidazole may not be attributable to an enhancement of an \(\cdot\text{OH}\) induced base alteration, like 8-oxodG.

**Effect of doranidazole on \(\cdot\text{OH}\)-induced immediate strand breaks of colE1 plasmid DNA**

The concentration of \(\cdot\text{OH}\) that actually reacted with plasmid ccDNA under the conditions described above was estimated according to the following reactions in addition to reaction (2):

\[
\begin{align*}
\cdot\text{OH} + \text{ccDNA} & \rightarrow \text{ccDNA radicals} \rightarrow \text{strand breaks} \\
\cdot\text{OH} + \text{ocDNA} & \rightarrow \text{ocDNA radicals} \rightarrow \text{damaged DNA} \\
\text{ccDNA radicals} + \text{doranidazole} & \rightarrow \text{strand breaks} \\
& \rightarrow \text{base damage}
\end{align*}
\]

Fig. 3. Formation of 8-oxodG when \(\text{N}_2\text{O}\)-saturated aqueous solution of 2'-deoxyguanosine in the presence ( □) or absence ( ●) of doranidazole was exposed to X-rays. The amounts of 8-oxodG detected by reverse-phase HPLC were plotted against the concentration of \(\cdot\text{OH}\) that actually reacted with 2'-deoxyguanosine. The error bars are the S.D. from three to five experiments.

Fig. 4. Effects of doranidazole on \(\cdot\text{OH}\)-induced strand breaks of colE1 plasmid DNA. An \(\text{N}_2\text{O}\)-saturated aqueous solution of DNA was exposed to X-rays in the absence of doranidazole ( □ ) and in the presence of doranidazole at the concentration ratios of [doranidazole]/[DNA]=0.1 ( □) and [doranidazole]/[DNA]=1 ( □). Band intensities of ccDNA before (\(S_0\)) and after (\(S\)) X irradiation were plotted against the concentration of \(\cdot\text{OH}\) that actually reacted with DNA on a semilogarithmic scale. The insert shows a plot of \(\ln(S/S_0)\) against the X-ray dose (Gy) when irradiated in the presence ( □) or absence ( □) of doranidazole without formate.
where ocDNA means open-circular and other forms of DNA. The concentrations of ccDNA and ocDNA were estimated by measuring their band intensities of gel electrophoretic patterns at irradiation dose = 0. By assuming $k_{ccDNA \cdot OH} = k_{ocDNA \cdot OH}$ and applying competitive reactions among (2), (7) and (8), the fraction of 'OH that actually reacted with ccDNA was calculated. When $\ln(S/S_0)$ of ccDNA was plotted as a function of the X-ray dose, the sensitization of 'OH-induced immediate strand breaks by doranidazole was not clear because some 'OH were scavenged by doranidazole (the insert in Figure 4). However, when $\ln(S/S_0)$ of ccDNA was plotted as a function of ['OH] that actually reacted with ccDNA, a marked sensitization by doranidazole was observed (Fig. 4), suggesting that it has the ability to enhance the 'OH-induced immediate strand breaks of DNA in the dilute aqueous state.

Fig. 5. PAGE patterns of oligonucleotide fragments when 5'-32P-oligo(dA)12/oligo(dT)12 duplex was exposed to X-rays under various conditions. (dA)2-P and (dA)2-PG indicate dimers of 2'-deoxyadenylic acid with 3'-phosphate and 3'-phosphoglycolate termini, respectively.
Influence of doranidazole on the formation of 3'-termini at strand breaks

To examine the influence of doranidazole on the formation of 3'-phosphate and 3'-phosphoglycolate termini, the 5'-32P-end-labeled oligo(dA)12/oligo(dT)12 duplex was exposed to X-rays under N2 and O2 conditions and both the presence and absence of doranidazole. Figure 5 shows the PAGE patterns of oligonucleotides. Irradiation produced oligonucleotide fragments ranging from dimer to 11-mer. Each fragment consisted of a band with a pair of spots and a single spot when exposed to X-rays under O2 and N2 conditions, respectively. When the oligonucleotides were treated with hot piperidine after irradiation, the intensity of the upper spot of the pair became stronger than that before a piperidine treatment (data not shown). Since a piperidine treatment can be used to visualize the strand breaks with a simple phosphate at the 3' terminus, the upper spot was regarded as being a fragment with a simple phosphate group at the 3' terminus. Henner et al. and Buchko and Weinfeld reported that γ irradiation of DNA under N2 conditions exclusively produced stand breaks with a simple phosphate at the 3' terminus, and under O2 conditions with two types of 3' termini, a simple phosphoryl group and a phosphoglycolate attached to the 3' terminus. Therefore, the results obtained here were regarded as suggesting that the fragments were cleaved with a simple phosphate group at the 3' terminus under N2 conditions and a simple phosphate group and a phosphoglycolate group at 3' termini under O2 conditions. Similar results were reported in our previous papers. A 2.5-fold increase in the total quantity of fragments was observed under O2 conditions. X irradiation under N2 conditions in the presence of 10 or 100 µM doranidazole also generated fragments with both types of termini. The doranidazole gave rise to a 1.6-fold increase in the total quantity of fragments.

The adduct formation between doranidazole and DNA radicals

Since this experiment used 12 mM DNA (nucleotide concentration) and 120 µM 14C-labeled doranidazole, OH exclusively reacted with DNA to induce DNA radicals. Therefore, this experimental system could be used to examine the ability of doranidazole to form adducts with OH-induced DNA radicals. Figure 6 shows a plot of the radioactivity of 14C-doranidazole that was bound to DNA against the X-ray dose. The dose-dependent addition of doranidazole to DNA confirmed that doranidazole certainly reacted with DNA radicals to form adducts. The adduct formation presumably occurred at the base moiety of DNA because the interaction between doranidazole and deoxyribose radicals resulted in an increase of strand breakage, as described above.

DISCUSSION

DNA was selected as a target in the present radiation-chemical model systems. This was due to the fact that the quantitative structure-activity relationships of nitroaryl radiosensitizers gave no evidence for a membrane target, but evidence for nuclear DNA. In fact, we recently observed that etanida-
zole sensitized X-irradiated HL-60 cells, in which DNA is a target, and did not sensitize X-irradiated MOLT-4 cells, in which DNA is not a target\textsuperscript{24}. Recent studies\textsuperscript{25,26} using another type of hypoxic cell sensitizer, sanazole, which has a one-electron reduction potential of $-0.33$ V, similar to that of doranidazole ($-0.308$ V)$^6$, also indicated that the preferential increase in DNA damage by sanazole was responsible for its radiosensitizing ability.

Several papers have dealt with the effects of reduced misonidazole on the induction of DNA damage, and show that the stable end products of misonidazole do not induce immediate strand breaks of DNA\textsuperscript{27–29}. The present study indicated that short-lived intermediates of doranidazole formed in its reduction process did not induce immediate strand breaks of DNA either, though the chemical structures of the intermediates were not analyzed in the present study.

Fuciarelli et al. reported that the yield of radiation-induced 8-oxodG under N$_2$O/O$_2$ conditions was higher than that under N$_2$O conditions, suggesting that the $\cdot$OH-induced formation of 8-oxodG was enhanced by O$_2$\textsuperscript{30}. We also reported an enhancement in the formation of 8-oxodG by the electron-affinic compound $\alpha$-phenyl-N-tert-butylnitrone\textsuperscript{14}. The electron transfer reaction from the $\cdot$OH-induced precursor radical of 8-oxodG to an electron-affinic molecule, like O$_2$ or $\alpha$-phenyl-N-tert-butylnitrone during irradiation, was inferred to explain this phenomenon. Therefore, a similar effect on the formation of 8-oxodG by doranidazole was expected because of its high electron affinity. However, a tendency for a protective effect rather than a sensitizing effect on the $\cdot$OH-induced formation of 8-oxodG was observed (Fig. 3). Using experimental data concerning radiation protection and sensitization, Wardman inferred that radiosensitization was due to fast, free-radical reactions between target radicals and the oxidant, and proposed, as critical reactions, the competitive reactions between target radicals and the oxidant. This may be due to the difference in the plot between $\ln(S/S_0)$ vs. radiation dose in those authors’ studies and $\ln(S/S_0)$ vs. [OH] in the present study.

The present study showed that not only O$_2$ (2.5-fold), but also doranidazole (1.6-fold), enhanced $\cdot$OH-induced strand breaks of DNA with 3'O-phosphate and 3'-phosphoglycolate termini (Figs. 4 and 5). This differs from a report by Buchko and Weifeld showing that while irradiation in the presence of O$_2$ increased

\begin{equation}
\log(k) = b_0 + b_1E,
\end{equation}

he calculated a $b_1$ for one-electron oxidation by nitroaryl compounds and compared it with that for the radical addition reaction, and found that $b_1$ for radical addition was much smaller than that for electron-transfer, thus suggesting the possibility that one-electron reduction may proceed via a radical addition reaction. In fact, he observed adduct formation and subsequent dissociation of the $\cdot$CH$_2$OH/misonidazole radical adduct. Furthermore, it was also reported that the base radicals do not apparently react with nitroaryl compounds\textsuperscript{31,32}. However, the present results in Fig. 6 showed that doranidazole formed adducts with DNA, proving that some DNA radicals react with the sensitizer to form adducts. From these results it was inferred that the interaction of doranidazole with $\cdot$OH-induced base radicals was a radical addition reaction, rather than an electron-transfer reaction.
the yield of strand breaks consisting of 3′-phosphoglycolate and 3′-phosphate 3-fold, misonidazole under anoxic conditions did not affect the level of strand breaks, but enhanced only the formation of 3′-phosphoglycolate\(^{23}\). This discrepancy may be attributable to the difference in the experimental conditions, the (oligo(dA))\(_{12}\)/oligo(dT))\(_{12}\) duplex used in the present study and the oligo(dA))\(_{12}\)/poly(dT) duplex in their study and the ratios of the sensitizer/oligonucleotide, though the precise reasons are not known. It is noted that doranidazole protected the oligonucleotide duplex against an \(\cdot OH\) attack at concentrations higher than 100 \(\mu M\), probably because doranidazole served as a scavenger of \(\cdot OH\) rather than a modifier. These results proved that doranidazole at a low concentration, like \(O_2\), increased the level of strand breakage and significantly enhanced the formation of 3′-phosphoglycolate end groups.

The following mechanism is proposed for the formation of 3′-phosphoglycolate by both \(\cdot OH\) and \(O_2\): H-abstraction at the C4′ of the deoxyribose moiety by \(\cdot OH\), subsequent addition of \(O_2\) to form the C4′-peroxyl radical, the reduction of the radical by glutathione to form hydroperoxide followed by the Criegee rearrangement, giving a 6-ring orthoacid, and 3′- and 5′-cleavages giving 5′-phosphate, 3′-phosphoglycolate ends and released base propenal\(^{35}\). In analogy to this mechanism, the formation of 3′-phosphoglycolate ends by doranidazole may be explained as follows: the addition of the nitro group of doranidazole to the C4′-radical, a rearrangement of the adduct to form 6-ring orthoacid, followed by the release of the side chain at the N1 of doranidazole, and hydrolysis giving 5′-phosphate, 3′-phosphoglycolate ends, released base propenal and 2-hydroxylaminimidazole. Further study is needed to confirm this mechanism.

To sum up, doranidazole has radiation-chemical properties quite similar to those of \(O_2\), except for being highly reactive toward \(\cdot OH\), high reactivity toward \(e_{aq}\), as proved by the reaction rate constants, low reactivity of its reduced form to DNA (the reactivity of \(O_2^−\) toward DNA is also low\(^{36}\)), and a radical addition reaction rather than electron transfer reaction to bring about adduct formation and an increase in the strand breakage with 3′-phosphoglycolate termini under conditions in which \(\cdot OH\) are mainly involved with the reactions. These data will be helpful for a better understanding of radiosensitizing mechanisms at the level of radiation-chemical reactions when this compound is applied to clinical use.

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