Original Research

Potent inhibition of peroxynitrite-induced DNA strand breakage and hydroxyl radical formation by dimethyl sulfoxide at very low concentrations

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Abstract

Dimethyl sulfoxide (DMSO) is frequently used as a solvent for many water-insoluble drugs in biological studies at concentrations often up to 1%. However, little is known about its effects on oxidatively generated DNA damage at very low concentrations (0.005–0.5%). This study was undertaken to investigate the effects of DMSO on peroxynitrite-induced DNA strand breaks, a critical event leading to peroxynitrite-elicited cytotoxicity. Incubation of φX-174 plasmid DNA, with 3-morpholinosydnonimine (SIN-1), a peroxynitrite generator, led to the formation of DNA strand breaks in a concentration- and time-dependent manner. The presence of DMSO at concentrations of 0.005–0.5% was found to significantly inhibit SIN-1-induced DNA strand breaks in a concentration-dependent manner. However, DMSO at the above concentrations showed no affect on SIN-1-mediated oxygen consumption, indicating that DMSO did not affect the auto-oxidation of SIN-1 to form peroxynitrite. It is observed that incubation of the plasmid DNA with authentic peroxynitrite resulted in significant formation of DNA strand breaks, which could also be dramatically inhibited by the presence of DMSO at 0.005–0.5%. Electron paramagnetic resonance spectroscopy, using 5,5-dimethylpyrroline-*N*-oxide (DMPO) as a spin trap demonstrated the formation of DMPO-hydroxyl radical adduct from the SIN-1 and authentic peroxynitrite. DMSO at the concentrations ranging from 0.01% to 0.5% significantly inhibited the adduct signal. Taken together, these studies demonstrate, for the first time, that DMSO at extremely low concentrations (0.005–0.5%) can potently inhibit peroxynitrite-mediated DNA strand breakage and hydroxyl radical formation. The results of this study suggest that, where DMSO is applied as a solvent, caution should be observed when evaluating the actions of drugs in experiments involving DNA damage.

Keywords: DMSO, peroxynitrite, DNA strand breaks, solvent

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Introduction

Dimethyl sulfoxide (DMSO) is an amphipathic molecule with a polar domain and two apolar methyl groups making it soluble in both aqueous and organic media. Due to its excellent solvating properties, DMSO is frequently used as a very efficient solvent for numerous water-insoluble drugs and chemical reactions in the biological studies. However, it has a wide range of biological effects that could confound the effects of drug administration. In cryobiology, DMSO has been routinely used as a cryoprotectant to preserve organs, tissues and cell suspensions in a mixture of 10% DMSO and 90% fetal bovine serum. It is also widely employed in cell biology to induce cell fusion[1] and cell differentiation.[2,3] In medicine, DMSO has been used in numerous human therapeutic situations. Due to its anti-inflammatory therapeutic effects on reactive oxygen species scavenger actions, DMSO has been used as a remedy for several gastrointestinal disorders.[4,5] In 1978, DMSO was approved by the Food and Drug Administration for the palliative treatment of interstitial cystitis, but with the effect that is not related to detectable mast cell release of histamine.[6] DMSO has also been suggested for the treatment of dermatological disorders,[7] intractable urinary frequency,[8] pulmonary adenocarcinoma[9] and manifestations of amyloidosis.[10] Furthermore, DMSO can cross the blood–brain barrier and has a beneficial effect in the treatment of traumatic brain edema[11] and Alzheimer's disease.[12]

Although DMSO is known as a potent scavenger of hydroxyl radicals and widely used as an agent for therapeutic intervention in many clinically related situations, its

action on peroxynitrite-induced DNA damage still remains unclear. Peroxynitrite is a potent electrophile that is generated from the radical–radical reaction of nitric oxide and superoxide at a diffusion-limited rate.[13] There is a considerable body of evidence suggesting that peroxynitrite is crucially involved in the pathogenesis of various diseases, including stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer and neurodegenerative disorders.[14–18] Multiple mechanisms have been proposed to account for the cytotoxicity elicited by peroxynitrite. Among them, induction of DNA strand breaks and the subsequent activation of poly(ADP-ribose) polymerase have been demonstrated to be critical events leading to peroxynitrite-elicited cytotoxicity.[19] To our knowledge, direct evidence for the effects of very low concentrations of DMSO on peroxynitrite-induced DNA strand breaks is lacking in the literature. DMSO at the concentration of 1% is often used as the vehicle for various water-insoluble drugs in many oxidatively generated DNA damage studies.[20–23] However, largely driven by empiricism, the use of DMSO as a solvent at concentrations up to 1% has been suggested to be safe, even in the absence of critical examination of its own effects in these and most other past experimental applications.[20–23] Recent studies demonstrate that DMSO itself, at concentrations as low as 0.02% could have its own effect on neurotransmission.[24] Whether DMSO at such low concentrations has potential protective effects on peroxynitrite-mediated DNA damage is still unknown and has been largely overlooked in previous studies. The lack of a deep understanding of the action of DMSO at very low concentrations could ultimately serve to confound the effects of drug administration and hinder DMSO's application as a drug solvent.

In this study using φX-174 plasmid DNA as an *in vitro* system, we have investigated the effects of DMSO on peroxynitrite-induced DNA strand breaks. Our results demonstrate for the first time that DMSO, at extremely low concentrations (0.005–0.5%), potently inhibits peroxynitrite-mediated DNA strand breakage and formation of hydroxyl radicals in a concentration-dependent manner.

Materials and methods

Materials

φX-174 RF I plasmid DNA was from New England Biolabs (Beverley, MA, USA). Authentic peroxynitrite (sodium salt) was from Calbiochem (San Diego, CA, USA). DMSO (≥99.9%), 3-morpholinosydnonimine (SIN-1) and other chemicals were from Sigma Chemical (St Louis, MO, USA).

Preparation of SIN-1 and peroxynitrite

SIN-1, an effective peroxynitrite generator, was dissolved in phosphate-buffered saline (PBS), pH 5 and stored at −80°C. The concentration of authentic peroxynitrite was determined spectrophotometrically at 302 nm (extinction coefficient = 1670/mmol/L/cm). The peroxynitrite was aliquoted and stored at −80°C under nitrogen.

Assay for DNA strand breaks

DNA strand breaks were measured by the conversion of supercoiled φX-174 RF I double-stranded DNA to open circular and linear forms.[25] Briefly, 0.25 μg DNA was incubated with SIN-1 or authentic peroxynitrite in the presence or absence of DMSO at 37°C (for SIN-1) or room temperature (25°C, for authentic peroxynitrite) in PBS (pH 7.4) at a final volume of 24 μL. Following incubation, the samples were immediately loaded in a 1% agarose gel containing 40 mmol/L Tris, 20 mmol/L sodium acetate and 2 mmol/L EDTA, and electrophoresed in a horizontal slab gel apparatus in Tris/acetate/EDTA gel buffer. After electrophoresis, the gels were stained with 0.5 μg/mL solution of ethidium bromide for 30 min, followed by another 30 min destaining in water. The gels were then photographed under ultraviolet illumination and quantified using Alpha Innotech Imaging system (San Leandro, CA, USA).

Measurement of oxygen consumption

Oxygen consumption caused by SIN-1 auto-oxidation was monitored with a Clark oxygen electrode (YSI 5300, Yellow Springs, OH, USA) upon mixing SIN-1 with DMSO in 2.5 mL air-saturated PBS (pH 7.4) at 37°C, as described previously.[25] The oxygen consumption was expressed as the percentage of saturation oxygen.

Electron paramagnetic resonance spin-trapping assay

For 5,5-dimethylpyrroline-*N*-oxide (DMPO) spin-trapping measurement of reactive oxygen species, electron paramagnetic resonance (EPR) spectra were recorded at room temperature with a spectrometer (Bruker D-200 ER, Billerica, MA, USA), operating at X-band with a TM cavity and capillary cell, as described previously.[26] The spectrometer settings were: modulation frequency, 100 kHz; microwave frequency, 9.5 GHz; microwave power, 20 mW; modulation amplitude, 1 G; and scan time, 200 s. Reactants were mixed in test tubes in a final volume of 0.1 mL following transference to a capillary cell for EPR spectral studies at room temperature under conditions described above. Spectral simulations were performed on the EPR data by matching directly to the spectra as described previously.[27]

Statistical analyses

Analysis of variance was used. Further statistical analyses for *post hoc* comparisons were performed using the Tukey–Kramer test. All differences of $P \leq 0.05$ were considered significant.

Results

Induction of DNA strand breaks by SIN-1

Induction of single-strand breaks from the supercoiled double-stranded φX-174 RF I plasmid DNA leads to the formation of open circular DNA, and the formation of a linear form of DNA is indicative of double-strand breaks.[28] At a physiological pH, SIN-1 is known to undergo

auto-oxidation to produce equal molar nitric oxide and superoxide, leading to the formation of peroxynitrite mimicking the *in vivo* situation.[29,30] As shown in Figure 1, incubation of the plasmid DNA with 25–1000 μmol/L SIN-1 resulted in a significant formation of open circular form of DNA in a concentration- and time-dependent manner. Treatment of plasmid DNA with SIN-1 for 120 min also led to the formation of a linear form of DNA, indicating that SIN-1 was also able to elicit double-strand breaks in this plasmid DNA system.

Inhibition of SIN-1-induced DNA strand breaks by DMSO

A significant formation of open circular form of DNA was observed following incubation of the plasmid DNA with 250 μmol/L SIN-1 for 60 min (Figure 1). As such, the above SIN-1 concentration and incubation time were selected for investigation of the protective effects of DMSO. As shown in Figure 2, the SIN-1-mediated single-strand DNA breaks were remarkably inhibited by DMSO in a concentration-dependent manner. Notably, a significant inhibition of SIN-1-induced DNA strand breakage was observed with DMSO at a concentration as low as 0.005%. Increasing the concentrations of DMSO led to further decreases in the induction of DNA breaks elicited by SIN-1 treatment.

Effects of DMSO on SIN-1-mediated oxygen consumption

Because oxygen consumption is a critical step involved in SIN-1 auto-oxidation leading to the formation of peroxynitrite, we investigated the effects of DMSO on SIN-1-mediated oxygen utilization. As shown in Figure 3a, addition of SIN-1 at the indicated concentrations (25–1000 μmol/L) to PBS (pH 7.4) resulted in oxygen consumption in a concentration-dependent manner. Notably, SIN-1 at 500 and 1000 μmol/L led to depletion of oxygen by 50% and 70%, respectively, in PBS 30 min after the addition of SIN-1. However, the presence of DMSO at the concentrations ranging from 0.005% to 0.5% showing protective

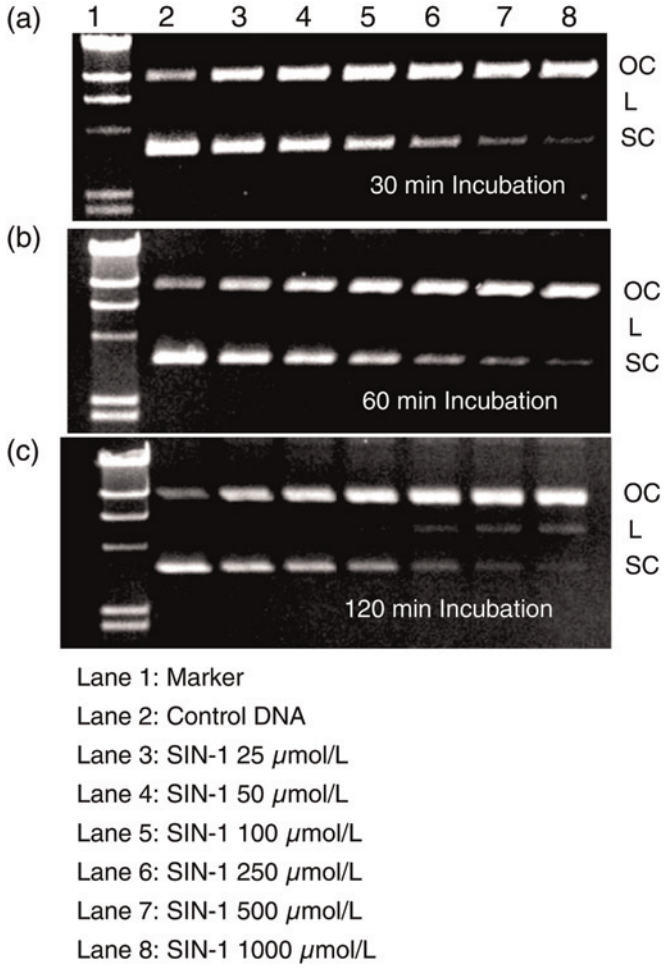

Figure 1 Concentration- and time-dependent induction of DNA strand breaks by SIN-1 in the φX-174 plasmid DNA. Shown are the representative gel pictures of the φX-174 plasmid DNA following incubation with the indicated concentrations of SIN-1 for 30, 60 and 120 min. The marker used was Lambda DNA-*Hind*III digest. OC, L and SC stand for open circular, linear and supercoiled DNA, respectively. SIN, 3-morpholinosydnonimine

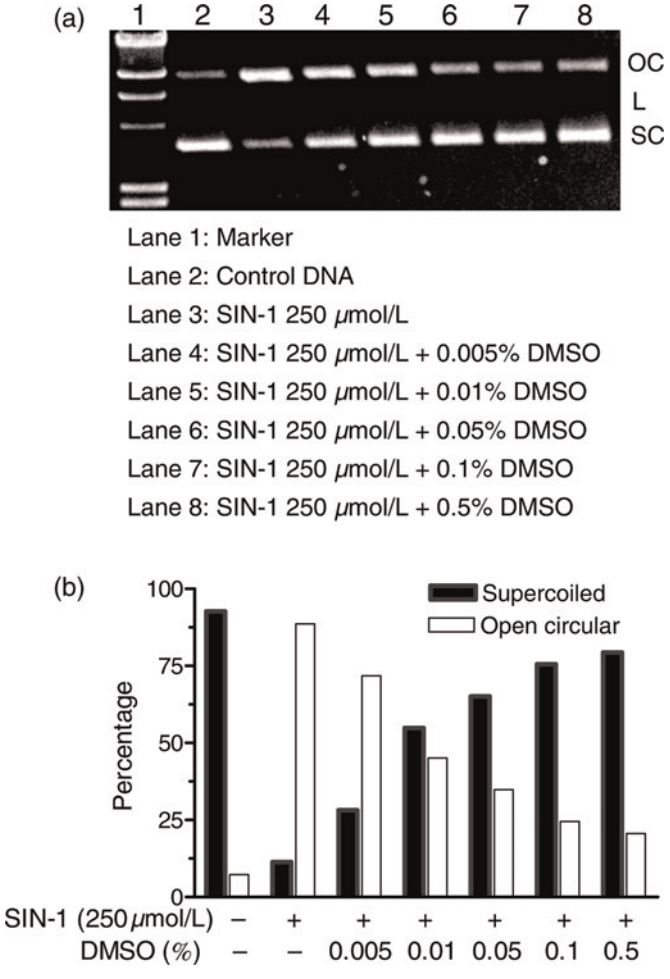

Figure 2 Inhibitory effects of DMSO on SIN-1-induced DNA strand breaks in the φX-174 plasmid DNA. The plasmid DNA was incubated with SIN-1 in the presence or absence of the indicated concentrations of DMSO for 60 min. (a) A representative gel picture. (b) Concentration of SIN-1 was 250 μmol/L; data represent the averages of two independent measurements. SIN, 3-morpholinosydnonimine; DMSO, dimethyl sulfoxide

effects on SIN-1-induced DNA damage did not significantly affect the oxygen consumption caused by 250 μmol/L SIN-1 (Figure 3b). This observation indicated that DMSO at the 0.005–0.5% was unable to affect the auto-oxidation of SIN-1 to form peroxynitrite.

Induction of DNA strand breaks by authentic peroxynitrite and the protective effects of DMSO

To further demonstrate the protective effects of DMSO on peroxynitrite-mediated DNA strand breaks, authentic peroxynitrite was used to elicit strand breaks in the plasmid DNA according to the published procedures.[29] As shown in Figure 4, incubation of the plasmid DNA with authentic peroxynitrite at room temperature for 60 min resulted in an increased formation of open circular form of DNA in a concentration-dependent manner, indicating that addition of authentic peroxynitrite to plasmid DNA could cause single-stranded breaks. Since a markedly increased formation of open circular form of DNA was observed following incubation of the plasmid DNA with 100 μmol/L authentic peroxynitrite for 60 min at room temperature (Figure 4, lane 5), this concentration of authentic peroxynitrite was selected for investigation of the protective effects of DMSO. As shown in Figure 5, the authentic peroxynitrite-mediated single-stranded DNA breaks were remarkably inhibited by DMSO in a concentration-dependent manner. Notably, a significant protection was observed with DMSO at a concentration as low as 0.005% (Figure 5).

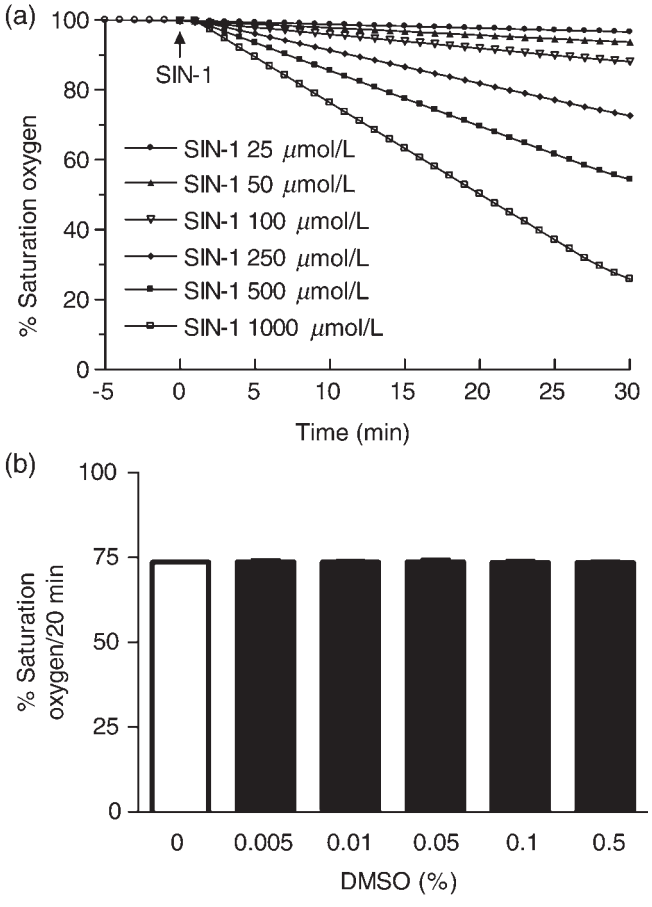

Figure 3 Effects of DMSO on SIN-1-mediated oxygen consumption. The oxygen consumption was measured following incubation of SIN-1 in the presence or absence of the indicated concentrations of DMSO for 30 min. (a) Representative oxygen consumption curves with 25–1000 μmol/L SIN-1. (b) Effects of DMSO on the oxygen consumption caused by 250 μmol/L SIN-1; data represent mean $\pm$ SEM from three independent experiments. SIN, 3-morpholinosydnonimine; DMSO, dimethyl sulfoxide; SEM, standard error of mean

Effects of DMSO on both authentic peroxynitrite and SIN-1-mediated generation of hydroxyl radicals

A number of mechanisms have been proposed to account for peroxynitrite-mediated DNA damage. Peroxynitrite decomposition has been suggested to generate oxidant/radical species that can damage a wide array of molecules in cells, including nucleic acids. As such, the EPR spin-trapping technique was employed to study the effects of DMSO on peroxynitrite-mediated free radical generation. Direct trapping of oxygen radicals in aqueous media was performed using the spin trap DMPO, which reacts with oxygen radicals, including superoxide and hydroxyl radicals to form more stable spin adducts.[31] As shown in Figure 6a, line b, decomposition of peroxynitrite in the presence of DMPO led to the formation of a DMPO-spin adduct with the hyperfine splitting constants of $a_N = a_H = 14.9$ G characteristics of DMPO-hydroxyl adduct (DMPO-OH).[27,31] DMPO itself did

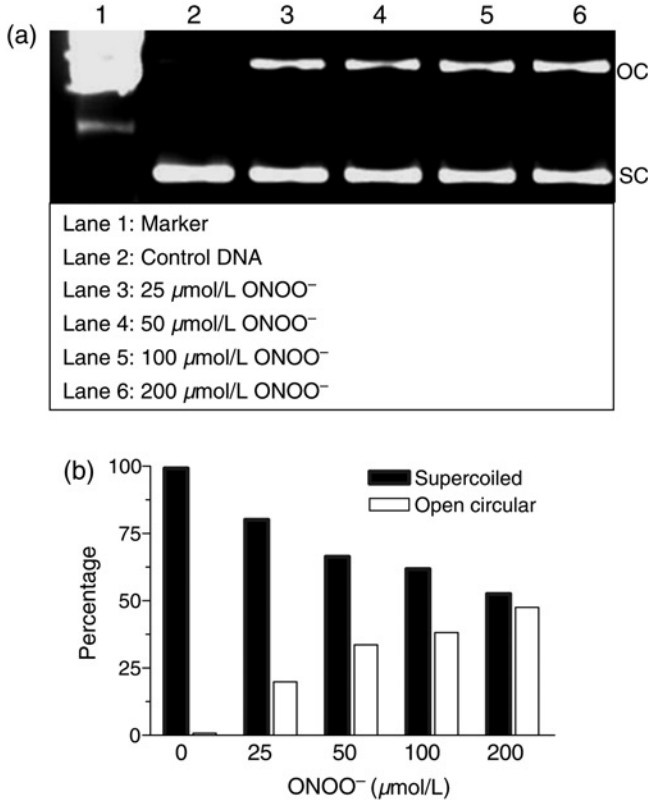

Figure 4 Concentration- and time-dependent induction of DNA strand breaks by authentic peroxynitrite in the φX-174 plasmid DNA. Shown are the representative gel pictures of the φX-174 plasmid DNA following incubation with the indicated concentrations of authentic peroxynitrite ($ONOO^-$) for 60 min. The marker used was Lambda DNA-*Hind*III digest. OC and SC stand for open circular and supercoiled DNA, respectively

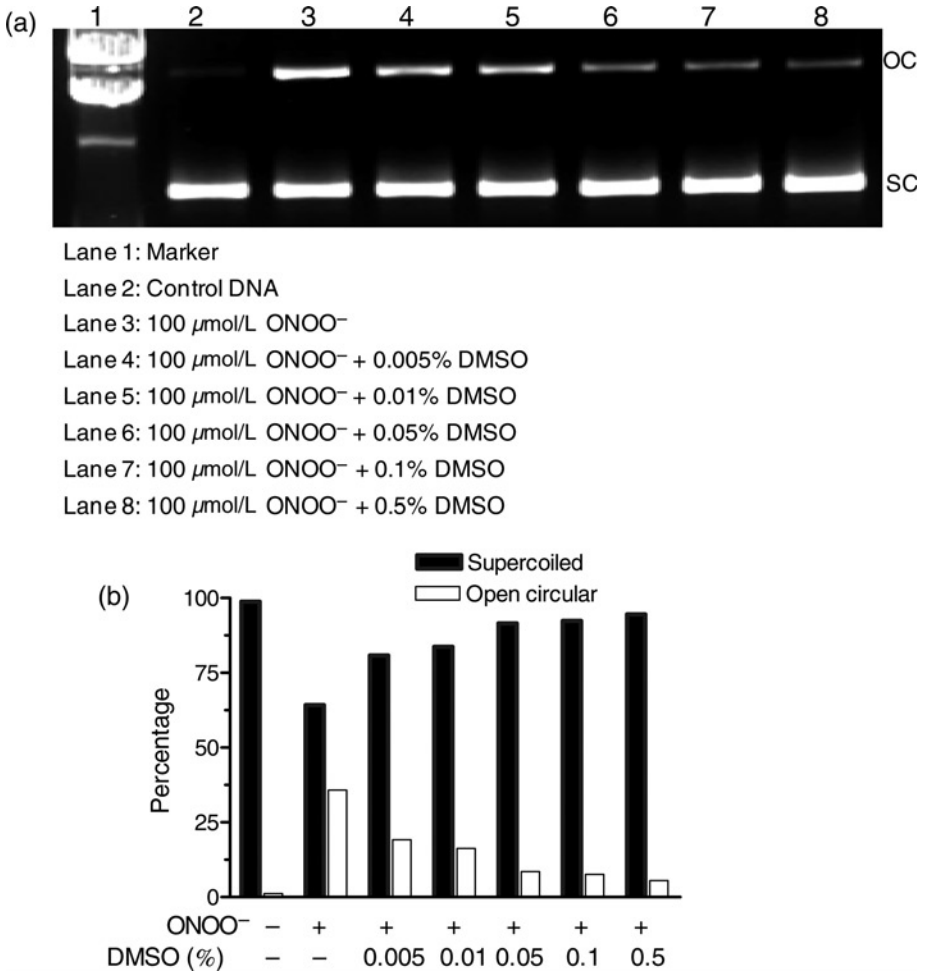

Figure 5 Inhibitory effects of DMSO on authentic peroxynitrite-induced DNA strand breaks in the φX-174 plasmid DNA. The plasmid DNA was incubated with authentic peroxynitrite ($ONOO^-$) in the presence or absence of the indicated concentrations of DMSO for 60 min. (a) A representative gel picture. (b) Concentration of authentic peroxynitrite was 100 μmol/L; data represent the averages of two independent measurements. DMSO, dimethyl sulfoxide

not give rise to the formation of any detectable spin adducts (Figure 6a, line a), indicating the high purity of the spin trap used in this study. It was found that the hydroxyl radical spin-adduct, DMPO-OH could also be derived from decomposition of the superoxide spin adduct.[32–34] The later pathway of formation of DMPO-OH can be readily identified using superoxide dismutase (SOD).[35] The addition of SOD did not affect the EPR signal intensity of DMPO-OH, indicating that the EPR spectra observed in this experiment arose from the trapping of hydroxyl radicals by DMPO during the decomposition of authentic peroxynitrite (data not shown). As shown in Figure 6b, EPR radical spectrum signal heights were significantly reduced by DMSO (0.01–0.5%) in a concentration-dependent manner ($\rho \leq 0.05$). The possibility of release of hydroxyl radicals by SIN-1, a peroxynitrite generator that lead to simultaneous production of superoxide and nitric oxide,[36] and the effects of DMSO on SIN-1-mediated hydroxyl radical formation were further investigated by the EPR spin-trapping technique. As shown in Figure 7, SIN-1 is also capable of producing DMPO-OH and DMSO at 0.01–0.5% significantly inhibited the adduct signal. These results suggest that the hydroxyl radicals involved in the reactions of peroxynitrite could, at least partly, account for the protective role of DMSO in attenuating peroxynitrite-dependent DNA damage.

Discussion

DMSO is one of the most common solvents for dissolving various water-insoluble drugs both *in vivo* and *in vitro* studies. However, it has a wide range of cellular effects that could confound the effects of drug administration. The possible effects of DMSO at the low concentrations (0.005–0.5%) on the peroxynitrite-mediated oxidatively generated DNA damage have not been thoroughly examined. The results of the present study demonstrated for the first time that DMSO, at a concentration as low as 0.005% (200 times less than the 1% concentration commonly used as a vehicle control), is able to inhibit the SIN-1- (Figure 2) or authentic peroxynitrite (Figure 5)-mediated DNA damage in φX174 plasmid DNA system.

Peroxynitrite is a highly reactive nitrogen species, which has been implicated in the pathogenesis of various diseases, including cardiovascular diseases and neurodegenerative

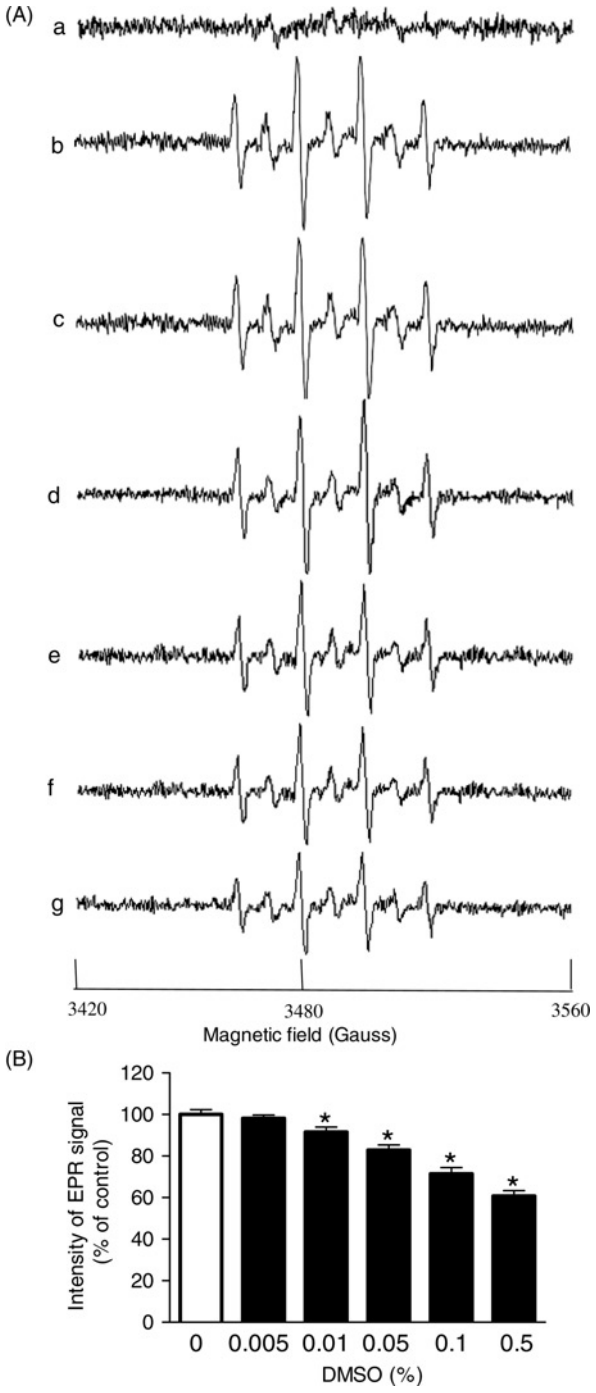

Figure 6 EPR evidence of the ability of DMSO in scavenging authentic peroxynitrite-mediated free radical generation. EPR spectroscopy in combination with the spin probe DMPO and authentic peroxynitrite were used to examine the free radical scavenging ability of DMSO. EPR spectra were recorded for 400 s after the initiation of reaction containing 80 mmol/L DMPO and the following concentrations of peroxynitrite and DMSO: (A) line a, without peroxynitrite and DMSO; line b, 100 μmol/L peroxynitrite only (control); line c, 0.005% DMSO + 100 μmol/L peroxynitrite; line d, 0.01% DMSO + 100 μmol/L peroxynitrite; line e, 0.05% DMSO + 100 μmol/L peroxynitrite; line f, 0.1% DMSO + 100 μmol/L peroxynitrite; line g, 0.5% DMSO + 100 μmol/L peroxynitrite. The EPR spectrometer settings were as follows: 200 KHz, X band microwave frequency, 9.5 GHz; microwave power, 20 mW (milliWatts); modulation amplitude, 6.3 G (gauss); time constant, 160 s; scan time, 200 s; and receiver gain, 4×10^5. (B) Signal intensity at 3480 G was expressed as mean ± SEM from three separate experiments. *Significantly different from control. EPR, electron paramagnetic resonance; DMSO, Dimethyl sulfoxide; DMPO, 5,5-dimethylpyrroline-*N*-oxide; SEM, standard error of mean

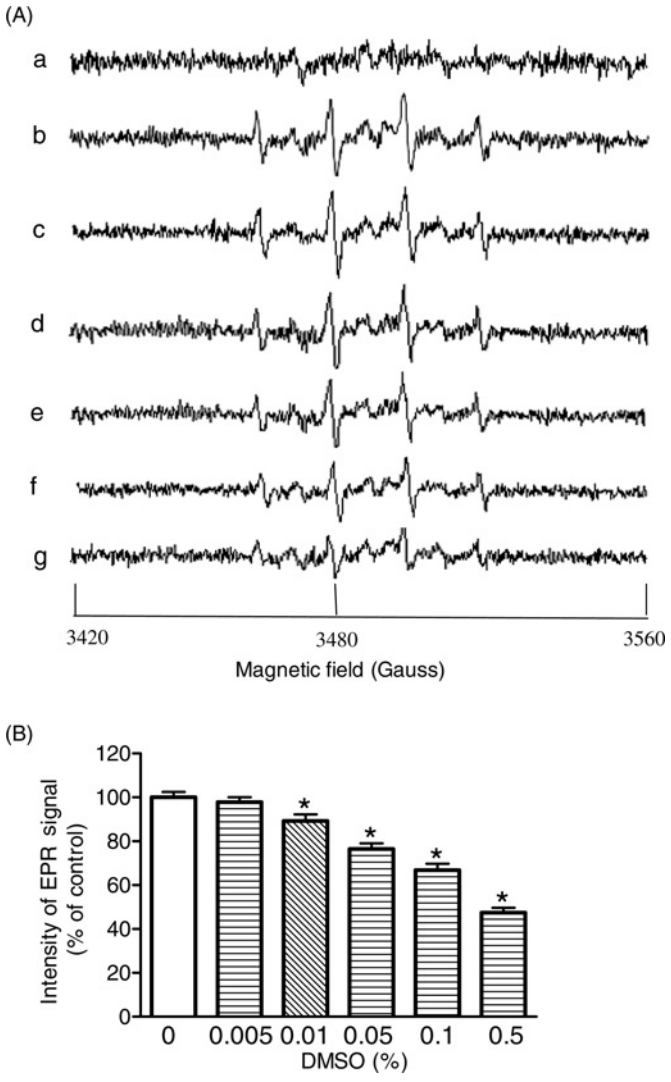

Figure 7 EPR evidence of the ability of DMSO in scavenging SIN-1-mediated free radical generation. EPR spectroscopy in combination with the spin probe DMPO and SIN-1 were used to examine the free radical scavenging ability of DMSO. Experimental conditions are described in the legend to Figure 6. EPR spectra were recorded for 400 s after the initiation of reaction containing 80 mmol/L DMPO and the following concentrations of SIN-1 and DMSO: (A): line a, without SIN-1 and DMSO; line b, 250 μmol/L SIN-1 only (control); line c, 0.005% DMSO + 250 μmol/L SIN-1; line d, 0.01% DMSO + 250 μmol/L SIN-1; line e, 0.05% DMSO + 250 μmol/L SIN-1; line f, 0.1% DMSO + 250 μmol/L SIN-1; line g, 0.5% DMSO + 250 μmol/L SIN-1. (B) Signal intensity at 3480 G was expressed as mean ± SEM from three separate experiments. *Significantly different from control. SIN, 3-morpholino-sydnonimine; EPR, electron paramagnetic resonance; DMPO, 5,5-dimethyl-pyrroline-*N*-oxide; DMSO, dimethyl sulfoxide

diseases.[37,38] Due to its potent oxidative properties, peroxynitrite is capable of reacting with a wide range of biological molecules. The peroxynitrite-mediated deleterious alterations include oxidation of thiol-containing biomolecules, oxidation and nitration of proteins, lipid peroxidation as well as base modifications and DNA single-stranded breaks.[19,39] It is well known that SIN-1 undergoes autooxidation at a physiological pH to produce equal molar nitric oxide and superoxide, which react at a diffusion-limited rate to form peroxynitrite. The generation of

peroxynitrite from SIN-1 auto-oxidation mimics the *in vivo* formation of peroxynitrite. Because peroxynitrite is highly unstable under the physiological pH, SIN-1 is widely used as a peroxynitrite generator in biological systems for studying the biological effects of peroxynitrite.[29,30] A significant formation of both open circular and linear forms of the plasmid DNA (Figure 1) indicated that SIN-1 was able to elicit both single- and double-stranded breaks in this plasmid DNA system. These observations are consistent with our previous report.[40]

In the presence of DMSO at the concentrations ranging from 0.005% to 0.5%, the SIN-1-mediated conversion of supercoiled DNA to open circular form was markedly reduced (Figure 2). This observation indicated that DMSO was able to protect against SIN-1-induced DNA strand breaks. Because auto-oxidation of SIN-1 is a critical step in the formation of peroxynitrite, one potential mechanism involved in the inhibitory effects of DMSO on SIN-1-induced DNA strand breaks might be that DMSO can directly inhibit the auto-oxidation of SIN-1, thereby diminishing peroxynitrite-induced DNA damage. To investigate this possibility, we determined the effects of DMSO on SIN-1-mediated oxygen consumption. DMSO (0.005–0.5%) showing protective effects on SIN-1-induced DNA damage (Figure 2) did not significantly affect the oxygen consumption, indicating that DMSO-mediated DNA protection was not due to its inhibition of SIN-1 auto-oxidation. One possible reason for this observation could be due to the less reactivity of DMSO with peroxynitrite generated from auto-oxidation of SIN-1, in contrast with the fact that DMSO is highly reactive with the yield of hydroxyl radicals produced in the decomposition of peroxynitrite. As shown in Figure 5, DMSO significantly inhibited single-strand breaks induced by authentic peroxynitrite in a concentration-dependent manner (0.005–0.5%), indicating direct protective effects of DMSO on peroxynitrite-mediated DNA cleavage.

The decomposition of peroxynitrite at physiological pH is known to yield free radicals, including the highly reactive hydroxyl radicals that critically participate in the induction of DNA damage.[41] Therefore, a possible mechanism underlying the inhibitory effects of DMSO on peroxynitrite-induced DNA strand breaks could be attributed to the diminished hydroxyl radical formation from peroxynitrite. Although it is well known that DMSO is a potent scavenger of hydroxyl radicals, the link and the mechanism by which peroxynitrite induces DNA strand breaks has not been well established. In the present studies, the formation of hydroxyl radicals from peroxynitrite as demonstrated by DMPO spin-trapping was remarkably reduced in the presence of DMSO (Figure 5), thereby supporting the above proposed link and the mechanism. Interestingly, Repine and colleagues[42] have reported that DMSO at 280 mmol/L (around 2%) completely eliminated the formation of DNA single-strand breaks by a chemical system (iron/hydrogen peroxide) that is believed to generate hydroxyl radicals. In contrast, DMSO prevented only 80% of the ionizing radiation-mediated DNA cleavage, indicating that hydroxyl radicals might only be responsible for 80% of the ionizing radiation-induced DNA strand breaks.[42] Consistent with this report, DMSO at concentrations higher than 0.01% significantly inhibited the hydroxyl radical signal heights of EPR spectrum in a concentration-dependent manner (Figures 6 and 7), indicating that the hydroxyl radicals could, at least partly, account for the protective role of DMSO against peroxynitrite-dependent DNA damage as shown in Figure 8. However, DMSO at 0.005% showing protective effects on SIN-1- and authentic peroxynitrite-induced DNA damage did not significantly affect the hydroxyl radical signal heights of EPR spectrum, indicating that mechanisms other than scavenging hydroxyl radicals might also be responsible for the protective effect of DMSO against peroxynitrite-induced DNA strand breaks. In addition to hydroxyl radicals, it has been suggested that the decomposition of peroxynitrite upon protonation also yields NO_2 radicals.[43] As such, it is possible that NO_2 radicals, which could be quenched by DMSO, might also play a role against peroxynitrite-induced DNA strand breaks. Further studies are essential in order to elucidate clearly the mechanisms of the protective effect of DMSO against peroxynitrite-induced DNA strand breaks.

It is important to note that the concentrations of DMSO (0.005–0.5%) used in our studies are significantly lower than those used in many applications, which have used DMSO as the vehicle control at the concentrations ranging from 0.02% up to 1% to dissolve entirely various water-insoluble drugs for studying their potentiating activity against oxidatively generated DNA damage.[20–23] These studies concluded that the protective effects against DNA damage were from the actions of drugs being investigated. However, as discussed above, the concentrations of DMSO used in those studies (0.02–1%) may, alone, be the cause of the protective effect on DNA damage. Thus, the absence of a complete understanding of the effects of DMSO precludes accurate conclusions, because any experimental artifacts caused by the vehicle control DMSO can lead to the erroneous interpretation of the action of drug administration.

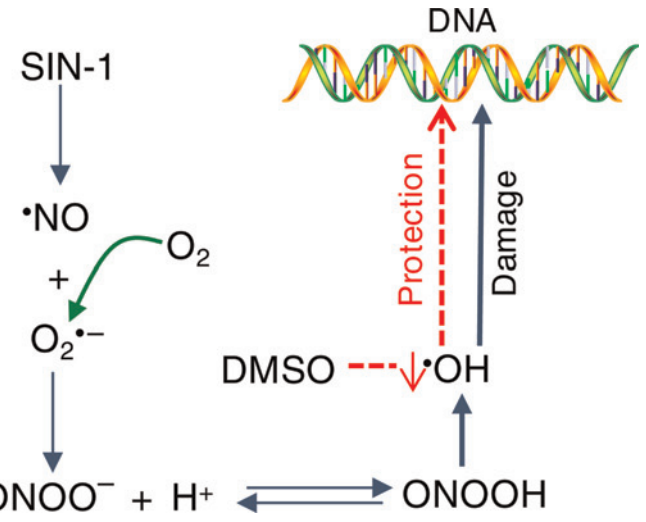

Figure 8 A schematic illustration of the possible mechanism of the protective effect of DMSO against peroxynitrite ($ONOO^-$)-induced DNA strand breaks. DMSO, dimethyl sulfoxide (A color version of this figure is available in the online journal)

In summary, our studies demonstrated for the first time that a DMSO concentration as low as 0.005% potently inhibits peroxynitrite-mediated DNA strand breaks (Figures 2 and 4), whereas DMSO at concentrations ranging from 0.02% up to 1% is often applied as a solvent for various water-insoluble drugs in many biological experiments. Therefore, the results of this study suggest that, where DMSO is applied as a solvent, caution should be observed when evaluating the actions of drugs in all experiments that involve DNA damage. As such, our studies could provide valid contributions toward avoiding and minimizing problems associated with the use of DMSO as a drug solvent that might otherwise confound the ability to evaluate accurately the actions of drugs.

Author contributions: ZJ contributed to literature search, figures, study design, data collection, data analysis, data interpretation and writing; HZ contributed to literature search, figures and data collection; YL contributed to study design, data analysis, figure and writing; and HPM contributed to data interpretation and data analysis.

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