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Production of Hydroxyl Free Radical in the Xanthine Oxidase System with Addition of 1-methyl-3-nitro-1-nitrosoguanidine

TOMIKO MIKUNI* and MASAHARU TATSUTA

Department of Gastrointestinal Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, 1-3-3, Higashinari-ku, Osaka 537-8511, Japan

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We have examined the mechanism of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG)-induced gastric cancer with respect to the production of hydroxyl free radical (·OH). Nucleophilic attack by H_2O_2 on the nitroso group of MNNG produces 1-methyl-3-nitroguanidine (MNG) and the intermediate peroxynitric acid (ONOOH), which splits into hydroxyl free radical (·OH) and nitrogen dioxide leading to the formation of nitric and nitrate ions in water. Xanthine oxidase (XO) induces the production of $O_2^{\cdot-}$ or H_2O_2 from molecular oxygen, depending on the overall level of enzyme reduction. In this study, we examined ·OH production by the reaction of MNNG with H_2O_2 derived from the XO–HX system containing XO and the purine substrate hypoxanthine by ESR using the spin trapping reagent 5,5′-dimethyl-1-pyrroline-*N*-oxide (DMPO). ·OH was produced in the XO–HX–DMPO system with addition of MNNG (the MNNG–XO–HX–DMPO system) under aerobic conditions, but was not in the XO–HX–DMPO system, and production of ·OH was inhibited by catalase but not by superoxide dismutase, suggesting that ·OH was produced by the reaction of MNNG with H_2O_2 derived from the XO–HX system. The production of ·OH was significantly increased with increase in the reducing activity of XO, though that of $O_2^{\cdot-}$ was not, also suggesting the $O_2^{\cdot-}$-independent ·OH production. The productions of nitrite ion and MNG in the MNNG–XO–HX system were determined by the colorimetric method and HPLC, respectively. Based on these findings, we conclude that ·OH was produced by homolytic split of the intermediate ONOOH formed by nucleophilic attack of H_2O_2 derived from the XO–HX system on MNNG.

Keywords: MNNG; Xanthine oxidase; Peroxynitrous acid; H_2O_2; ·OH; Gastric cancer

INTRODUCTION

MNNG (Fig. 1A) induces gastric cancer in rats within 1 year after its administration in drinking water.[1] Some investigators have suggested that methylation of nucleic acids or proteins by MNNG may be responsible for this carcinogenesis,[2,3] but such reactions cannot account for the entire mechanism of MNNG-induced carcinogenesis.[4] We have already found that butylated hydroxytoluene (BHT) inhibits the development of MNNG-induced gastric cancer,[5] and further that BHT scavenged free radicals produced by the reaction of hydrogen peroxide (H_2O_2) and MNNG.[6] We concluded that free radicals may be related to the carcinogenic activity of MNNG.[6] Among free radicals, ·OH results in mutagenic and carcinogenic damage to membrane lipids and nucleic acids.[7,8] We therefore decided to examine the mechanism of carcinogenesis by MNNG with respect to ·OH production. It has been reported that the nitroso nitrogen of MNNG is susceptible to nucleophilic attack to form MNG (Fig. 1B) and a labile nitroso compound intermediate, which homolyticaly splits into two free radicals,[9] and we have already reported the formation of ·OH and NO_2 by the reaction of MNNG with the nucleophilic reagent H_2O_2.[10] Xanthine oxidase (XO) induces the production of H_2O_2 and/or $O_2^{\cdot-}$ from molecular oxygen, depending on the overall

*Corresponding author. Fax: +81-6-6981-4067. E-mail: xmikuni@iph.pref.osaka.jp

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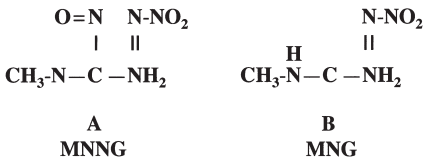

FIGURE 1 Chemical structures of 1-methyl-3′-nitro-1-nitrosoguanidine (MNNG, A) and 1-methyl-3′-nitro-guanidine (MNG, B).

level of enzyme reduction.[11] In inflammation lesions of the gastric tract in which cancers may be induced,[12] H_2O_2 and $O_2^{\cdot-}$ are readily formed, and XO is one of their important sources,[13–15] suggesting that administration of MNNG to lesions in the stomach with elevated XO activity results in gastric carcinogenesis. In the present study, we found using ESR spin trapping technique that $^{\cdot}OH$ was produced via the intermediate ONOOH formed by addition of MNNG to the XO–HX system (the MNNG–XO–HX system).

MATERIALS AND METHODS

Chemicals

XO (grade I) from buttermilk, hypoxanthine, xanthine, diethylenetriaminepentacetic acid (DTPA), copper–zinc superoxide dismutase from bovine erythrocyte (SOD), and catalase from mouse liver were purchased from Sigma Chemical, Co., (St. Louis, MO). MNNG was from Aldrich Chem. Co., (Milwaukee, WI), and the spin trapping reagent 5,5′-dimethyl-1-pyrroline-*N*-oxide (DMPO) from Labotec Co. (Tokyo, Japan). 1-Methyl-3-nitroguanidine (MNG) was prepared by the methods of McKay and Wright.[16] MNNG was dissolved in distilled water at a concentration of 12.5 mM in a test tube covered with aluminum foil to avoid its denaturation by light and stored at 5°C, and all procedures containing MNNG were performed in glassware covered with aluminum foil. One unit of the enzyme is defined as the amount of enzyme that catalyzes an increase in absorbance of 0.001/30 min at 290 nm with xanthine as a substrate.[17] Although catalase is often contaminated by SOD,[18] the catalase used in our experiments did not affect the production of $O_2^{\cdot-}$ in the XO–HX-DMPO system. The concentration of DMPO stock solution was determined from its optical absorbance ($\varepsilon_{234} = 7700\,M^{-1}\,cm^{-1}$).[19] The distilled water used in the experiments and stock solutions of 500 mM sodium phosphate buffer were passed through columns of Chelex-100 resin to reduce polyvalent metal ion impurities.

$^{\cdot}OH$ Production

A typical experiment was carried out as follows. The reaction mixture (the MNNG–XO–HX–DMPO system) contained, in a final volume of 100 μl, 50 mM sodium-phosphate buffer (pH 7.4), 0.03 mM DTPA, 0.15 mM hypoxanthine, 300 mM DMPO, 1.25 mM MNNG, and 2.9 mu/ml XO. Reactions were initiated by adding XO. The mixture was vortexed briefly and transferred rapidly to a quartz flat cell. Unless otherwise noted, the ESR spectrum was recorded 1.5 min after the reaction was started. The hyperfine splitting constant of the signal obtained from the MNNG–XO–HX–DMPO system was compared with that originating from $^{\cdot}OH$ added to DMPO.[20] The conditions for obtaining ESR spectra were as follows: ESR spectrometer, model JES-FEIIX (JEOL, Tokyo, Japan); field modulation, 100 kHz; magnetic field, 329 ± 10 mT; microwave power, 10 mW; modulation amplitude, 0.1 mT; scan time, 2 min; response, 1 s; amplitude of gain, 1×10^3 and room temperature.

The production of $^{\cdot}OH$ in the MNNG–XO–HX–DMPO system was confirmed by addition to the system of ethanol (800 mM) as a $^{\cdot}OH$ scavenger.[21] The effect of molecular oxygen on $^{\cdot}OH$ production was examined by comparison of the systems under aerobic conditions with those in which argon gas was bubbled for 3 min to replace the air in the reaction mixture before addition of XO to the systems. The production of $O_2^{\cdot-}$ in the XO–HX–DMPO system was examined by addition of SOD at 20 u/ml to the system. The effects of MNNG and XO on the production of $^{\cdot}OH$ were examined varying the concentration of MNNG between 0 and 1.25 mM at constant XO activity (2.9 mu/ml) and varying the XO activity between 0 and 2.9 mu/ml at constant concentration of MNNG (1.25 mM), respectively. The time course of production of $^{\cdot}OH$ in the MNNG–XO–HX–DMPO system was examined every 0.75 min from 1.5 until 3.75 min after start of the reaction.

Effects of H_2O_2 and $O_2^{\cdot-}$ on the Production of $^{\cdot}OH$

Effects of H_2O_2 and $O_2^{\cdot-}$ on the production of $^{\cdot}OH$ were examined in the MNNG–XO–HX–DMPO system with addition of catalase or superoxide dismutase (SOD) as scavengers of H_2O_2 and $O_2^{\cdot-}$, which were each used at a concentration of 500 units/ml, respectively. Further, to confirm that $^{\cdot}OH$ production was independent on that of $O_2^{\cdot-}$, it was examined whether the production of $O_2^{\cdot-}$ was increased with increase in the XO activity from 2.1 to 2.9 mu/ml when the production of $^{\cdot}OH$ was increased with the increase in the XO activity in the MNNG–XO–HX–DMPO system keeping the concentration of MNNG constant. The amounts of

DMPO adducts of ·OH (DMPO-(·OH)) and $O_2^{\cdot-}$ (DMPO-(·OOH)) were, respectively calculated as the relative intensity of the signal that appeared in the same magnetic field as the second peak of DMPO-(·OH) and as the first peak of DMPO-(·OOH) which was affected by the signal of ·OH less than any other peaks of $O_2^{\cdot-}$, from the lower field using Mn^{2+} as a standard.

Nitrite Ion Production

Since the intermediate ONOOH rapidly decays to form ·OH and NO_2, which reacts immediately with water to produce nitrite and nitrate ions,[21] the production of NO_2 was examined by the detection of nitrite ion with a slight modification of the diazotization procedure described previously.[10] That is, 1 ml of 50 mM sodium phosphate buffer at pH 5.0, at which MNNG is stable (half-life: 50 h),[22] was added to 100 μl of the MNNG–XO–HX system 1.5 min after start of reaction to stop the reaction, and absorbance at 540 nm was measured in a spectrophotometer (Model 200-20; Hitachi Ltd., Tokyo) after 300 μl of the colorimetric reagent of 3% ammonium amidosulfate plus 0.13% N-1-naphthylethylenediamine dihydrochloride plus 3.3% metaphosphoric acid was also added to the MNNG–XO–HX system. The standard curve was prepared using 5 μg/ml sodium nitrite as a standard solution. The effects of MNNG and XO on the production of nitrite ion were, respectively examined by varying the concentration of MNNG between 0 and 1.25 mM with the activity of XO kept constant, and by varying the XO activity between 0 and 2.9 mu/ml with the concentration of MNNG kept constant. The time course of production of nitrite ion was measured every 45 s after start of the reaction.

Identification of MNG

The production of MNG in the MNNG–XO–HX system was examined by HPLC. That is, 20 μl of the system containing, 1.25 mM MNNG, 2.9 mu/ml XO plus 0.15 mM hypoxanthine plus 50 mM phosphate buffer (pH 7.4), and a 20 ml aliquot of phosphate buffer containing 1.25 mM MNNG or MNG were injected 2 min after start of the reaction. HPLC analysis was performed using an LKB 2150 HPLC pump (Tosoh Co, Tokyo, Japan), an LKB controller (Tosoh), and a 4.6 mm × 15 cm TSKgel ODS-120A (Tosoh) at a flow rate of 1 ml/min. The eluent consisted of a 95:5 (V/V) mixture of 10 mM potassium phosphate buffer (pH 7.4) and methanol. Absorption was measured at 280 nm by a Toso UV-8010 detector.

Statistical Analysis

Values are expressed as means ± S.E., and results were analyzed by Student's *t*-test.[23] Findings were considered significant when *p*-values were less than 0.05.

RESULTS

·OH Production

The ESR spectrum of the MNNG–XO–HX–DMPO system obtained immediately after start of the reaction is shown in Fig. 2A. The spectrum consists of a 1:2:2:1 quartet with hfcs of $A_{NO}^{H} = A_{\beta}^{H} = 1.48$ mT, identical to those previously reported for DMPO-(·OH),[20] but did not include the signal of DMPO-(·OOH). Further evidence of ·OH production was obtained on addition of ethanol to the MNNG–XO–HX–DMPO system (Fig. 2B). A signal corresponding to α-hydroxyethyl radical adduct was detected, indicating that DMPO-(·OH) signal had been formed from the direct reaction of ·OH but not from the breakdown of DMPO-(·OOH), since α-hydroxyethyl radical was generated via the reaction between ·OH and ethanol. The signal of ·OH was inhibited by replacement of air gas by argon in the MNNG–XO–HX–DMPO system (Fig. 2C), indicating that ·OH production was induced from molecular oxygen. The spectrum of the XO–HX–DMPO system in the absence of MNNG showed the signal of DMPO-(·OOH) (Fig. 2D), and on addition of SOD to the XO–HX–DMPO system, all signals

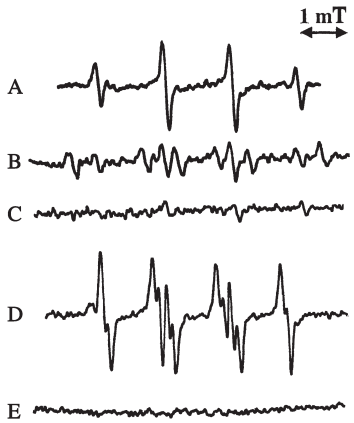

FIGURE 2 Typical ESR spectra of the MNNG–XO–HX–DMPO system. A: the MNNG–XO–HX–DMPO system exhibiting DMPO-(OH) signal but not DMPO-(OOH) signal; B: MNNG–XO–HX–DMPO system with addition of ethanol exhibiting signal for DMPO-(CH(CH$_3$)OH) superimposed on that for DMPO-(·OH); C: MNNG–XO–HX–DMPO system after bubbling of argon gas, exhibiting disappearance of the signal for OH; D: XO–HX–DMPO system exhibiting signal for DMPO-(·OOH); E: XO–HX–DMPO system with addition of SOD, the XO–MNNG–DMPO system in the absence of the substrate, and further the MNNG–HX–DMPO system in the absence of MNNG served as controls.

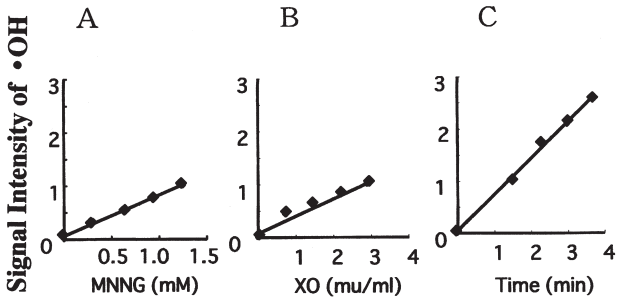

FIGURE 3 A: Effect of MNNG at concentrations from 0 to 1.25 mM, keeping XO activity constant; B: Effect of XO at activities from 0 to 2.9 mu/ml, keeping the concentration of MNNG constant; C: time courses of production of ·OH at 1.25 mM MNNG and at 2.9 mu/ml XO in the MNNG–XO–HX–DMPO system. The signal intensity of ·OH trapped by DMPO was measured every 45 or 90 s after start of the reaction by addition of XO to the system. Values are means of results of triplicate experiments.

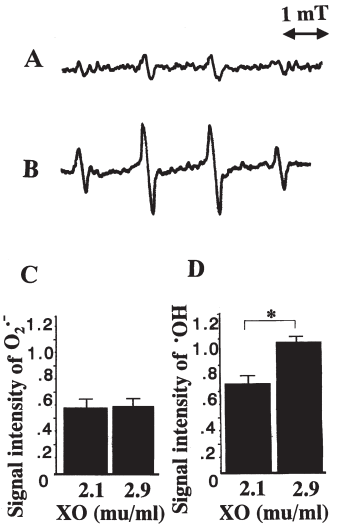

FIGURE 4 H_2O_2-dependent but $O_2^{\cdot-}$-independent ·OH production. A: MNNG–XO–HX–DMPO system with addition of catalase. Compared to the intensity of the signal of DMPO-(OH) in Fig. 1A, that in the MNNG–XO–HX–DMPO system with addition of catalase was extremely decreased; B: MNNG–XO–DMPO system with addition of SOD. The intensity of the signal of DMPO-(OH) in the MNNG–XO–HX–DMPO system (Fig. 1A) was not affected by addition of SOD to the this system; C: Effect of variation of XO activity from 2.1 to 2.9 mu/ml on production of $O_2^{\cdot-}$ in the XO–HX–DMPO system, and D: Effect of variation of XO activity from 2.1 to 2.9 mu/ml on production of ·OH in the MNNG–XO–HX–DMPO system, with the concentration of MNNG kept constant. The relative signal intensities of $O_2^{\cdot-}$ were compared in the XO–HX–DMPO systems containing XO activities of 2.1 and 2.9 mu/ml, and those of ·OH were compared in the MNNG–XO–HX–DMPO system containing XO activities of 2.1 and 2.9 mu/ml. Bars show S.E. of five experiments. Significant difference in values between the MNNG–XO–HX–DMPO systems containing XO at 2.1 and 2.9 mu/ml: $^*p = 0.011$.

disappeared, indicating that the signal of DMPO-(·OOH) alone was detected in the XO–HX–DMPO system, but any other signal was not superimposed on that of DMPO-(·OOH) (Fig. 2E). The spectra of the DMPO solutions containing MNNG plus XO in the absence of the substrate HX and containing MNNG plus HX in the absence of the enzyme included no signals (Fig. 2E). These results suggested that ·OH was produced by the reaction of MNNG and the oxygen product induced from the XO–HX system.

MNNG-, XO-, and Reaction Time-dependent ·OH Production

As shown in Fig. 3A,B, in the MNNG–XO–DMPO system the production of ·OH was increased with increase in the concentration of MNNG for concentrations from 0 to 1.25 mM keeping XO activity constant (2.9 mu/ml) and also increased with increase in XO activity for activities from 0 to 2.9 mu/ml keeping the concentration of MNNG constant (1.25 mM). Further, Fig. 3C shows that the production of ·OH in the MNNG–XO–HX–DMPO system linearly increased with increase in reaction time for 3.75 min from start of the reaction. These results again suggested that ·OH was produced by the reaction of MNNG and the product derived from the XO–HX system.

H_2O_2-dependent but $O_2^{\cdot-}$-independent ·OH Production

From which of the oxygen products in the XO–HX system, H_2O_2 or $O_2^{\cdot-}$, ·OH was derived was examined using the scavenging enzymes catalase and SOD. With addition of catalase to the MNNG–XO–HX–DMPO system, the signal intensity of ·OH was extremely decreased (Fig. 4A), but there was no effect on ·OH production by addition of SOD to this system (Fig. 4B), suggesting that the production of ·OH was derived from H_2O_2 but not from $O_2^{\cdot-}$. As already described, the production of $O_2^{\cdot-}$ is kept constant but that of H_2O_2 is increased with increase in reducing activities of XO at high reducing activities of XO. Then to confirm that ·OH production was independent on that of $O_2^{\cdot-}$ derived from the XO–HX system, whether production of $O_2^{\cdot-}$ was kept constant but that of ·OH was increased when the reducing activity of XO was increased. As shown in Fig. 4C,D, when the reducing activity of XO was increased from 2.1 to 2.9 mu/ml, the production of $O_2^{\cdot-}$ in the XO–HX–DMPO system was not significantly increased, however, that of ·OH in the MNNG–XO–HX–DMPO system at a constant concentration of MNNG was significantly increased, suggesting that the production of ·OH was independent on that of $O_2^{\cdot-}$. Based on these findings, we concluded that ·OH was produced by the reaction of MNNG with H_2O_2 but not with $O_2^{\cdot-}$ derived from the XO–HX system.

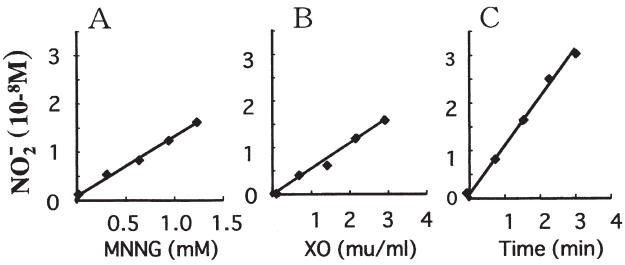

FIGURE 5 A: Effect of MNNG on production of nitrite ion of variation of concentration of MNNG between 0 and 1.25 mM, keeping XO activity constant, B: Effect of XO on production of nitrite ion of variation of reducing activity of XO between 0 and 2.9 mu/ml, keeping the concentration of MNNG constant, and C: the time course of changes in amount of nitrite ion in the MNNG–XO–HX system. Values are means of results of triplicate experiments.

MNNG-, XO-, and Reaction Time-dependent Nitrite Ion Production

We examined the effects of the concentration of MNNG, the reducing activity of XO, and time of reaction on the formation of peroxynitrous acid (ONOOH) by detection of nitrite ion in the MNNG–XO–HX system. Fig. 5A,B show that in the MNNG–XO–HX system the amount of nitrite ion increased with increase in the concentration of MNNG, keeping XO activity constant, and also increased with increase in reducing activity of XO, keeping the concentration of MNNG constant. As shown in Fig. 5C, the production of nitrite ion was time-dependently increased in the MNNG–XO–XO system. These results suggested that nitrite ion was also produced by the reaction of MNNG with the product derived from the XO–HX system.

Identification of MNG

As shown in Fig. 6A,B, MNG was identified by HPLC in the MNNG–XO system but not in the MNNG solution, indicating that MNG was produced by the reaction of MNNG in the XO–HX system.

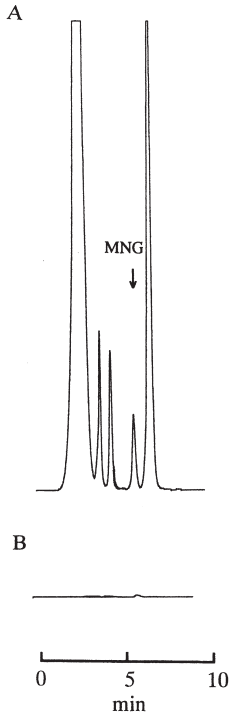

FIGURE 6 Detection of MNG with HPLC in the MNNG–XO–HX system. A, the MNNG–XO–HX system; B, MNNG solution.

DISCUSSION

The findings of the present study suggest that ·OH was produced via the intermediate ONOOH formed by the reaction of MNNG and H_2O_2 derived from the XO–HX system.

·OH alone was detected and $O_2^{\cdot-}$ was not in the MNNG–XO–HX–DMPO system in the presence of MNNG under aerobic conditions, and the production of ·OH was inhibited by catalase but not by SOD, suggesting that ·OH was derived from H_2O_2 but not from $O_2^{\cdot-}$ in the MNNG–XO–HX–DMPO system. Though Kuppusamy and Zweier[24] using ESR, reported the direct production of ·OH as well as $O_2^{\cdot-}$ in the XO–HX–DMPO system, in our study, on addition of SOD to the XO–HX–DMPO system, all signals in the system disappeared, indicating that ·OH was produced by the MNNG–XO–HX–DMPO system in the presence of MNNG but not directly produced by the XO–HX–DMPO system. We have already reported that the NO group of MNNG is susceptible to nucleophilic attack by H_2O_2 to form MNG and ONOOH, which is split into ·OH and NO_2 forming nitrate and nitrite ions with water. In the present study, we demonstrated MNNG- and XO-dependent production of ·OH and nitrite ion as well as the production of MNG, suggesting production of the intermediate ONOOH in the MNNG–XO–HX system. We therefore propose the following reactions for the production of ·OH in the MNNG–XO–HX system.

$$HX + O_2 + 2H_2O \xrightarrow{XO}, \text{ uric acid} + 2H_2O_2$$

$$CH_3N(NO)C(NH_2)NNO_2 + H_2O_2$$

$$\rightarrow CH_3NHC(NH_2)NNO_2 + ONOOH$$

$$ONOOH \longrightarrow, {}^{\cdot}OH + NO_2$$

$$2NO_2 + H_2O \longrightarrow, NO_2^- + NO_3^- + 2H^+$$

$$CH_3N(NO)C(NH_2)NNO_2 : \text{MNNG}$$

$$CH_3NHC(NH_2)NNO_2 : \text{MNG}$$

Lawley[2] reported that nucleic acids are alkylated at specific points by the methyl group of MNNG. Moreover, Nagao *et al.*[3] reported that the formation and also persistence of O^6-alkylguanine appear to be correlated with carcinogenicity. However, Kleihues *et al.*[4] reported that the persistence of O^6-methylguanine in cerebral DNA of Mongolian gerbils is similar to that in rat brain, though Mongolian gerbis differ markedly from rats in susceptibility to the neuro-oncogenic effect of alkylnitrsoureas. Kleihues *et al.*[4] also suggested that the tissue-specific carcinogenicity of these compounds does not depend solely on the formation and persistence of O^6-alkylguanine in DNA. As noted above, we previously found that BHT inhibits the development of MNNG-induced gastric cancer in rats,[6] and, further, that it scavenges free radicals derived from the reaction of MNNG and H_2O_2.[7] Consistent with our reports, Timmins and Davies[25] demonstrated that all the peroxides in his study produced free radicals in keratinocytes, and that BHT, which are known to inhibit peroxidase-induced tumor promotion *in vivo*, decreased the amounts of radicals trapped and suggested that BHT partly inhibits carcinogenesis through its antioxidant activity. We therefore suggested that free radicals are related to the carcinogenic activity of MNNG, though Kong *et al.* reported that BHT inhibits tumor formation via the inducing activity of phase II detoxifying enzymes such as glutathione *S*-transferase and quinone reductase, which may protect cells against cellular injury.[26] In this study, we demonstrated the production of $^{\cdot}OH$ by the reaction of MNNG and H_2O_2 derived from the XO–HX system. In inflammatory lesions, which was induced by ischemia followed by reperfusion in the gastrointestinal tract, large amounts of H_2O_2 as well as $O_2^{\cdot-}$ are readily formed by the enzymatic machinery available, and XO is one of their important sources.[13–15] We therefore suggest that MNNG reacts with H_2O_2 derived from the XO–HX system containing a high level of XO activity in inflammatory lesions to form $^{\cdot}OH$ exceeding the capacity of antioxidant defenses, thereby damaging DNA and resulting in carcinogenesis. Perry *et al.*[15] reported that the level of XO in the antrum was twice that in the corpus during postischemic reperfusion in the stomach of rats, and also that the concentration of XO in lesions following postischemic reperfusion of the stomach (5.7 mu/mg tissue $\times 10^3$) was close to that used in this study. In addition, Sugimura *et al.* reported that MNNG-induced gastric cancers often occur in the antrum and rarely in the corpus in rats.[1] These reports support our hypothesis that MNNG reacts with H_2O_2 derived from the high reducing activity of the XO–HX system to form $^{\cdot}OH$, resulting in the gastric carcinogenesis. High catalase activity was required for scavenging enzymes in our study, and Kuppusamy and Zweier[24] already demonstrated the production of H_2O_2 in the XO–HX system with catalase at the same or ten times the activity we used. Catalase might be difficult to attack on H_2O_2 produced in the XO–HX system. Further we suggest the reason that MNNG does not form $^{\cdot}OH$ via its reaction with $O_2^{\cdot-}$ though the signal of DMPO–($^{\cdot}OOH$) disappeared in the MNNG–XO–HX–DMPO system as follows; Nitroso group of MNNG would react as a spin trap with $O_2^{\cdot-}$. The nitroso spin traps, 2-methyl-2-nitrosopropane (MNP) and 3,5-dibromo-4-nitrosobenzenesulfonic acid (DBNBS), react much more rapidly with $O_2^{\cdot-}$ ($4.4 \times 10^7\,M^{-}\,s^{-1}$)[27] than does DMPO ($10\,M^{-1}\,s^{-1}$),[28] but do not form stable spin adducts.[27] The nitroso group of MNNG could easily out compete DMPO for $O_2^{\cdot-}$, but apparently does not form a stable adduct nor does this adduct decay to $^{\cdot}OH$.

These results suggest that when MNNG is given to rats in drinking water, $^{\cdot}OH$ is formed by the reaction of MNNG and H_2O_2 derived from the XO system containing hypoxanthine or xanthine in inflammatory lesions, exceeding the capacity of the antioxidant defense, and induces damage to DNA resulting in gastric carcinogenesis. Further study will enable more precise characterization of the mechanism of gastric carcinogenesis induced by the administration of MNNG to rats in drinking water.

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