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MECHANISM OF DNA STRAND BREAKS BY MITONAFIDE, AN IMIDE DERIVATIVE OF 3-NITRO-1,8-NAPHTHALIC ACID

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Abstract—The metabolism and the mechanism of action of 5-nitro-2-(2-dimethylaminoethyl)-benzo(de) isoquinoline-1,3-dione (mitonafide), a nitro-containing antitumor drug, have been studied. Incubation of mitonafide under anaerobic conditions with rat liver microsomes and NADPH formed the fully reduced amine metabolite, 5-aminomitonafide. The formation of the amine metabolite was not inhibited by SKF-525A, metyrapone or piperonyl butoxide, indicating that the cytochrome P-450 was not involved in this reduction. Incubation of mitonafide with rat liver microsomes and NADPH under aerobic conditions stimulated oxygen consumption; piperonyl butoxide, SKF-525A, superoxide dismutase and catalase had no effect on this stimulation. Both mitonafide and 5-aminomitonafide were found to bind to DNA in a similar manner. However, in inducing single-stand breaks in the DNA of L1210 cells mitonafide was 10-fold more potent than 5-aminomitonafide. These results suggest that metabolic activation of mitonafide to species other than that of the amine metabolite may play a significant role in the induction of DNA damage and the biological activity of the drug.

Imide derivatives of 3-nitro-1,8-napthalic acid have been shown to be cytotoxic to numerous tumor cell lines *in vitro* [1]. 5-Nitro-2-(2-dimethylaminoethyl)-benzo(de)isoquinoline-1,3-dione (mitonafide, Fig. 1) is one such example of this class of anticancer agents [2]. Mitonafide has been reported to bind to double-stranded DNA through intercalation [3], to inhibit DNA synthesis, and to induce single-strand breaks in the DNA of Chinese hamster ovary cells [4]. Nishio and Uyeki [4] have suggested that mitonafide does not require metabolic activation in order to disrupt DNA synthesis and induce DNA damage.

Nitro-containing compounds such as nitroaromatics and nitroheterocyclics undergo reductive metabolism, and the reduction of the nitro group is believed to play a major role in their antitumor activity and toxicity [5–9]. Thus, it seems possible that bioactivation of mitonafide to toxic intermediates may play a role in the induction of DNA damage. We have, therefore, studied the metabolism of mitonafide under both aerobic and anaerobic conditions in the presence of rat liver microsomes and NADPH. We have identified 5-aminomitonafide (Fig. 1) as a metabolite of mitonafide and compared the relative ability of the two compounds to bind to, and induce single-strand breaks in, the DNA of L1210 leukemia cells.

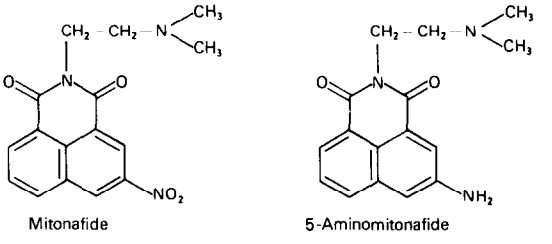

Fig. 1. Structures of mitonafide and 5-aminomitonafide.

MATERIALS AND METHODS

Mitonafide (NSC 300288) and 5-aminomitonafide (NSC 308847) were obtained from the Drug Synthesis Branch, National Cancer Institute, NIH, Bethesda, MD. NADP, NADPH glucose-6-phosphate, glucose-6-phosphate dehydrogenase (torula

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yeast), calf thymus DNA (highly polymerized, type IV), superoxide dismutase (SOD, bovine blood) catalase, Proteinase K (*Tritirachium album*), and *N*-2-hydroxyethylpiperazine-*N*-2-ethansulfonic acid (HEPES) were obtained from the Sigma Chemical Co., St. Louis, MO. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) and metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone) were obtained from Aldrich Chemicals, Milwaukee, WI. SKF-525A (β-diethyl-aminoethyl-diphenylpropyl acetate · HCl) was a gift from Smith, Kline & French, Inc., Philadelphia, PA. Piperonyl butoxide was obtained from Fluka Chemicals. [^{14}C]Thymidine (56 mCi/mmole) was purchased from New England Nuclear, Boston, MA. Polycarbonate filters were obtained from Nuclepore, Pleasanton, CA, and tetrapropylammonium hydroxide was from the RSA Corp., Elmsford, NY.

Rat liver microsomes and phenobarbital-induced (PB, 80 mg/kg, i.p. × 3 daily) liver microsomes were prepared from male rats (Sprague–Dawley) according to a published method [10]. Food and water were provided *ad lib*. The protein concentration was determined according to the method of Sutherland *et al.* [11] with bovine serum albumin as standard. Oxygen uptake studies with mitonafide and 5-aminomitonafide were carried out with a Clarke-type electrode at 37° in 150 mM KCL-50 mM Tris · HCl buffer (pH 7.4) as described previously [12].

The metabolic studies with mitonafide were carried out by incubating the drug (5 mM) with microsomes (4 mg/ml) in 150 mM KCl–10 mM Tris · HCl (pH 7.4) in the presence or absence of an NADPH-generating system (NADP 1.0 mM, glucose-6-phosphate 1 mM, and glucose-6-phosphate dehydrogenase 5.0 units/ml) at 37° in a total volume of 2.5 ml. The incubations were carried out under an atmosphere of 100% oxygen or nitrogen. At the end of the incubation (30 min), the mixtures were extracted with equal volumes of *n*-butanol (2 ml) and centrifuged (2000 *g*, 10 min). The *n*-butanol layer was collected and evaporated under a stream of nitrogen. The residues were then dissolved in 250 μl of methanol, and the metabolites were analyzed by gas chromatography/mass spectrometry (GC/MS) or high pressure liquid chromotography (HPLC). Identification of the 5-aminomitonafide metabolite of mitonafide was accomplished with a Rivermag R-10-10-C mass spectrometer equipped with a Series 32 Girdel gas chromatograph, Delsi Nermag, Houston, TX. Separation of the compounds contained in the microsomal extract was achieved with a 1.2 m × 1 mm i.d. glass column packed with 1% OV-7 on Supelcoport, Sueplco Inc., Bellefonte, PA. The column temperature was 250°, and helium at a flow rate of 20 ml/min was used as the carrier gas. Under these conditions, mitonafide and the 5-aminomitonafide metabolite eluted at 4.7 and 6.8 min respectively. Mass spectra were obtained using electron impact at 70 eV. The HPLC analysis for the separation and identification of the metabolites contained in the microsomal extracts was carried out with a Waters Associates liquid chromatograph containing a dual wavelength detector set at 254 and 420 nm for the detection of mitonafide and 5-aminomitonafide respectively. The chromatography was carried out with a μ-Bondapac Phenyl column using acetonitrile–water (20:80; adjusted to pH 4.0 with formic acid–ammonium hydroxide) as the mobile phase, and the flow rate was 2.0 ml/min. Under these experimental conditions, mitonafide and 5-aminomitonafide eluted at 11.6 and 5.9 min respectively.

The binding of mitonafide and 3-aminomitonafide to double-stranded DNA was examined spectrophotometrically (Cary model 14) according to the method described by Waring *et al.* [3]. The drugs were dissolved in dimethylformamide (DMF) and added to HEPES buffer containing 10 μM EDTA

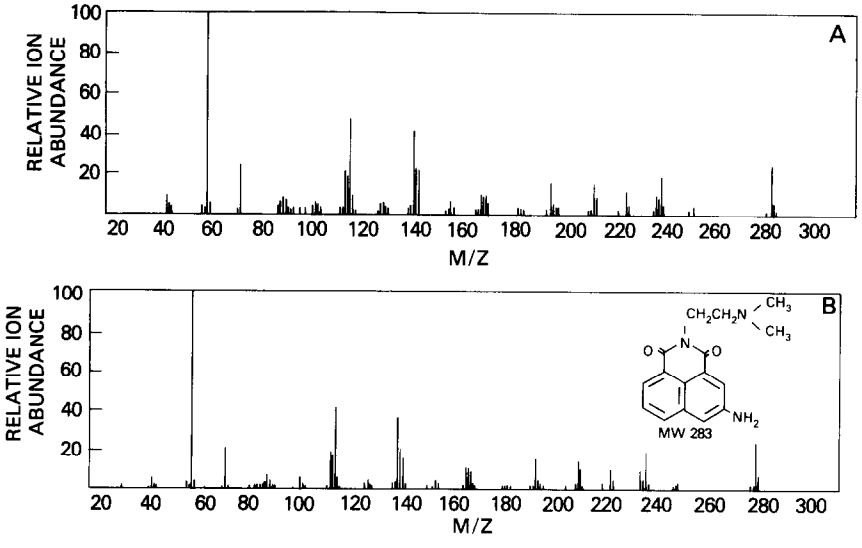

Fig. 2. Mass spectrum obtained from (A) mitonafide (5 mM) following incubation with the microsomes (4 mg/ml) in the presence of an NADPH-generating system under anaerobic conditions and (B) mass spectrum of the authentic sample of 5-aminomitonafide.

and 9.4 mM NaCl, pH 7.4, to obtain a final concentration of 50 μM. The final volume of DMF was 0.5%. The DNA concentration (nucleotide) was based on the molar extinction coefficient of 7000 at 260 mm [13]. The thermal denaturation studies were carried out as described previously [14, 15].

The electron spin resonance (ESR) studies were carried out on a Varian E-104 spectrometer operating at 9.5 GHz. Mitonafide (1–2 mM) was incubated with rat liver microsomes (2–4 mg/ml) and NADPH (1 mM) either aerobically or anaerobically in the KCl–Tris · HCl buffer and then was transferred to quartz flat cells; the resulting spectrum was recorded at 22°. The spin-trapping studies for the detection of superoxide and hydroxyl radicals were carried out aerobically as described above in the presence of DMPO (100 mM). DMPO, used in this study, was purified by two vacuum distillations.

The DNA of L1210 cells used in alkaline elution assays was radioactively labeled by growing 3×10^5 cells for 24 hr in the presence of [^{14}C]thymidine, 0.02 μCi/ml. The alkaline elutions for the detection of the single-stranded breaks in DNA induced by mitonafide, 5-aminomitonafide and X-ray were carried out as described previously [16, 17]. Briefly, after drug treatment cells were gently layered on 0.8 μm pore size polycarbonate filters. Immediately after layering, the cells were lysed with 5 ml of a

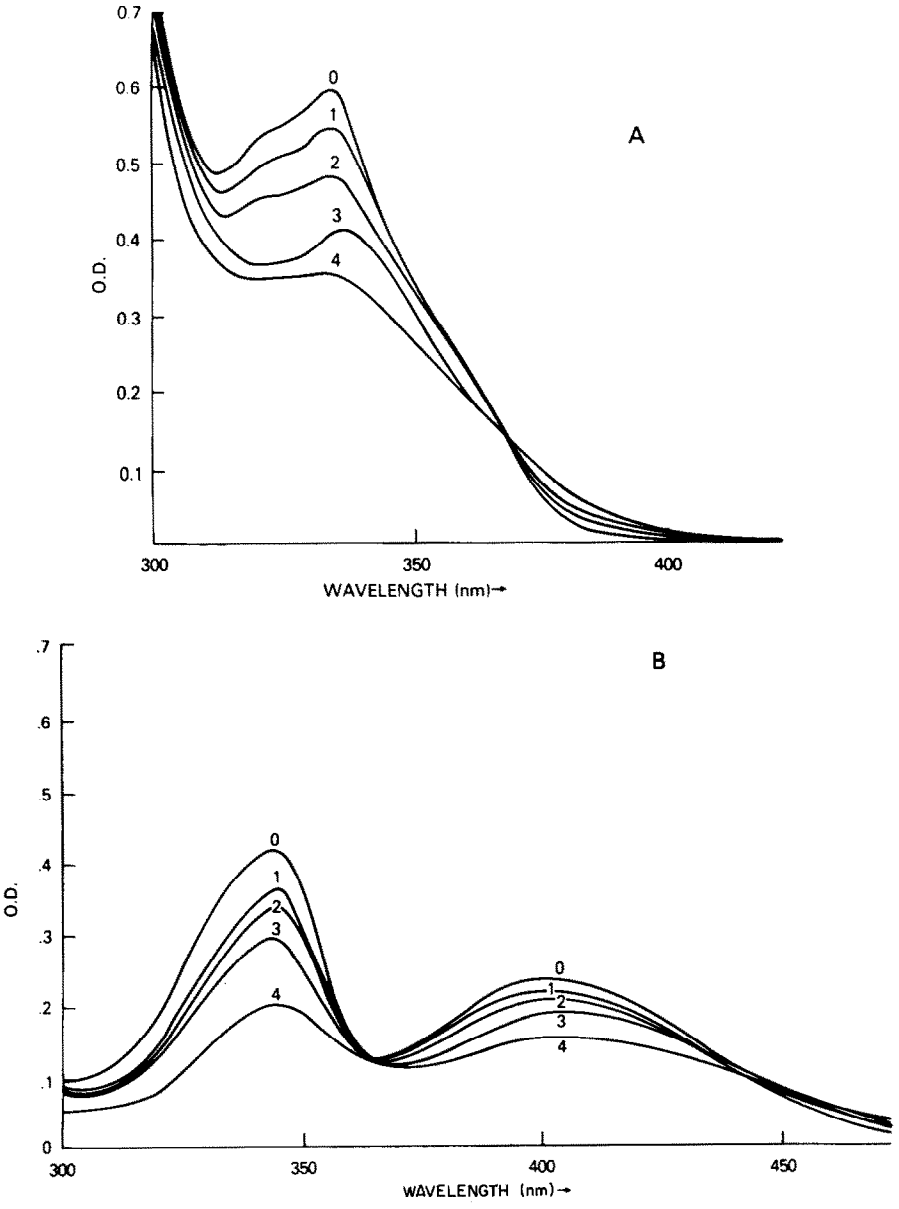

Fig. 3. Absorption spectra of mitonafide (A) or 5-aminomitonafide (B) (50 μM in HEPES buffer, pH 7.4) in the absence of DNA (curve 0) or with calf thymus DNA added at a final drug/nucleotide ratio of 1.0 (curve 1), 0.5 (curve 2), 0.33 (curve 3) and 0.1 (curve 4).

solution containing 2% sodium dodecyl sulfate, 0.02 M disodium EDTA, and 0.1 M glycine (pH 10). Following the lysis, 2 ml of 2% sodium dodecyl sulfate–0.02 M EDTA–0.1 M glycine, with or without 0.5 mg/ml proteinase K, was added to a reservoir over polycarbonate filters and pumped through the filter. DNA was eluted from filters by pumping 0.02 M EDTA solution adjusted to pH 12.1 with tetrapropylammonium hydroxide that contained 0.1% sodium dodecyl sulfate. Fractions were collected every 3 hr and processed as described [16]. The effects of oxygen on the DNA degradation in the presence of the drugs were examined as described by Potmesil *et al.* [18].

Table 1. T_m determination*

	T_m (°C)	ΔT_m (°C)
DNA	73.5 ± 0.5	
DNA + mitonafide	78.6 ± 0.5	5.1 ± 0.5
DNA + 5-aminomitonatide	78.0 ± 0.5	4.5 ± 0.5

* The drugs were used at a concentration of 1.0×10^{-5} M in a solution containing 30 μg/ml calf thymus DNA in 5 mM phosphate buffer containing 15 mM NaCl at pH 7.4. The melting temperature was determined at 260 nm by means of a Hewlett–Packard 8450A UV/Vis spectrophotometer with 891000A Hewlett–Packard temperature controller. The data represent the average of three determinations ± S.E.

RESULTS

The incubation of mitonafide with rat liver microsomes and an NADPH-generating system under anaerobic conditions resulted in the formation of a metabolite that was identified as 5-aminomitonafide by comparison with an authentic standard using GC-MS and HPLC analysis. Figure 2B is the electron impact mass spectrum obtained from reference 5-aminomitonafide and Fig. 2A is the electron impact mass spectrum following the incubation of mitonafide with microsomes that were incubated with mitonafide and NADPH. Both spectra show molecular ions at m/z 283. The most abundant ion, $C_3H_8N^+$, observed at m/z 58, results from β cleavage to the alkyl nitrogen and is characteristic of alkylamines. The ion fragments, $C_{14}H_{11}N_2O_2^+$, $C_{13}H_9N_2O_2^+$ and $C_{12}H_8N_2O_2^+$ observed at m/z 239, m/z 225 and m/z 212, respectively, are probably due to fragmentation of the alkylamine side chain. The formation of the amine required NADPH and the microsomes; SKF-525A (1 mM), piperonyl butoxide (1 mM) and metyrapone (1 mM), inhibitors of cytochrome P-450 [19, 20], did not inhibit its formation. When the incubations were carried out in the presence of oxygen, no amine could be detected.

Since intercalation of mitonafide into DNA has been reported to be involved in its biological activity [1, 4], we have examined whether the 5-aminomitonafide metabolite could also bind to DNA by a similar mechanism. The addition of calf thymus DNA to mitonafide caused a small bathochromic shift (Fig. 3A). More importantly, a large (50%) reduction in absorbance of the free drug was observed in the presence of excess DNA. In addition, an isobestic point at 374 nm was present, which represents the presence of two species of drugs [21], the free and the bound form. These results are identical to those obtained by Waring *et al.* [3]. Under the identical conditions, 5-aminomitonafide also showed a small shift to red in the presence of DNA (Fig. 3B). Furthermore, there was significant hypochromism (50%) at 342 and 405 nm in the presence of DNA, representing the free and bound drugs (Fig. 3B). Moreover, there were two isobestic points at 364 and 440 nm in the presence of DNA, representing the free and the bound drug.

The interactions of mitonafide and 5-aminomitonafide with DNA were also examined by evaluating the changes in the T_m (the temperature at which 50% hyperchromicity is induced by heat denaturation of native DNA). The thermal denaturation data (Table 1) show that each compound stabilized the DNA helix to the same extent ($\Delta T_m = 5.0°$). Thus, identical T_m values and similar spectroscopic behavior in the presence of DNA suggest that the binding of these compounds to DNA is similar.

The abilities of mitonafide and 5-aminomitonafide to induce DNA single-strand breaks in L1210 cells were examined using the alkaline elution technique [16, 17]. Results presented in Fig. 4A show that single-strand breaks in L1210 cells were induced by mitonafide in greater quantity than by 3000 rads of X-ray (Fig. 4C). In contrast, 5-aminomitonafide at equimolar concentrations was significantly less effective in inducing these breaks (Fig. 4B). Moreover, the DNA breaks induced by these compounds were not protein-associated since the presence of proteinase K had no effect on their frequency [16] (data not shown). This is interesting since intercalating agents have been shown to activate topoisomerase and induce mainly protein associated breaks [16]. Although the reasons are not clear, it may be that mitonafide and the amino compound, unlike other intercalating agents, do not bind sufficiently to topoisomerase to form the ternary, drug–topoisomerase–DNA, complex which is thought to be necessary for protein associated DNA breaks [22].

Since mitonafide is metabolized to 5-aminomitonafide only under anaerobic conditions, we have examined the ability of each compound to induce DNA damage in the presence and absence of oxygen. In the presence of oxygen, mitonafide induced significantly more single-strand breaks in DNA of L1210 cells than did 3000 rads of X-ray or 5-aminomitonafide (Fig. 5, A and B). Under anaerobic conditions, mitonafide also induced more DNA damage in L1210 cells than did 5-aminomitonafide (Fig. 6, A and B).

The incubation of mitonafide with rat liver microsomes in the presence of NADPH stimulated the oxygen consumption by 122% over the basal rate (Table 2). The addition of either piperonyl butoxide (1 mM) or SKF-525-A (1 mM), which are the inhibitors of cytochrome P-450 [19, 20], to the incubation mixtures had no effect upon oxygen consumption. Furthermore, neither superoxide dismutase nor catalase affected oxygen consumption by mitonafide.

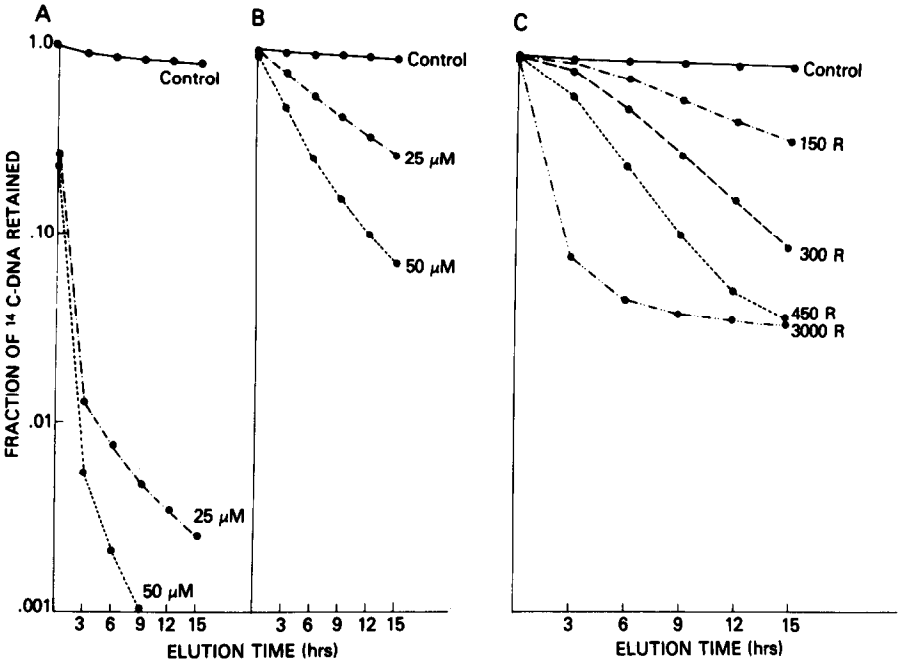

Fig. 4. DNA single-strand break frequency produced in L1210 cells in the absence of proteinase K by (A) mitonafide, (B) 5-aminomitonafide under identical conditions, and (C) X-ray. The cells were treated with various doses of X-rays or the drugs for 1 hr at 37°.

The 5-amino derivative, on the other hand, did not appreciably affect the basal rate. To understand this oxygen activation by mitonafide, spin-trapping studies were initiated. Aerobic incubation of mitonafide with microsomes and NADPH in the presence of DMPO as a spin-trap resulted in a 2-fold increase in the formation of a DMPO adduct over the basal values (data not shown). The hyperfine constants for the adduct were $a^N = 14.3G$; $a_\beta^H = 11.7G$; $a_\gamma^H = 1.2G$ which are characteristics [23] for the

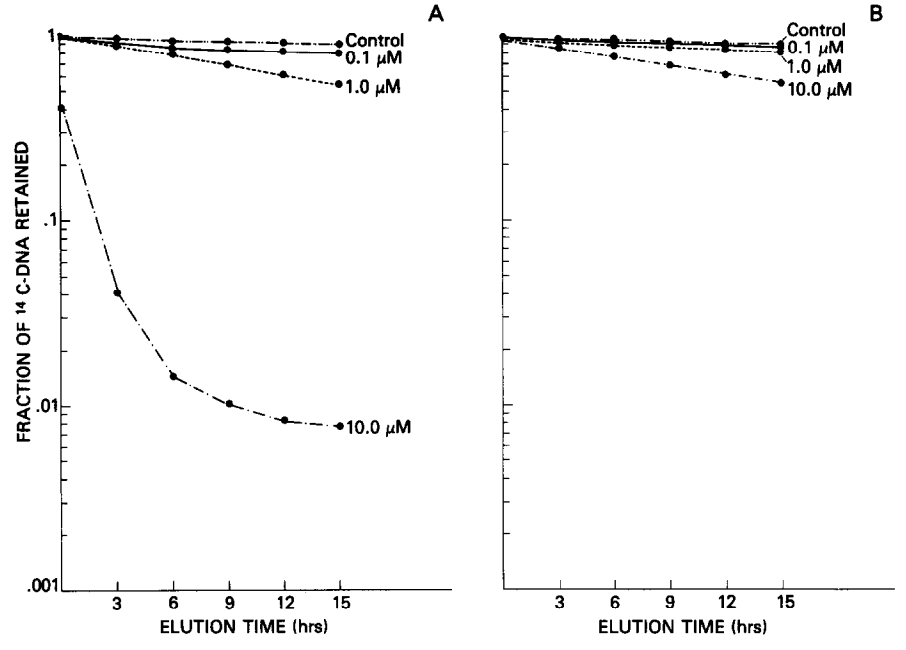

Fig. 5. DNA single-strand break frequency produced in L1210 cells under aerobic conditions by (A) mitonafide and (B) 5-aminomitonafide in the absence of proteinase K.

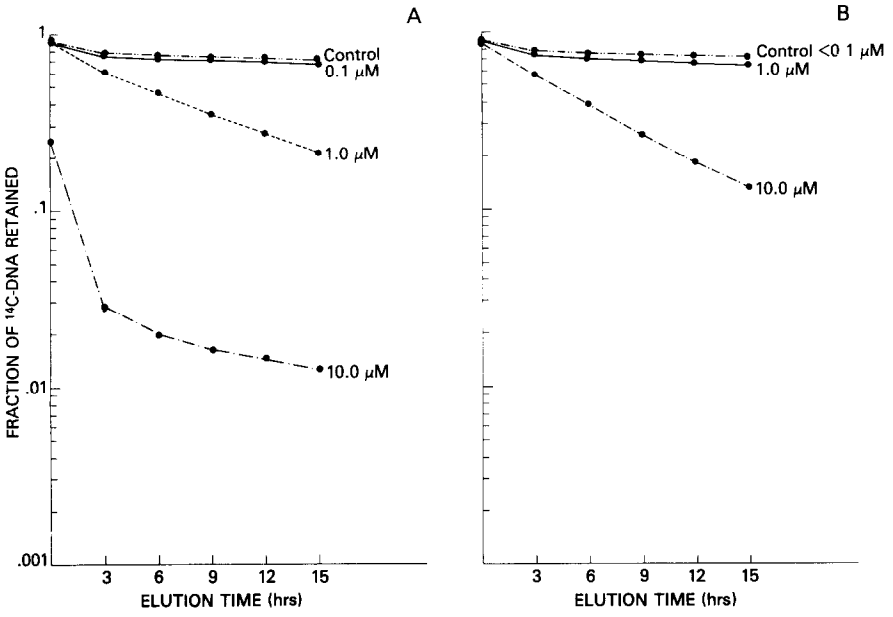

Fig. 6. DNA single-strand break frequency produced in L1210 cells under anaerobic conditions by (A) mitonafide and (B) 5-aminomitonafide in the absence of proteinase K.

DMPO adduct of superoxide (DMPOOOH) resulting from the trapping of $O_2^{\cdot}$ formed. The formation of $O_2^{\cdot}$ required the microsomes, NADPH and oxygen.

DISCUSSION

Previous studies of Waring *et al.* [3] have shown that mitonafide intercalates into DNA. Our studies show that 5-aminomitonafide also bound to double-stranded DNA and that the binding was similar to that of mitonafide. However, equimolar concentrations of mitonafide induced a greater quantity of single-strand breaks than did 5-aminomitonafide. It has been reported previously that the intercalative binding of mitonafide to DNA may be responsible for its ability to induce single-strand breaks [4]. Thus, we would have expected both mitonafide and 5-aminomitonafide to induce the same amount of single-strand breaks because they appear to bind to DNA to the same extent.

The difference in DNA damage caused by these two compounds may indicate that mitonafide is metabolized to other reactive species that are responsible for the increased DNA damage. Mitonafide at concentrations of 10 μM or greater produced a similar amount of DNA damage when L1210 cells were incubated either anaerobically or aerobically. However, at lower concentrations, mitonafide (1 μM)

Table 2. Effects of mitonafide on oxygen consumption by rat liver microsomes and NADPH*

Additions	O_2 uptake (nmoles/mg protein/min)
None	18.0 ± 3.0
Mitonafide	40.6 ± 2.0
Mitonafide + SKF-525A (1 mM)	41.0 ± 1.5
Mitonafide + piperonylbutoxide (1 mM)	39.5 ± 2.0
Mitonafide + superoxide dismutase (100 μg/ml)	38.3 ± 1.5
Mitonafide + catalase (100 μg/ml)	37.6 ± 1.8
5-Aminomitonafide	21.0 ± 3.0
Mitonafide + NADPH† (1 mM)	
Mitonafide + heat-denatured microsomes‡ + NADPH	

* Mitonafide (250 μM) and 5-aminomitonafide (250 μM) were incubated with rat liver microsomes (1 mg/ml) and NADPH (1 mM) at 37°. Oxygen uptake was determined as described in Materials and Methods. The values represent averages of triplicate incubations ± standard errors.

† Mitonafide was treated with NADPH in the absence of microsomes.

‡ The microsomes were heated to 80° for 20 min.

induced more DNA damage under hypoxia than in the presence of oxygen.

Our studies show that mitonafide stimulated oxygen consumption that was not inhibited by SKF-525A and piperonyl butoxide. This would suggest the involvement of NADPH-cytochrome P-450-reductase in the reduction of the nitro group of mitonafide and is consistent with that observed for other nitro compounds [24–27]. The stimulation of oxygen required the presence of both microsomes and NADPH. In contrast, 5-aminomitonafide did not appreciably affect the basal rate of oxygen consumption, indicating that the intact nitro group was essential to the formation of the reactive intermediate(s). Although the nitro-anion radical, the one-electron reduction product, is believed to be an obligate intermediate in the microsomal reduction pathway of nitro compounds [28–30] and in the catalytic formation of $O_2^{\cdot}$, we were unable to detect this anion radical through our ESR studies during either microsomal or xanthine-xanthine oxidase [32] activation of mitonafide. Thus, it appears that the mitonafide nitro-anion radical concentration is too low for ESR detection. Microsomal reduction of mitonafide in the presence of NADPH formed only the amine derivative which was not inhibited by inhibitors of cytochrome P-450 (e.g. SKF-525A, metyrapone, piperonyl butoxide), indicating that cytochrome P-450 was not involved in this reduction. Moreover, nitroso and the hydroxylamine derivatives were not detected by mass spectral analysis, suggesting that the nitroso and the hydroxylamine derivatives are of limited stability and may, possibly, bind to microsomal proteins or may undergo further metabolism to the amine. Since the radioactive mitonafide was not available, no covalent binding studies could be carried out. However, during the microsomal metabolism of mitonafide, a small portion of drug bound to the microsomal proteins (yellow color). The bound drug was not removed by exhaustive extractions with *n*-butanol and methanol-ethylacetate, suggesting that reactive intermediates (such as nitroso and hydroxylamine derivatives) formed during reductive activation of mitonafide were irreversibly bound to the microsomal proteins. Recently, Howard *et al.* [33] showed that *in vitro* enzymatic reduction of 1-nitropyrene resulted in the formation of a hydroxylamine derivative which bound to DNA.

Studies presented in this manuscript show that mitonafide was metabolized by rat liver microsomes to reactive intermediates. Since tumor cells also contain metabolizing enzymes, e.g. reductases capable of activating nitro groups [34], it is reasonable to suggest that the metabolic activation of mitonafide may be responsible for the DNA damage observed in L1210 cells. Under aerobic conditions, it is also possible that oxygen radicals formed during futile redox cycling of the nitroanion radical and other reactive intermediates capable of binding to the cellular macromolecules may contribute more to the significantly enhanced DNA damage than those observed with 5-aminomitonafide. The increased DNA damage observed under hypoxia may result from the increased rate of formation of reactive species resulting from the enzymatic reduction of the nitro group. In this regard, Mohindra and Rauth [35] reported that metronidazole and nitrofurazone are more toxic to mammalian cells under hypoxic conditions. Moreover, metabolites formed by partial reduction of nitrofuran were shown to bind to cellular proteins and to cause DNA strand breaks [36, 37]. McManus *et al.* [27] showed that bioactivation of misonidazole with rat liver microsoes resulted in the formation of metabolite(s) that bound covalently to the microsomal proteins. LaRusso *et al.* [38] reported the covalent binding of metronidazole to DNA during reduction with dithionite. Thus, increased DNA damage observed under hypoxia with mitonafide may then result from such binding of toxic intermediate to cellular macromolecules.

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REFERENCES

1. M. F. Brana, J. M. Castellano, A. Jimenez, A. Llombart, F. P. Rabadan, M. Roldan, C. Roldan, A. Santos and D. Vazques, in *Current Chemotherapy* (Proceedings of the Tenth International Congress of Chemotherapy, Zurich, Sept 18–23, 1977) (Eds. W. Siegenthaler and R. Luthry), Vol. 2, pp. 1216–7. American Society for Microbiology, Washington, DC (1978).
2. M. F. Brana, J. M. Catellano, C. M. Roldan, A. Santos, D. Vazquez and A. Jimenez, *Cancer Chemother. Pharmac.* **4**, 61 (1980).
3. M. J. Waring, A. Gonzalez, A. Jimenez and D. Vazquez, *Nucleic Acid Res.* **7**, 217 (1979).
4. A. Nishio and E. M. Uyeki, *J. natn. Cancer Inst.* **70**, 1097 (1983).
5. S. M. Cohen, E. Erturk, J. M. Price and G. T. Bryan, *Cancer Res.* **30**, 897 (1970).
6. D. R. McCalla and D. Voutsinos, *Mutation Res.* **26**, 3 (1974).
7. K. A. Kennedy, B. A. Teicher, S. Rockwell and A. C. Sartorelli, *Biochem. Pharmac.* **29**, 1 (1980).
8. P. D. Josephy and R. P. Mason, in *Bioactivation of Foreign Compounds* (Ed. M. W. Anders), pp. 451–483. Academic Press, New York (1985).
9. R. P. Mason, in *Free Radicals in Biology* (Ed. W. A. Pryor), Vol. 5, pp. 161–222. Academic Press, New York (1982).
10. T. E. Gram, C. L. Litterst and E. G. Mimnaugh, *Drug Metab. Dispos.* **2**, 254 (1974).
11. E. W. Sutherland, C. F. Cori, R. Hayes and N. S. Olsen, *J. biol. Chem.* **180**, 825 (1949).
12. B. K. Sinha and M. G. Cox, *Molec. Pharmac.* **17**, 432 (1980).
13. B. K. Sinha, *Chem. Biol. Interact.* **30**, 67 (1980).
14. V. E. Márquez J. W. Cranston, R. W. Ruddon and J. H. Burckhalter, *J. med. Chem.* **17**, 856 (1974).
15. B. K. Sinha, R. M. Philen, R. Sato and R. L. Cysyk, *J. med. Chem.* **20**, 1528 (1977).
16. L. A. Zwelling, S. Michaels, D. Kerrigan, Y. Pommier and K. W. Kohn, *Biochem. Pharmac.* **31**, 3261 (1982).
17. N. W. Gibson, L. C. Erickson and J. A. Hickman, *Cancer Res.* **44**, 1767 (1984).
18. M. Potmesil, S. Kirschenbaum, M. Israel, M. Levin, V. K. Khetarpal and R. Silber, *Cancer Res.* **43**, 3528 (1983).
19. M. R. Franklin, *Biochem. Pharmac.* **21**, 2387 (1972).
20. J. R. Bend, G. R. Hook, R. E. Easterling, T. E. Gram and J. R. Fouts, *J. Pharmac. exp. Ther.* **183**, 206 (1972).
21. R. W. Estabrook, J. Peterson, J. Baron and A. Hildebradt, in *Methods in Pharmacology* (Ed. C. F. Chignell), Vol. 2, pp. 303–50. Plenum Press, New York (1972).

22. K. M. Tewey, T. C. Rowe, L. Yang, B. D. Halligan and L. F. Liu, *Science* **226**, 466 (1984).
23. E. Finkelstein, G. M. Rosen, E. J. Rauckman and J. Paxton, *Molec. Pharmac.* **16**, 676 (1979).
24. E. Perez-Reyes, B. Kalyanaraman and R. P. Mason, *Molec. Pharmac.* **17**, 239 (1980).
25. C. Y. Wang, B. C. Behrens, M. Ichikawa and G. T. Bryan, *Biochem. Pharmac.* **23**, 3395 (1974).
26. N. Harda and T. Omura, *J. Biochem.* **87**, 1539 (1980).
27. M. E. McManus, M. A. Lang, K. Stuart and J. Strong, *Biochem. Pharmac.* **31**, 547 (1981).
28. R. P. Mason and J. L. Holtzman, *Pharmacologist* **16**, 277 (1974).
29. R. P. Mason and J. L. Holtzman, *Biochemistry* **14**, 1626 (1975).
30. R. DoCampo, R. P. Mason, C. Motly and R. P. A. Muniz, *J. biol. Chem.* **256**, 10930 (1981).
31. R. P. Mason and J. L. Holtzman, *Biochem. biophys. Res. Commun.* **67**, 1267 (1975).
32. P. D. Josephy, B. Palcic and L. D. Skarsgard, *Biochem. Pharmac.* **30**, 849 (1981).
33. P. C. Howard, R. H. Heflich, F. E. Evans and F. A. Beland, *Cancer Res.* **43**, 2052 (1983).
34. S. R. Keyes, P. M. Fracasso, D. C. Heimbrook, S. Rockwell, S. G. Sligar and A. C. Sartorelli, *Cancer Res.* **44**, 5638 (1984).
35. J. K. Mohindra and A. M. Rauth, *Cancer Res.* **36**, 930 (1976).
36. D. R. McCalla, P. L. Olive, Y. Tu and M. Fan, *Can. J. Microbiol.* **21**, 1484 (1975).
37. P. L. Olive and D. R. McCalla, *Chem.Biol. Interact.* **16**, 223 (1977).
38. N. F. LaRusso, M. Tomasz, M. Muller and R. Lipman, *Molec. Pharmac.* **13**, 872 (1977).