0026-895X/92/050969-06$03.00/0

MOLECULAR PHARMACOLOGY, 41:969-974

Effects of L-Histidine on Hydrogen Peroxide-Induced DNA Damage and Cytotoxicity in Cultured Mammalian Cells

ORAZIO CANTONI, PIERO SESTILI, ANDREA GUIDARELLI, PAOLO U. GIACOMONI, and FLAMINIO CATTABENI

Istituto di Farmacologia e Farmacognosia and Centro di Farmacologia Oncologica Sperimentale, Università degli Studi di Urbino, 61029, Urbino, Italy (O.C., P.S., A.G., F.C.), and Laboratoire de Recherche Fondamentale de L'Oreàl, 93600, Aulnay Sous Bois, France (P.U.G.)

Received August 26, 1991; Accepted January 20, 1992

SUMMARY

L-Histidine markedly increased the growth- and DNA synthesis-inhibitory effects elicited by hydrogen peroxide in cultured Chinese hamster ovary cells. DNA single-strand breakage was also higher in the presence of the amino acid and, in addition, these breaks were characterized by a slower rate of repair, compared with that of the breaks generated by the oxidant alone. In the presence of L-histidine, hydrogen peroxide also produced DNA double-strand breakage, a lesion that cannot be detected in cells treated with even exceedingly high concentrations of the oxidant alone. Data reported herein suggest that the L-histidine-mediated increase of the cytotoxic response of cultured Chinese hamster ovary cells to hydrogen peroxide may be at least partially dependent on the formation of DNA double-strand breaks.

Hydrogen peroxide is a cellular metabolite present in many tissues, and its concentration can rise locally from physiological levels to higher values in various pathological conditions, e.g., inflammation (1), or after exposure to an array of chemical or physical agents (1–4). H_2O_2 may, therefore, reach cytotoxic levels, and cell injury seems to depend on the formation of hydroxyl radicals (5–7). Several events occur under these conditions, including DNA single-strand breakage (5–16), decreases in cellular ATP and NAD^+ levels (10, 16), oxidation of acid-soluble and protein thiols (17), elevation of cytosolic free calcium ion levels (9–11), and morphological alterations (9). Recently, various authors have investigated the effect of specific components of the extracellular milieu on the toxicity of hydrogen peroxide (18–24), because the composition of the biological fluids with which target cells are bathed can be markedly affected by various pathological conditions, and this may significantly change the susceptibility of the cells to oxidative attack. Interesting results have been obtained in these studies, and a critical role has been suggested for glucose (18) and, importantly, L-histidine, an amino acid that markedly sensitizes cells to oxidative attack (18–24). It has been reported that L-histidine increases the fragmentation of purified DNA induced by hydrogen peroxide (19, 20, 24), as well as other effects produced by the oxidant in cultured mammalian cells, i.e., cytotoxicity (19, 20), chromosomal aberrations (21), sister chromatid exchanges (19, 20, 22), and the formation of micronuclei (19, 20, 22). The mechanism involved in the enhancing effect of L-histidine on the deleterious actions of hydrogen peroxide is still controversial, although various hypotheses have been proposed (18, 21, 22). The present study was undertaken to investigate the effects of L-histidine on DNA damage produced by hydrogen peroxide in cultured mammalian cells.

This study was supported by a grant from the Associazione Italiana per la Ricerca sul Cancro (AIRC).

Experimental Procedures

Materials. L-Histidine, sodium dodecyl sulfate, tetraethylammonium hydroxide, and EDTA (free acid) were obtained from Sigma Chemical Company (St. Louis, MO). Hydrogen peroxide was purchased as a 30% stock solution from J. T. Baker Chemicals BV (Deventer, Holland). [^{14}C]- and [^{3}H]thymidine were from NEN (Boston, MA).

Cell culture, radioactive labeling, and treatment conditions. CHO cells were routinely grown in McCoy's 5a medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin, in an atmosphere of 5% CO_2 in air, at 37°. All experiments were performed with logarithmic phase cells (5×10^5/35-mm dish). Experimental cultures for the filter elution assay were labeled overnight with [*methyl*-^{14}C]thymidine (0.05 μCi/ml), plated in 35-mm tissue culture dishes, and then grown for 6 hr in label-free medium. Treatments were performed at either 37° or 4°, in 2 ml of saline A (0.14 M NaCl, 5 mM KCl, 4 mM $NaHCO_3$, 5 mM glucose).

Cell growth-inhibition studies. Exponentially growing CHO cells were treated with increasing concentrations of H_2O_2 for 30 min, in the presence or absence of L-histidine. Monolayers were then rinsed with saline A and allowed to grow in fresh culture medium and, at specific time intervals, cells were harvested by trypsinization and counted with a Coulter particle counter ZM.

DNA synthesis assay. Cells were treated for 30 min with various concentrations of H_2O_2, in the presence or absence of L-histidine. Cells

ABBREVIATION: CHO, Chinese hamster ovary.

were rinsed with saline A and incubated for 15 min in complete culture medium containing 1 μCi/ml [^{3}H]thymidine. Monolayers were then rinsed twice with ice-cold saline A and incubated with 10% trichloroacetic acid on ice for 15 min. This step was repeated twice. The trichloroacetic acid-precipitable fraction was then dissolved in 1 ml of 0.4 N NaOH and, after addition of 0.4 ml of 1 N HCl, the solution was collected in counting vials, containing 8 ml of Lumagel, and processed for scintillation counting.

Alkaline elution assay. Cells containing ^{14}C-DNA were exposed to H_2O_2, in the presence or absence of L-histidine, and then analyzed for DNA damage either immediately or after various time intervals. Cells were removed from the dishes by trypsinization (1% trypsin for 5 min on ice). The filter elution assay was carried out by a procedure virtually identical to that described by Kohn *et al.* (25), with minor modifications (12). Briefly, 5×10^5 cells were gently loaded onto 25-mm, 2-μm-pore size polycarbonate filters and then rinsed twice with 10 ml of ice-cold saline A containing 5 mM EDTA (disodium salt). Cells were then lysed with 5 ml of 2% sodium dodecyl sulfate, 0.025 M EDTA (tetrasodium salt), pH 10.1. Lysates were rinsed with 7 ml of 0.02 M EDTA (tetrasodium salt), and the DNA was eluted overnight in the dark with 1.5% tetraethylammonium hydroxide, 0.02 M EDTA (free acid), 0.1% sodium dodecyl sulfate (pH 12.1), at a flow rate of ~30 μl/min. Fractions of approximately 3 ml were collected and counted in 7 ml of Lumagel containing 0.7% glacial acetic acid. DNA remaining on the filters was recovered by heating for 1 hr at 60° in 0.4 ml of 1 N HCl, followed by the addition of 0.4 N NaOH (2.5 ml), and was, again, determined by scintillation counting. DNA was also recovered from the interior of the membrane holders after vigorous flushing with 3 ml of 0.4 N NaOH. This solution was processed for scintillation counting as described above.

Strand scission factors were calculated from the resulting elution profiles by determining the absolute logarithm of the ratio of the percentage of DNA retained in the filters of the drug-treated sample to that retained from the untreated control sample (both after 8 hr of elution).

Neutral elution assay. DNA double-strand breaks were estimated by neutral elution, as described by Kohn *et al.* (25); the procedure was similar to that described above, except that a pH 9.6 eluting solution, which does not denature the DNA, was used.

Results

Growth-inhibition studies. The effect of L-histidine on the growth-inhibitory action of hydrogen peroxide has been investigated. Note that the amino acid, at the concentrations and under all the experimental conditions described below, did not produce any detectable effect on cell replication. We found that L-histidine greatly enhanced the sensitivity of cultured CHO cells to hydrogen peroxide. Indeed, data displayed in Fig. 1 indicate that the growth rate of cells treated for 30 min at 37° with 10, 25, or 37.5 μM H_2O_2 and 1 mM L-histidine was markedly slower, compared with that of cells exposed to the oxidant alone. Note that the proliferation of cells that had been treated with 37.5 μM H_2O_2 and 1 mM L-histidine was totally suppressed, because no increase in cell number was detected after 48 hr of post-challenge growth. The effect of the amino acid became apparent at concentrations as low as 10–30 μM and remained linear for up to 1 mM (Fig. 2). At higher concentrations, no further increase in the activity was detectable (data not shown). Fig. 2 also shows that the slopes of the three curves, which display the growth-inhibitory effect of 10, 25, and 37.5 μM H_2O_2 at increasing concentrations of L-histidine, were a function of the concentration of hydrogen peroxide. The steepness of the curves was progressively higher at increasing concentrations of hydrogen peroxide (slope = −3.93 at 10 μM, slope = −13.57 at 37.5 μM, and slope = −17.71 at 37.5 μM). In marked contrast to the results obtained at 37°, the toxicity elicited by the oxidant at 4° was not increased further by L-histidine (data not shown).

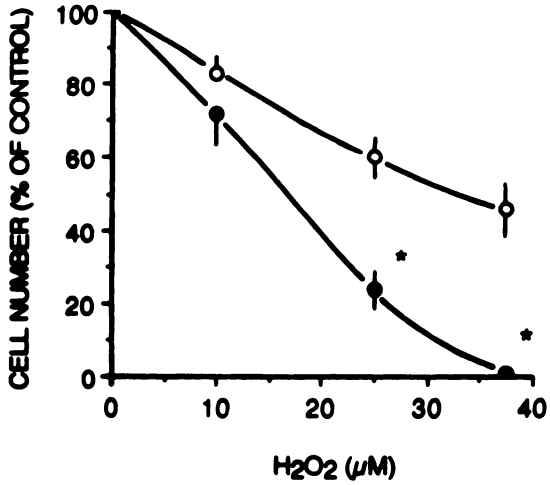

Fig. 1. Effect of L-histidine on the growth inhibition of CHO cells by H_2O_2. Exponentially growing CHO cells were exposed for 30 min at 37° to increasing concentrations of H_2O_2, in the absence (○) or presence (●) of 1 mM L-histidine. Cells were then allowed to grow in fresh culture medium, and after 48 hr the cell number was estimated with a Coulter counter. The growth rate of cells receiving L-histidine alone was not different from that of untreated cells. Data represent the mean ± standard error calculated from three separate experiments, each performed in duplicate. *, Different from cells not receiving L-histidine, by Student's *t* test ($p < 0.0001$).

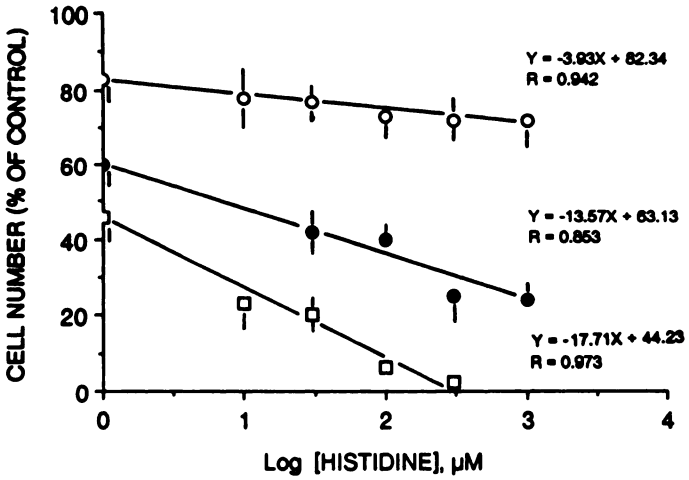

Fig. 2. Effect of various concentrations of L-histidine on the proliferation of CHO cells treated with H_2O_2. CHO cells were exposed to 10 (○), 25 (●), or 37.5 (□) μM H_2O_2, in the absence or presence of 30 μM, 100 μM, 300 μM, or 1 mM L-histidine. Cells were then allowed to grow in fresh, drug-free, culture medium, and after 48 hr cell number was determined with a Coulter counter. The data are expressed on the *abscissa* as log [L-histidine] (in μM) and, therefore, *1*, 2, and 3 correspond to 10, 100, and 1000 μM, respectively. Data represent the mean ± standard error of three separate experiments, each performed in duplicate.

DNA synthesis-inhibition studies. The effect of L-histidine on the DNA synthesis-inhibitory action of hydrogen peroxide was also tested. It was found that the amino acid, at a 1 mM concentration, resulted in an approximately 2-fold increase in the sensitivity of the cells to DNA synthesis inhibition induced by the oxidant (Fig. 3). In these experiments, treat-

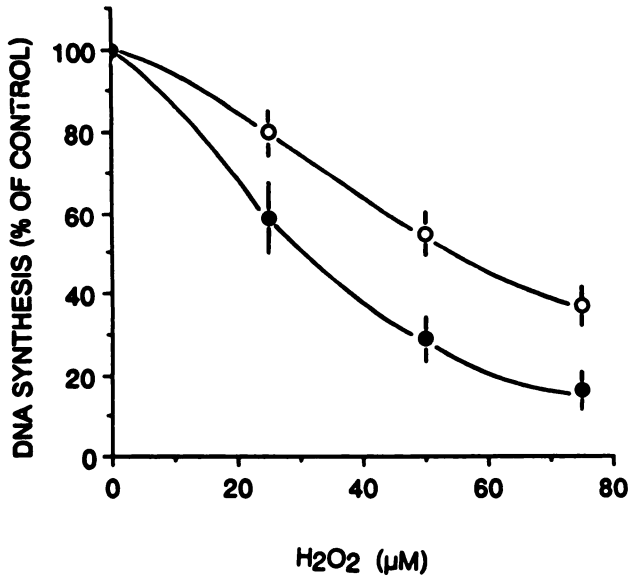

Fig. 3. Effect of L-histidine on DNA synthesis inhibition by hydrogen peroxide. Cells were exposed for 30 min to increasing concentrations of H_2O_2, in the absence (○) or presence (●) of 1 mM L-histidine. The DNA synthesis rate was assayed as detailed in Experimental Procedures. Data represent the mean of two separate experiments, each performed in duplicate.

ments were performed at 37° and the amino acid alone did not affect CHO cell DNA synthesis.

Studies on the formation and repair of DNA single-strand breaks. Fig. 4 shows the effect of 1 mM L-histidine on DNA single-strand breakage induced by increasing concentrations of H_2O_2. The incubation was for 30 min at physiological temperature. Cells were about 2 times more sensitive to the DNA-damaging effect of H_2O_2 in the presence of the amino acid. Experiments were designed to investigate the effect of L-histidine on the rate of formation (over a 30-min period) of H_2O_2-induced DNA single-strand breaks, and it was found that the kinetics were similar under conditions of exposure to the oxidant alone or in the presence of the amino acid (data not shown). Thus, L-histidine increased the net amount of DNA single-strand breaks produced by H_2O_2 without affecting the rate at which these lesions were induced. Data illustrated in Fig. 4, *inset*, were obtained from experiments similar to the ones discussed above, and displayed in Fig. 4, with the exception that the temperature of cell exposure was 4°. Under these experimental conditions, L-histidine also increased the susceptibility of the cells to the DNA-damaging effect of hydrogen peroxide, and the degree of potentiation was actually higher (about 3-fold), compared with that observed with treatments at physiological temperature (2-fold).

The effect of increasing concentrations of L-histidine on DNA single-strand breakage produced by 30 μM H_2O_2 at 37° was also investigated. Data shown in Fig. 5 indicate that the effect of the amino acid was apparent at concentrations as low as 30 μM and was concentration dependent up to 300 μM. At 1 mM, or higher levels (data not shown), no further increase was detectable. A similar L-histidine concentration dependence for DNA single-strand break induction by hydrogen peroxide was obtained in experiments where treatments were performed on ice (Fig. 5, *inset*).

Finally, we investigated the kinetics of repair of DNA single-strand breaks generated by the oxidant in the presence or absence of the amino acid. In those experiments where treatments were performed at 37°, H_2O_2 was used at 37.5 μM when added to the cultures alone and at 15 μM when used in conjunction with L-histidine (1 mM). Under these experimental conditions, similar levels of initial damage to the DNA, as measured by the filter elution assay, could be produced (see data shown in Fig. 4). After treatments, cells were allowed to repair in fresh prewarmed medium for various time intervals and were assayed for the residual DNA damage. The rate of removal of DNA lesions generated by H_2O_2 plus L-histidine was much slower than that of breaks produced by H_2O_2 alone, with $t_{1/2}$ values of 40 and 15 min, respectively (Fig. 6). It should be noted that L-histidine itself does not delay the repair of hydrogen peroxide-induced DNA single-strand breakage, because similar repair kinetics were observed in cells treated with hydrogen peroxide and then allowed to repair the damage in the presence or absence of 1 mM L-histidine (data not shown). In contrast to the results indicating different rates of removal of DNA lesions produced at 37°, superimposable kinetics of DNA single-strand break repair were obtained in cells treated at 4° (Fig. 6, *inset*). It should be noted that, in the latter set of experiments, the initial damage was obtained by treating the cells at 4° for 30 min with either 100 μM H_2O_2 alone or 37.5 μM H_2O_2 plus 1 mM L-histidine. Under these experimental condi-

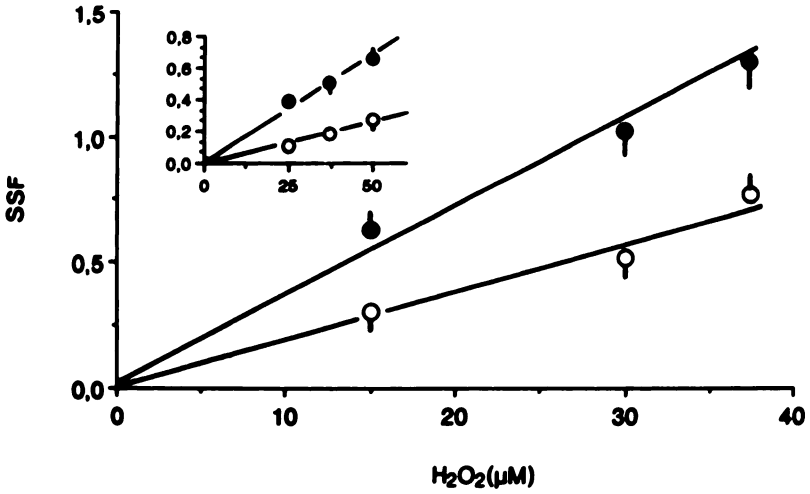

Fig. 4. Effect of L-histidine on H_2O_2-induced DNA single-strand breakage. Cells were exposed for 30 min at 37° to various concentrations of H_2O_2, in the absence (○) or presence (●) of 1 mM L-Histidine, and were then analyzed for DNA damage by the filter elution assay. Strand scission factor (*SSF*) values were calculated from the alkaline elution profiles, as detailed in Experimental Procedures. *Inset*, results from similar experiments, except that the treatments were performed at 4°. Values represent the mean of three or four independent experiments, each performed in duplicate.

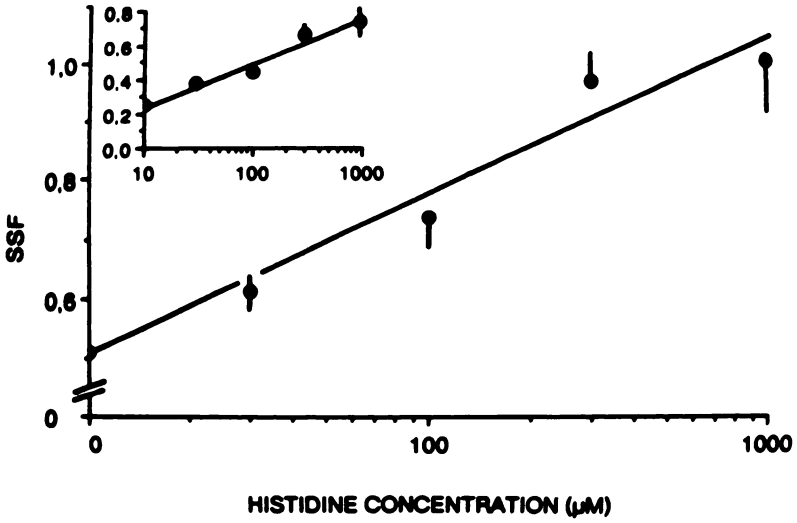

Fig. 5. Effect of increasing concentrations of L-histidine on H_2O_2-induced DNA single-strand breaks. CHO cells were treated for 30 min in saline A either at 37° with 15 μM H_2O_2 or at 4° with 30 μM H_2O_2 (*inset*), in the presence of increasing concentrations of L-histidine (0–1 mM). The DNA from these cells was analyzed as described in the legend to Fig. 4 and in Experimental Procedures. Values represent the mean of three separate experiments, each performed in duplicate.

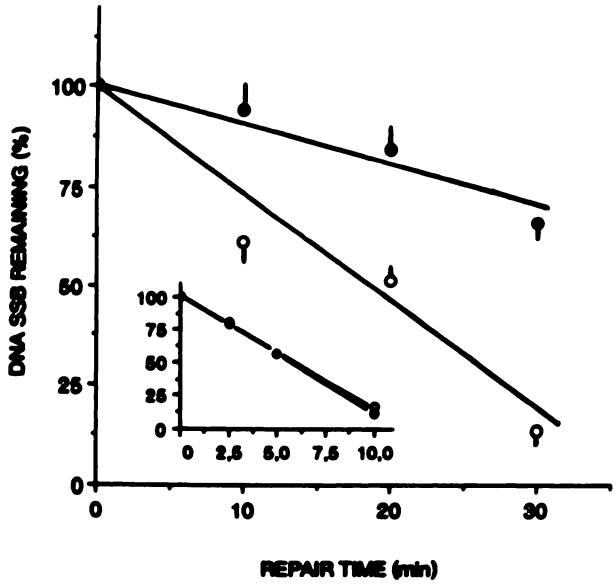

Fig. 6. Kinetics of removal of DNA single-strand breaks (*SSB*) produced by H_2O_2 alone or with L-histidine. Cells were exposed for 30 min at 37° to 37.5 μM H_2O_2 (○) or 15 μM H_2O_2 plus 1 mM L-histidine (●). After treatments, cells were rinsed and allowed to repair in a complete culture medium. At specific time intervals, cells were analyzed for DNA damage. Also shown (*inset*) are the results from experiments where the initial damage was obtained by treating the cells at 4° for 30 min with 100 μM H_2O_2 (○) or 37.5 μM H_2O_2 plus 1 mM L-histidine (●). Values represent the mean ± standard error of three to five separate experiments, each performed in duplicate.

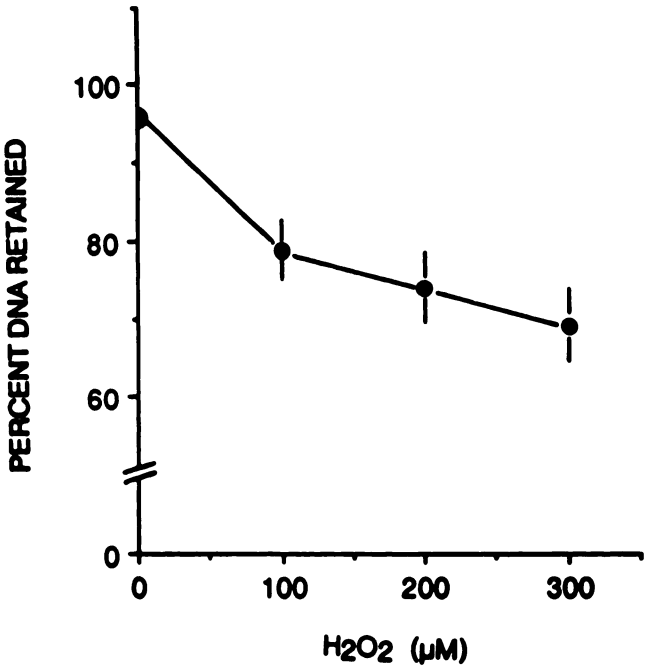

Fig. 7. Induction of DNA double-strand breaks by the cocktail H_2O_2/L-histidine. Cells were exposed to increasing concentrations of H_2O_2 in the presence of 1 mM L-histidine, and after treatments the DNA from these cells was analyzed by the neutral elution assay, as detailed in Experimental Procedures. Values represent the fraction of DNA retained on the filter after 12 hr of elution and are the mean ± standard error of three separate experiments, each performed in duplicate.

tions, similar levels of DNA single-strand breakage were obtained.

Studies on the formation of DNA double-strand breaks. Various studies have previously demonstrated that hydrogen peroxide does not induce DNA double-strand breakage in cultured mammalian cells (12–14). We have obtained similar results in this investigation, and DNA double-strand breaks were not detectable even after treatment with millimolar levels of the oxidant (data not shown). In the presence of L-histidine, however, H_2O_2 was capable of inducing this type of lesion. Indeed, data shown in Fig. 7 indicate that the elutability of DNA, under neutral conditions, was progressively higher when cells were exposed to L-histidine and concentrations of hydrogen peroxide in the range of 100–300 μM. No DNA double-strand breaks could be detected in cells treated with the oxidant (at concentrations up to 1 mM) and 1 mM L-histidine at 4° (data not shown).

Discussion

In the present study, we have investigated the effect of L-histidine on DNA damage and growth inhibition caused by hydrogen peroxide in cultured CHO cells. Previous studies had examined the effects of L-histidine on a number of biological end-points in oxidatively injured cultured mammalian cells. It was shown that the amino acid markedly increased cytotoxicity

(19, 20), chromosomal aberrations (21), sister chromatid exchanges (19, 20, 22), and the formation of micronuclei (19, 20, 22) elicited by hydrogen peroxide. In addition, L-histidine also increased the fragmentation of purified DNA induced by the oxidant (19, 20, 24). We now demonstrate that L-histidine increases DNA single-strand breakage (Figs. 4 and 5) in H_2O_2-treated CHO cells and that this effect is associated with greater DNA synthesis-inhibitory (Fig. 3) and cell growth-inhibitory (Figs. 1 and 2) responses. Whether the higher level of DNA single-strand breakage is the cause of the increased lethality elicited by H_2O_2 in the presence of L-histidine remains to be established. It should be noted, however, that various reports have suggested that DNA single-strand breaks do not represent a lethal lesion in oxidatively injured cells. Ward *et al.* (13, 14) calculated that 0.4×10^6 DNA single-strand breaks are required to kill a cell after challenge with hydrogen peroxide and that DNA double-strand breaks cannot be detected under these conditions. We also showed that a very high number of DNA single-strand breaks were generated by hydrogen peroxide, compared with X-rays, under equitoxic conditions (15), and that the susceptibility of CHO cells to killing by H_2O_2 was only slightly augmented when the repair of DNA single-strand breaks was slowed by incubation of the cells in the presence of 3-aminobenzamide, an inhibitor of the enzyme poly(ADP-ribose) polymerase (16). Finally, in this study we have demonstrated that the level of DNA single-strand breakage produced by H_2O_2 (Figs. 4 and 5, *insets*), but not its lethality (data not shown), is increased by L-histidine, when cells are treated at 4°. These observations are in keeping with recently published data from Coleman *et al.* (26), indicating a dissociation of the accumulation of DNA single-strand breakage from the killing of hepatocytes treated with various agents that produce an oxidative stress and, therefore, suggesting that DNA single-strand breaks caused by oxygen-derived radicals do not represent a lethal lesion. Thus, it is unlikely that the higher level of DNA single-strand breakage detected in cells exposed to L-histidine and H_2O_2 is responsible for the increased cytotoxic response. Further experimental evidence supporting this inference will be discussed below. It is possible, however, that the amino acid, besides increasing the net amount of DNA single-strand breaks generated by the oxidant, also promotes the formation of different types of DNA single-strand breaks. One could, therefore, speculate that, although DNA single-strand breaks produced by H_2O_2 are not lethal, those, or at least some of those, produced by the oxidant in the presence of the amino acid may be capable of eliciting cellular death. Experimental data presented in this report apparently support this hypothesis, because we have found that the repair of the DNA strand breaks produced (at 37°) by the oxidant alone is significantly faster, compared with the repair of those breaks generated by the cocktail H_2O_2/L-histidine (Fig. 6). On the basis of these data, it may be suggested that qualitatively different lesions are generated by the oxidant in the presence of the amino acid, compared with the situation of exposure to H_2O_2 alone. It should be noted, however, that the alkaline elution technique does not allow the distinction of specific types of DNA lesions but only permits the detection of frank DNA single-strand breaks or alkali-labile sites (such as apurinic sites, which are converted to chain scission under conditions of alkaline pH, i.e., pH > 12) (25). In addition, DNA double-strand breaks would also be detected as single-strand breaks via this procedure. The filter elution assay, however, can be slightly modified (see Experimental Procedures) and, by adjusting the pH to values lower than 10, the DNA can be eluted in a double-stranded form, thus allowing the detection of DNA double-strand breaks (25). We have found that, in the presence of L-histidine, this type of DNA lesion was detectable in cells challenged with hydrogen peroxide (Fig. 7). This was surprising to us, because DNA double-strand breakage was not found in cells treated with millimolar concentrations of the oxidant (data from this paper and Refs. 12–14 and 16). Thus, it is possible that the delayed removal of DNA breaks shown in Fig. 6 was a consequence of a combination of the kinetics of repair of DNA single-strand (fast) and double-strand (slow) breaks. We have shown that the relative potency of L-histidine in increasing the toxicity of hydrogen peroxide is a direct function of the concentration of the oxidant (Fig. 2). This could be due to the recruitment of a specific type of damage that is potentially lethal, such as DNA double-strand breakage. Indeed, this lesion is thought to be more toxic than DNA single-strand breakage (27), and it can be speculated that the mechanism, or at least one of the mechanisms, whereby L-histidine augments the toxicity of hydrogen peroxide is dependent on the formation of this lesion. Experiments in progress in our laboratory seem to support this hypothesis, because, under various experimental conditions, the L-histidine-mediated enhancement of hydrogen peroxide-induced cytotoxicity was always associated with the appearance of DNA double-strand breakage. We found this association when cells were pretreated with the amino acid and then exposed to the oxidant.[1] It is worth noting that, under these conditions, no enhancing effect was noticed for DNA single-strand breakage. An association was also found when cells were exposed to the cocktail H_2O_2/L-histidine in the presence of L-glutamine, an amino acid that is taken up by the cells via the same transport system as is L-histidine and, therefore, inhibits L-histidine uptake (28). Under these conditions, the enhancing effect was apparent only for DNA single-strand breakage; cytotoxicity was not increased and no DNA double-strand breaks could be detected. Finally, we found an association when treatments were performed at 4°. Again, under these experimental conditions the enhancing effect of L-histidine was apparent for only DNA single-strand breakage (Figs. 4 and 5, *insets*), whereas cytotoxicity was not increased (data reported in the text) and no DNA double-strand breaks could be detected (data reported in the text). It is also worth noting that DNA single-strand breaks produced by the oxidant, alone or combined with L-histidine, at 4°, were repaired with superimposable rates (Fig. 6, *inset*).

In conclusion, the data presented in this paper strongly suggest that the L-histidine-mediated increase in the cytotoxic response of cultured mammalian cells to hydrogen peroxide is caused by the appearance of DNA double-strand breakage, whereas the higher level of DNA single-strand breaks appears totally unrelated to this phenomenon. Further studies are necessary to understand the mechanism whereby L-histidine induces the formation of DNA double-strand breaks in oxidatively injured cells. In principle, the amino acid may affect the relative rates of reaction (either chemical or enzymatic) that result in the generation or destruction of the various oxygen species. Experimental data reported in this paper indicate that

[1] Manuscript in preparation.

the formation of DNA double-strand breaks occurs only at physiological temperature, suggesting the involvement of energy-dependent events. Because the results obtained by exposing the cells to hydrogen peroxide at ice-bath temperature should reflect the exclusive action of chemical/free radical processes, it may also be concluded that L-histidine somehow increases the level of reactive oxygen species producing DNA single-strand breakage.

References

1. Freeman, B. A., and J. D. Crapo. Biology of disease: free radicals and tissue injury. *Lab. Invest.* **47**:412–426 (1982).
2. Hoffmann, M. F., and R. Meneghini. Action of hydrogen peroxide on human fibroblasts in culture. *Photochem. Photobiol.* **29**:299–303 (1979).
3. Parshad, R., K. K. Sanford, G. M. Jones, and R. F. Tarone. Fluorescent light-induced chromosome damage and its prevention in mouse cells in culture. *Proc. Natl. Acad. Sci. USA* **75**:1830–1833 (1978).
4. Halliwell, B. Superoxide and superoxide-dependent formation of hydroxyl radicals are important in oxygen toxicity. *Trends Biochem. Sci.* **7**:270–272 (1982).
5. Meneghini, R., and M. E. Hoffmann. The damaging action of hydrogen peroxide on DNA of human fibroblasts is mediated by a nondialyzable compound. *Biochim. Biophys. Acta* **608**:167–173 (1980).
6. Mello Filho, A. C., and R. Meneghini. *In vivo* formation of single-strand breaks in DNA by hydrogen peroxide is mediated by the Haber Weiss reaction. *Biochim. Biophys. Acta* **781**:56–63 (1984).
7. Mello Filho, A. C., M. E. Hoffmann, and R. Meneghini. Cell killing and DNA damage by hydrogen peroxide are mediated by intracellular iron. *Biochem. J.* **218**:273–275 (1984).
8. Mello Filho, A. C., and R. Meneghini. Correlation between cytotoxic effect of hydrogen peroxide and the yield of DNA strand breaks in cells of different species. *Biochim. Biophys. Acta* **781**:273–275 (1984).
9. Hyslop, P. A., D. B. Hinshaw, I. U. Schraufstatter, L. A. Sklar, R. G. Spragg, and C. G. Cochrane. Intracellular calcium homeostasis during hydrogen peroxide injury to cultured P388D1 cells. *J. Cell Physiol.* **129**:356–366 (1986).
10. Schraufstatter, I. U., P. A. Hyslop, D. B. Hinshaw, R. G. Spragg, L. A. Sklar, and C. G. Cochrane. Hydrogen peroxide-induced injury of cells and its prevention by inhibitors of poly(ADP-ribose) polymerase. *Proc. Natl. Acad. Sci. USA* **83**:4908–4912 (1986).
11. Cantoni, O., P. Sestili, F. Cattabeni, S. Pou, M. S. Cohen, and P. Cerutti. Calcium chelator quin2 prevents hydrogen-peroxide-induced DNA breakage and cytotoxicity. *Eur. J. Biochem.* **182**:209–212 (1989).
12. Cantoni, O., D. Murray, and R. E. Meyn. Effect of 3-aminobenzamide on DNA strand-break rejoining and cytotoxicity in CHO cells treated with hydrogen peroxide. *Biochim. Biophys. Acta* **867**:135–143 (1986).
13. Ward, J. F., W. F. Blakely, and E. I. Jonern. Mammalian cells are not killed by DNA single strand breaks caused by hydroxyl radicals from hydrogen peroxide. *Radiat. Res.* **103**:383–392 (1985).
14. Ward, J. F., J. W. Evans, C. L. Limoli, and P. M. Calabro-Jones. Radiation and hydrogen peroxide induced free radical damage to DNA. *Br. J. Cancer* **55** (Suppl. VIII):105–112 (1987).
15. Cantoni, O., P. Sestili, and F. Cattabeni. Randomly distributed DNA single strand breaks are not lethal for mammalian cells. *Xenobiotica* **18**:1481–1487 (1988).
16. Cantoni, O., D. Murray, F. Cattabeni, V. Stocchi, P. Cerutti, and R. E. Meyn. Hydrogen peroxide insult in cultured mammalian cells: relationships between DNA single-strand breakage, poly(ADP)-ribosylation, and cell killing. *Biochim. Biophys. Acta* **1014**:1–7 (1989).
17. Schraufstatter, I. U., D. B. Hinshaw, P. A. Hyslop, R. G. Spragg, and C. G. Cochrane. Glutathione cycle activity and pyridine nucleotide levels in oxidant-induced injury of cells. *J. Clin. Invest.* **76**:1131–1139 (1985).
18. Link, E. M. The mechanism of pH-dependent hydrogen peroxide cytotoxicity *in vitro*. *Arch. Biochem. Biophys.* **265**:362–372 (1988).
19. Tachon, P., and P. Giacomoni. Histidine a clastogenic factor? in *Light in Biology and Medicine* (R. H. Douglas, J. Moan, and F. Dall' Acqua, eds.), Vol. 1. Plenum Publishing Corporation, New York, 211–217 (1988).
20. Tachon, P., and P. Giacomoni. Histidine modulates the clastogenic effect of oxidative stress. *Mutat. Res.* **211**:103–109 (1989).
21. Oya, Y., and K. Yamamoto. The biological activity of hydrogen peroxide. IV. Enhancement of its clastogenic actions by coadministration of L-histidine. *Mutat. Res.* **198**:233–240 (1988).
22. Tachon, P. Intracellular iron mediates the enhancing effect of histidine on the cellular killing and clastogenicity induced by H_2O_2. *Mutat. Res.* **228**:221–228 (1990).
23. Shacter, E., R. L. Lopez, E. D. Beecham, and S. Janz. DNA damage induced by phorbol ester-stimulated neutrophils is augmented by extracellular cofactors. *J. Biol. Chem.* **265**:6693–6699 (1990).
24. Tachon, P. DNA single strand breakage by H_2O_2 and ferric and cupric ions: its modulation by histidine. *Free Radicals Res. Commun.* **9**:39–47 (1990).
25. Kohn, K. W., R. A. G. Ewig, L. C. Erickson, and L. A. Zwelling. Measurement of strand breaks and crosslinks by alkaline elution, in *DNA Repair: A Laboratory Manual of Research Procedures* (E. Friedberg and P. Hanawalt, eds.), Vol. 1. Marcel Dekker, Inc., New York, 379–401 (1981).
26. Coleman, J. B., D. Gilfor, and J. L. Farber. Dissociation of the accumulation of single-strand breaks in DNA from killing of cultured hepatocytes by an oxidative stress. *Mol. Pharmacol.* **36**:193–200 (1989).
27. Elkind, M. M., H. Utsumi, and E. Ben-Hur. Are single or multiple mechanisms involved in radiation-induced mammalian cell killing? *Br. J. Cancer* **55** (Suppl. VIII):24–31 (1987).
28. Kilberg, M. S., M. E. Handlogten, and H. N. Christensen. Characteristics of an amino acid transport system in rat liver for glutamine, asparagine, histidine, and closely related analogues. *J. Biol. Chem.* **255**:4011–4019 (1980).

Send reprint requests to: Dr. Orazio Cantoni, Istituto di Farmacologia e Farmacognosia, Università di Urbino, Via S. Chiara, 27, 61029 Urbino, Italy.