FEBS Letters 353 (1994) 75–78

FEBS 14623

The L-histidine-mediated enhancement of hydrogen peroxide-induced cytotoxicity is a general response in cultured mammalian cell lines and is always associated with the formation of DNA double strand breaks

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Received 29 August 1994

Abstract Micromolar concentrations of L-histidine increase the cytotoxicity of hydrogen peroxide in a number of cell lines including CHO (hamster), EAHY, McCoy's, U937 and CCRF-CEM (human), Vero (monkey) and SC-1 (mouse). Importantly, these cell lines displayed different degrees of sensitivity to H_2O_2 alone and the extent of enhancement elicited by the amino acid was more pronounced in resistant cell lines. The increased cytotoxicity was invariably associated with the formation of DNA DSBs and a remarkable correlation was found by plotting the level of DNA DSBs against the cytotoxic response. These results strongly support the hypothesis that the mechanism whereby L-histidine increases the toxicity elicited by H_2O_2 involves the formation of DNA DSBs and are consistent with the possibility that the amino acid might participate in the regulation of the physio-pathological response to oxidative stress in mammals.

Key words: L-Histidine; Hydrogen peroxide; Cytotoxicity; DNA double strand breaks

1. Introduction

L-Histidine enhances a number of deleterious effects elicited by hydrogen peroxide in cultured mammalian cells [1–11]. Recent work from our laboratory has indicated that, in the presence of L-histidine, H_2O_2 also produces DNA DSBs in cultured CHO cells [7–11]. Importantly, DNA DSBs are generally considered to be a lethal lesion [12] and cannot be detected in CHO cells treated with toxicologically relevant concentrations of hydrogen peroxide alone [7–11]. Experimental evidence was then collected indicating that the intracellular fraction of the amino acid was responsible for the enhanced formation of DNA DSBs and toxicity in oxidatively-injured cells and that these two events were always associated, suggesting a cause–effect relationship [11]. In marked contrast, the extracellular fraction of the amino acid seemed to mediate the increased formation of DNA single strand breaks, an event that appeared to be unrelated to cytotoxicity [9,10].

Since the above effects were produced by low levels of L-histidine [7], it is possible that the amino acid might modulate the impact of an oxidative stress in mammalian cells. We therefore investigated the level of DNA DSBs and cytotoxicity produced by the cocktail H_2O_2/L-histidine in various cultured mammalian cell lines, derived from different species and with a different origin, with the specific aims of assessing the generality of this response and gathering further information in support of the notion that DNA DSBs are the cause of cell death following challenge with H_2O_2/L-histidine.

2. Materials and methods

2.1. Cell culture and treatments

Cells were either grown in monolayer culture (CHO, Vero, EAHY, McCoys and SC-1) or in suspension (U937 and CCRF-CEM) in their appropriate growth media (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Seralab, Sussex, UK), penicillin (50 units/ml), and streptomycin (50 μg/ml), at 37°C in T-75 tissue culture flasks (Corning, Corning, NY) gassed with an atmosphere of 95% air–5% CO_2.

Stock solutions of hydrogen peroxide and L-histidine were freshly prepared in Saline A (8.182 g/l NaCl, 0.372 g/l KCl, 0.336 g/l $NaHCO_3$ and 0.9 g/l glucose). Cells (5×10^5 for cell lines growing in monolayer and 2×10^6 for cell lines growing in suspension) were treated for 30 min in Saline A (2 ml), washed with prechilled Saline A and processed for cytotoxicity or DNA damage assays.

2.2. Cytotoxicity assays

Cytotoxicity was determined using a growth-inhibition assay. After treatments, appropriate dilutions of cells ($1–2 \times 10^4$ in those cell lines growing in monolayer and $5–7 \times 10^4$in cells growing in suspension) were plated in triplicate into 60-mm tissue culture dishes and incubated at 37°C for 3–6 days, depending on the cell type. The cell number was then estimated with a Coulter counter. Results obtained with this growth-inhibition assay are basically superimposable to those obtained with the conventional plating-efficiency assay, at least up to the second logarithm of killing (Sestili, unpublished).

2.3 Measurement of DSBs by the CHEF assay

Cells were labelled overnight with [^{14}C]thymidine (0.05 μCi/ml) and incubated for a further 6 h in a medium containing unlabelled thymidine (1 μg/ml).

The CHEF assay was performed essentially as described by Blocher et al. [13]. To prepare agarose plugs, cells were sedimented at 1000 rpm for 5 min at 4°C and the pellet was resuspended in phosphate-buffered saline (PBS) containing 5 mM EDTA, pH 8.3. This procedure was repeated three times, and the final suspension in PBS was adjusted to give a density of 3.64×10^6 cells/ml. 300 μl of this suspension were mixed with 300 μl of melted agarose (low melt agarose, Biorad, Richmond, CA; 1% solution in PBS) in a 15 ml conical tube maintained at 45°C. The cell–agarose mixture was vortexed, transferred to a gel plug former on ice, and refrigerated for 15 min. The plugs (four per experimental point) were removed and incubated in 1 ml of ESP (0.5 M EDTA, 1% sarkosyl, 1 mg/ml proteinase K, pH 9) for 1–2 h at 4°C then at 45°C for 20 h. The plugs were washed five times for 1 h in sterile 0.5 × TE buffer (10 mM Tris-HCl, 1 mM Na_2EDTA; pH 7.5) and stored at 4°C in 0.5 M EDTA (pH 8).

The DNA in the plugs was separated by PFGE in a 0.5% agarose gel

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Abbreviations: DSBs, DNA double strand breaks; CHEF, contour clamped, homogeneous electric field.

SSDI 0014-5793(94)01010-2

(chromosomal grade, Bio-Rad, Richmond, CA) in 0.5 × TAE buffer (44.5 mM Tris base, 34.5 mM sodium borate, 1 mM Na_2EDTA, pH 7.2). The gel was run for 20 h on a CHEF-DR II system (Bio-Rad, Richmond, CA) operating at 1.21 V/cm with a switch time of 75 min. The buffer (0.5 × TAE) was maintained at 25°C. The gel was stained with ethidium bromide, viewed under a UV transilluminator and photographed. The distribution of radioactivity in the gel was determined by cutting portions of the gel containing the DNA (well and lane). These portions were then melted in glass scintillation vials in the presence of 50 μl of 1 N HCl and processed for liquid scintillation counting.

3. Results

3.1. Effect of L-histidine on the cytotoxic response of an array of cell lines challenged with increasing concentrations of hydrogen peroxide

In order to investigate and compare the effects of L-histidine on the toxicity of hydrogen peroxide in different cell lines, we adopted an experimental protocol involving a 30 min exposure (37°C) to the amino acid (300 μM) and increasing concentrations of H_2O_2. Under these conditions, the amino acid does not produce any toxic effect (not shown) but significantly potentiates the toxicity elicited by hydrogen peroxide. Results shown in Fig. 1 indicate that the toxicity of H_2O_2 changes significantly in the various cell lines and suggest that the enhancing effect of L-histidine might be a direct function of the level of resistance to the oxidant; in other words, the amino acid seems more effective in those cell lines exhibiting an intrinsic resistance to the insult elicited by hydrogen peroxide.

3.2. Effect of L-histidine on the level of DNA DSBs in various cultured mammalian cell lines challenged with increasing concentrations of hydrogen peroxide

In order to determine whether the enhanced cytotoxic response elicited by L-histidine in hydrogen peroxide-treated cells was accompanied by a more effective induction of DNA DSBs, we assessed the level of this lesion in cells treated under conditions similar to those utilized in toxicity studies. For this purpose we used the CHEF assay, a very sensitive technique that has recently been developed for measuring DNA DSBs [13]. Results illustrated in Fig. 2 indicate that addition of the amino acid always resulted in the formation of DNA DSBs in oxidatively-injured cells. Once again, L-histidine was more effective in promoting the formation of DNA DSBs in those cell lines displaying resistance to the toxicity elicited by H_2O_2 alone.

3.3. Correlation between the formation of DNA DSBs and cytotoxicity

Fig. 3 shows a correlation analysis curve obtained by plotting the level of DNA DSBs and cytotoxicity produced by the cocktail hydrogen peroxide/L-histidine in the various cell lines utilized in this study. It is of interest that all the experimental points fit in a single curve ($r = 0.931$).

4. Discussion

The L-histidine-mediated enhancement of hydrogen peroxide-induced cytotoxicity is a general response in mammalian cells since, as documented in this paper, it can be observed in a number of cell lines derived from different animal species (human and non-human primates and rodents) and, within the same animal species, from different tissues. It is important to emphasize that these cell lines were characterized by different levels of sensitivity to H_2O_2 alone. As an example, U937 cells were highly resistant since growth inhibition was elicited by

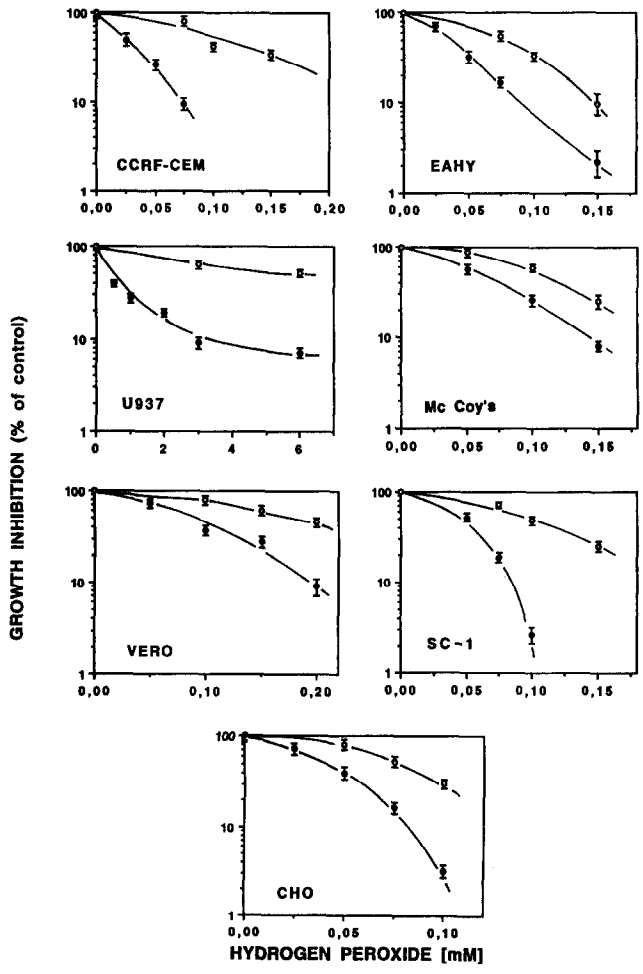

Fig. 1. Effect of L-histidine on the cytotoxicity produced by hydrogen peroxide in various cultured mammalian cell lines. Cells were exposed for 30 min in Saline A to increasing concentrations of hydrogen peroxide, in the absence or presence of 300 μM L-histidine. Following treatments cells were assayed for cytotoxicity, as detailed in the Methods section. Results are the mean ± S.E.M. calculated from 3–4 separate experiments, each performed in duplicate.

Table 1
Comparison of the IC_{50} values* for H_2O_2 alone or associated to L-histidine in various cell lines

Cell line	IC_{50} (μM)		Dose modifying factor
	− L-histidine	+ L-histidine	
CCRF-CEM	110	26	4.2
EAHY	80	37.5	2.1
U937	6200	600	10.3
Mc Coy's	105	60	1.75
VERO	190	90	2.1
SC-1	100	45	2.2
CHO	80	40	2

*IC_{50} values were calculated from the toxicity curves illustrated in Fig. 1.

millimolar levels of hydrogen peroxide whereas, in the remaining cell types, toxicity was elicited by oxidant concentrations in the micromolar range. Importantly, the L-histidine-mediated enhancement of hydrogen peroxide-induced cytotoxicity was much higher in U937 cells, as compared to the other cell lines (Table 1), and indeed, in the presence of the amino acid, the sensitivity of these human myeloid leukemic cells was shifted back to micromolar levels of the oxidant.

Thus, the enhancing effect of L-histidine would appear to be a direct function of the level of resistance of the specific cell line to H_2O_2. This inference, however, needs to be supported by additional data because of the limited number of cell lines that have been tested. Nevertheless, research in progress in our laboratory demonstrates that L-histidine increases the toxicity of hydrogen peroxide by a factor of 5 in AG8 cells (a cell line highly resistant to the oxidant that has been isolated in our laboratory) as compared to a factor of about 2 that was observed in the parental CHO cell line. In our opinion this evidence strongly supports the above inference, and might suggest that acquired resistance to hydrogen peroxide can be reverted by the addition of L-histidine.

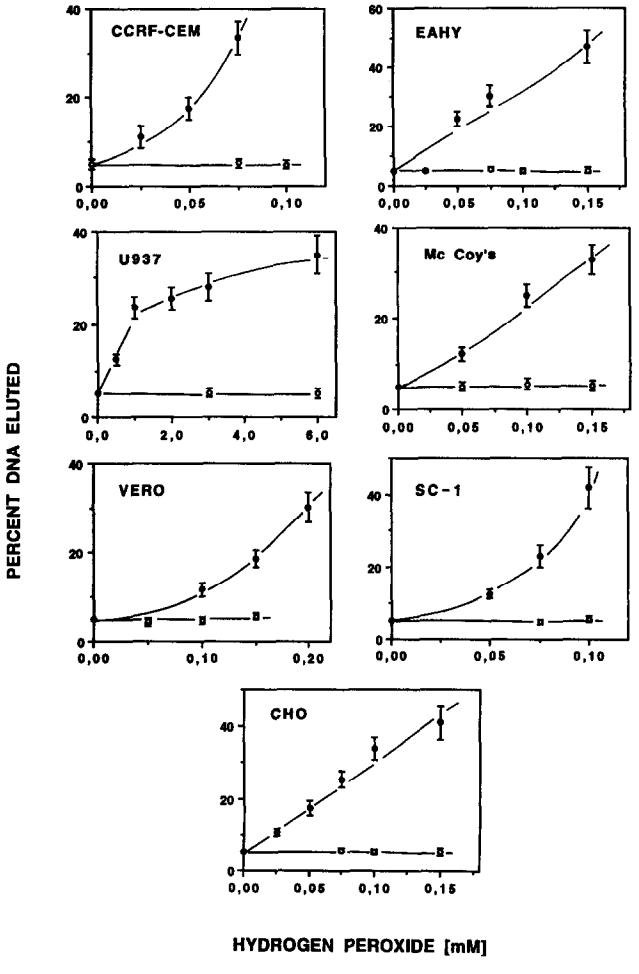

Fig. 2. Effect of L-histidine on the yield of DNA DSBs induced by hydrogen peroxide in various mammalian cell lines. Cells were treated as detailed in the legend to Fig. 1 and then analyzed for DNA double strand breakage by the CHEF assay. Data are expressed as 'Percent DNA eluted' which is the percent ratio of cpm in the lanes versus total cpm of the sample. About 93% of the DNA from untreated cells (in all the cell lines) remained in the plug after electrophoresis. Results are the mean ± S.E.M. calculated from three separate experiments, each performed in duplicate.

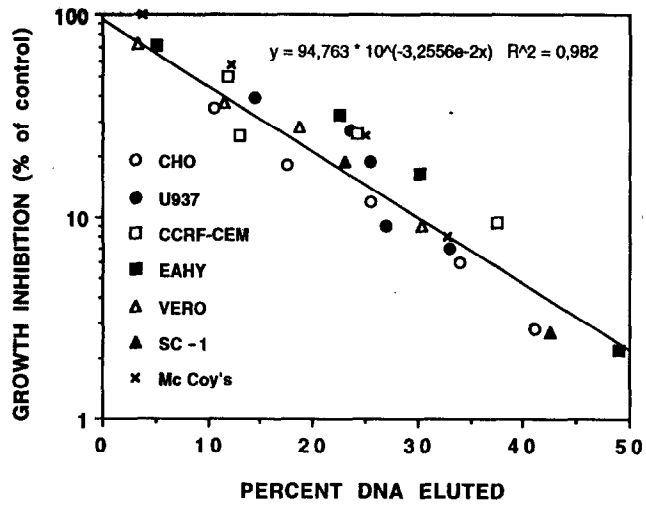

Fig. 3. Correlation analysis for DNA DSBs and cytotoxicity in various mammalian cell lines treated with hydrogen and L-histidine. Experimental results illustrated in Fig. 1 (cytotoxicity) were plotted against those displayed in Fig. 2 (DNA double strand breakage). Standard errors were omitted for the sake of clarity.

Experimental results reported in this paper also demonstrate that the increased cytotoxic response elicited by L-histidine in oxidatively-injured cells was paralleled by the appearance of DNA DSBs in all the cell types studied whereas, in accordance with previous investigations [7,14], no DNA DSBs could be detected following challenge with H_2O_2 alone (at levels utilized in toxicity studies).

The striking correlation that was found by plotting the level of DNA DSBs against the cytotoxic response indicates that the L-histidine-mediated enhancement of hydrogen peroxide-induced cytotoxicity is invariably associated with the formation of DNA DSBs in a number of cell lines and that, in these cell types, a given level of DNA lesions always corresponds to the same level of lethality. These observations strongly support the hypothesis that the mechanism whereby L-histidine increases the toxicity elicited by H_2O_2 involves the formation of DNA DSBs, and are consistent with the possibility that the amino acid might partecipate in the regulation of the physio-pathological response to oxidative stress in mammals.

Acknowledgments: The authors thank Mr. Andrea Guidarelli for technical assistance. This investigation was supported by a grant from CNR-Progetto Finalizzato Applicazioni Cliniche della Ricerca Oncologica.

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