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Nitric oxide co-operates with hydrogen peroxide in inducing DNA fragmentation and cell lysis in murine lymphoma cells

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We examined whether NO and H_2O_2 could interact in inducing DNA fragmentation and cell death. H_2O_2 and the NO-releasing compounds sodium nitroprusside (SNP) and *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) by themselves elicited lysis of YAC-1 murine lymphoma cells in a concentration-dependent manner. Exposure of the cells to a combination of sublytic concentrations of SNP (0.78 mM) plus H_2O_2 (7.8 μM) or SNAP (0.18 mM) plus H_2O_2 (7.8 μM) resulted in cell death which is mediated, in part, through apoptosis. Evidence for this direction is provided by fluorescence microscopic evaluation of the cells, which revealed the presence of changes in nuclear morphology characteristic of apoptosis in 30–40% of lymphoma cells and by the specific pattern of internucleosomal DNA fragmentation detected by gel electrophoresis. The cytotoxic effect of SNP plus H_2O_2 could be effectively inhibited by either oxyhaemoglobin, which binds NO, or catalase, which eliminates H_2O_2. Partial protection from SNP-plus-H_2O_2-induced cell lysis was observed with the poly(ADP-ribose) polymerase inhibitors, nicotinamide and 3-aminobenzamide, parallelling their ability to reverse depletion of cellular NAD^+ pools. These results indicate an interaction between NO and H_2O_2 which leads to a markedly enhanced cytotoxic activity, in part, via induction of apoptosis and suggest that poly(ADP-ribosylation) and subsequent NAD^+ depletion mediate, at least in part, this cytotoxic activity.

INTRODUCTION

Reactive oxygen intermediates, such as H_2O_2, can induce apoptosis in a variety of cell types [1,2], thereby directly establishing oxidative stress as a mediator of apoptosis [3]. Recently another free radical, NO [4,5], has been implicated as an inducer of apoptosis in monocytes/macrophages [6] and fibroblasts [7]. Activated phagocytes produce NO together with other oxygen radicals [8]. Presumably, the concurrent production of these radicals should provide an advantage to the host cell. NO and superoxide anion $O_2^{-\bullet}$ may form peroxynitrite ion ($ONOO^-$) [9], which has been implicated in the mediation of bactericidal and tumoricidal activities of macrophages [10]. Thus scavenging of $O_2^{-\bullet}$ by superoxide dismutase, yielding H_2O_2, could be expected to ameliorate these sections. However, superoxide dismutase has little or no effect on the cytotoxic effect of 3-morpholinosydnonimine-*N*-acetyl-carbamide, an NO donor that also releases $O_2^{-\bullet}$ [11], in rat hepatoma cells [12] and bacteria [13]. One possible prediction of these findings is that an interaction between NO and H_2O_2 is, at least in part, responsible for cell lysis. Indeed, recent studies suggested that H_2O_2, rather than $O_2^{-\bullet}$ enhances the cytolytic activity of NO against hepatoma cells [12] and endothelial cells [14]. Potentiation of H_2O_2-induced killing of *Escherichia coli* by NO has also been reported [15].

Previous studies have demonstrated that free oxygen radicals generated by macrophages contribute to killing of murine and human lymphoma cells *in vitro* [16]. Furthermore exposure of human lymphoblasts to NO gas results in DNA damage and mutation [17]. The present study demonstrates that a combination of sublytic concentrations of chemically generated NO and H_2O_2 leads to death of murine lymphoma cells, in part via induction of apoptosis.

EXPERIMENTAL

Materials

H_2O_2, catalase (from bovine liver; 2000 units/mg of protein), superoxide dismutase (4200 units/mg of protein), haemoglobin (from bovine erythrocytes), sodium nitroprusside (SNP), $NaNO_2$, $K_4Fe(CN)_6$, nicotinamide and 3-aminobenzamide were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *S*-Nitroso-*N*-acetyl-D,L-penicillamine (SNAP) was from Research Biochemicals International (Natick, MA, U.S.A.). All chemicals were dissolved in RPMI 1640 medium immediately before use. A stock solution of Acridine Orange (Fisher, Ottawa, Ontario, Canada) and ethidium bromide (Sigma), each at 100 μg/ml, was prepared in 0.9% NaCl solution.

Cell culture

YAC-1, a murine lymphoma cell line (American Type Cell Culture, Rockville, MD, U.S.A.) was grown as suspension cultures in RPMI 1640 medium supplemented with 10% fetal bovine serum (HyClone, Logan, UT, U.S.A.), 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin (culture medium).

Radiolabelling of tumour cells

YAC-1 cells [$(0.5–2)\times10^6$ cells] were labelled in their cytoplasm with $Na[^{51}Cr]O_4$ (100 μCi; New England Nuclear, Boston, MA, U.S.A.) and/or their DNA with $[^{125}I]$deoxyuridine ($[^{125}I]$dUrd; 10 μCi; ICN Radiochemicals, Irvine, CA, U.S.A.). The doubly labelled cells were used only if the incorporation level of

Abbreviations used: SNP, sodium nitroprusside; SNAP, *S*-nitroso-*N*-acetyl-D,L-penicillamine; $[^{125}I]$dUrd, $[^{125}I]$deoxyuridine.
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[^{125}I]dUrd exceeded 1 c.p.m./cell and if the $^{125}I/^{51}Cr$ radioactivity ratio was greater than 2.

Quantification of DNA fragmentation and cytolysis

Radiolabelled YAC-1 cells (2.5×10^3) were cultured with SNP, $NaNO_2$, $K_4Fe(CN)_6$, H_2O_2 or a combination of sublytic concentrations of SNP (0.78 mM) plus H_2O_2 (7.8 μM) or SNAP (0.18 mM) plus H_2O_2 (7.8 μM) in the absence and presence of oxyhaemoglobin (10 μM) or catalase (200 units/ml) at 37 °C in 5 % CO_2 in air for the times indicated. In experiments designed to test the effects of inhibitors of poly(ADP-ribosylation), the cells were preincubated with nicotinamide (0.6, 2.5 or 10 mM) or 3-aminobenzamide (4 mM) for 30 min before addition of SNP plus H_2O_2. Preliminary results showed that 30 min exposure of cells to these agents resulted in maximal inhibition of cell lysis. At the end of the culture period, the cells were centrifuged at 200 ***g*** for 10 min and the incubation medium was carefully withdrawn and saved. The cell pellet was lysed with 1 ml of 10 mM Tris/HCl, pH 7.5, containing 1 mM EDTA and 0.2 % Triton X-100. The lysates were then centrifuged at 13000 ***g*** for 10 min to separate fragmented from intact chromatin. The radioactivity present in the incubation medium, in the 13000 ***g*** supernatant and in the 13000 ***g*** pellet were quantified using a Wallac 1470 Wizard Automatic Gamma Counter (Turku, Finland). The system was programmed to correct for cross-talk and spillover between detectors and counting channels. The percentage ^{51}Cr release was calculated by using the following formula:

$$\%\ \text{lysis} = (\text{c.p.m.}_{\text{released}}/\text{c.p.m.}_{\text{total}})\times100$$

where $\text{c.p.m.}_{\text{released}}$ is the radioactivity present in the incubation medium and $\text{c.p.m.}_{\text{total}}$ is the total ^{51}Cr radioactivity present in the incubation medium, 13000 ***g*** supernatant and 13000 ***g*** pellet. The percentage fragmented DNA was calculated using the formula:

$$\%\ \text{fragmented DNA} = (\text{c.p.m.}_{\text{fragmented}}/\text{c.p.m.}_{\text{total}})\times100$$

where $\text{c.p.m.}_{\text{fragmented}}$ is the ^{125}I radioactivity present in the culture medium plus the 13000 ***g*** supernatant (i.e. non-sedimenting chromatin) and $\text{c.p.m.}_{\text{total}}$ is $\text{c.p.m.}_{\text{fragmented}}$ plus the ^{125}I radioactivity in the 13000 ***g*** pellet. Percentage specific fragmented DNA and lysis were calculated by using the following formula:

$$\%\ \text{specific fragmented DNA or lysis} = [(E-S)/(100-S)]\times100$$

where E is the experimental value for percentage fragmented DNA or lysis and S is the spontaneous value for percentage fragmented DNA or lysis.

DNA isolation and gel electrophoresis

Non-sedimenting [^{125}I]dUrd-labelled chromatin from 13000 ***g*** supernatants of hypotonically lysed cells was precipitated with 50 % propan-2-ol/0.5 M NaCl, washed with 75 % ethanol, air-dried, redissolved in 10 μl of 10 mM Tris/HCl/1 mM EDTA, pH 7.4, and subjected to electrophoresis in 0.8 % agarose for 90 min at 100 V with 40 mM Tris/20 mM acetic acid/1 mM EDTA as a running buffer. DNA was visualized by autoradiography.

Quantification of apoptotic morphology

The number of live and dead cells with normal and apoptotic nuclei was determined by fluorescence microscopy after staining of the cells with Acridine Orange and ethidium bromide by the method of Duke et al. [18]. A minimum of 200 cells was counted and classified as (a) live cells with normal nuclei (LN), (b) live cells with apoptotic nuclei (LA), (c) dead cells with normal nuclei (DN) and (d) dead cells with apoptotic nuclei (DA). The percentage of dead cells and cells with apoptotic nuclei was calculated as follows:

$$\%\ \text{dead cells} = [(\text{DN}+\text{DA})/\text{total number of cells counted}]\times100$$

$$\%\ \text{of apoptotic cells} = [(\text{LA}+\text{DA})/\text{total number of cells counted}]\times100$$

Intracellular NAD^+ content

YAC-1 cell NAD^+ content was measured by an enzymic cycling method using alcohol dehydrogenase after extraction with 0.5 M perchloric acid as described by Bernofsky and Swan [19]. Cell NAD^+ content is expressed as percentage of untreated cells.

Statistics

Values are reported as means ± S.E.M. Statistical analysis of the data was performed by a non-parametric analysis of variance (Kruskal–Wallis test) followed by Dunn's multiple contrast hypothesis test to identify differences between various treatments or by the Mann-Whitney U test for paired comparisons. A $P < 0.05$ level was considered significant for all tests.

RESULTS

Cytotoxicity of chemically generated NO and H_2O_2

Exposure of YAC-1 cells to increasing concentrations of either SNP or H_2O_2 resulted in a dose-dependent increase in chromium release with apparent EC_{50} values of 2.4 mM and 80 μM respectively (Figure 1). Neither $NaNO_2$, the auto-oxidation product of NO, nor $K_4Fe(CN)_6$, up to a concentration of 50 mM, evoked cell lysis (Figure 1).

NO-plus-H_2O_2-induced cell lysis and DNA fragmentation

Incubation of YAC-1 cells for up to 4 h with 0.78 mM SNP, 0.18 mM SNAP or 7.8 μM H_2O_2 did not enhance significantly

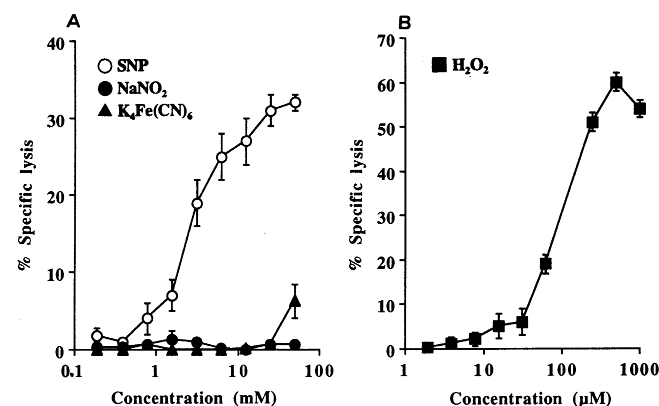

Figure 1 SNP- and H_2O_2-induced lysis of YAC-1 lymphoma cells

$Na[^{51}Cr]O_4$-labelled cells (2.5×10^3) were cultured at 37 °C for 4 h in 5% CO_2. Specific cell lysis was calculated as described in the Experimental section. Values are means ± S.E.M. from three to nine independent experiments carried out in triplicate.

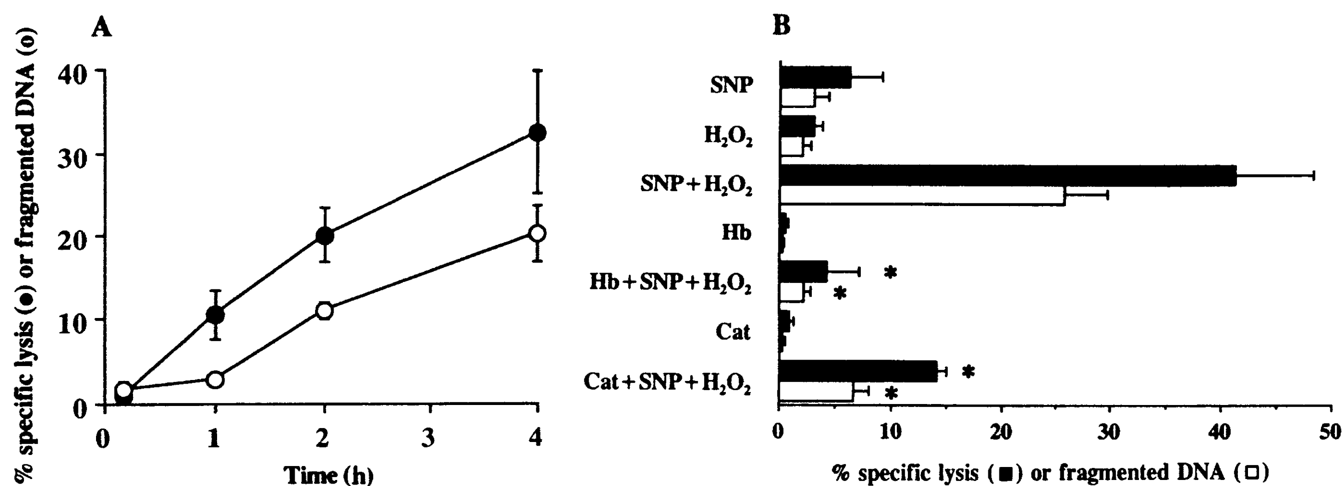

Figure 2 Co-operation of SNP with H_2O_2 in inducing cell lysis and DNA fragmentation in YAC-1 lymphoma cells

(**A**) Time course of DNA fragmentation (○) and cell lysis (●) induced by a combination of a sublytic concentration of SNP (0.78 mM) and H_2O_2 (7.8 μM). (**B**) Doubly labelled tumour cells (2.5×10^3) were cultured with SNP (0.78 mM), H_2O_2 (7.8 μM) or SNP plus H_2O_2 in the absence and presence of oxyhaemoglobin (Hb, 10 μM) or catalase (Cat, 200 units/ml) at 37 °C for 4 h in 5% CO_2. Percentage specific fragmented DNA and cell lysis was calculated as described in the Experimental section. Percentage spontaneous DNA fragmentation and cell lysis were 2–8% and 6–17% respectively. Values are means ± S.E.M. from four to six independent experiments performed in triplicate. $^*P < 0.05$ (compared with SNP-plus-H_2O_2-treated cells by Dunn's multiple contrast hypothesis test).

Table 1 Co-operation of SNAP with H_2O_2 in inducing cell lysis and DNA fragmentation in YAC-1 lymphoma cells

Doubly labelled tumour cells (2.5×10^3) were cultured with SNAP (0.18 mM), H_2O_2 (7.8 μM) or SNAP plus H_2O_2 at 37 °C for 4 h in 5% CO_2. Percentage specific cell lysis and fragmented DNA were calculated as described in the Experimental section. Percentage spontaneous DNA fragmentation and cell lysis were 2–6% and 5–13% respectively. Values are means ± S.E.M. from four independent experiments performed in triplicate.

	Specific cell lysis (%)	Specific fragmented DNA (%)
SNAP	4.1 ± 1.7	2.5 ± 0.9
H_2O_2	3.1 ± 0.7	2.0 ± 0.8
SNAP plus H_2O_2	33.1 ± 2.7	20.2 ± 1.7

Table 2 Effect of chemically generated NO and H_2O_2 on cellular and nuclear morphology of YAC-1 lymphoma cells

Tumour cells (2.5×10^4) were cultured with or without the NO donor SNP (0.78 mM) or SNAP (0.18 mM) and H_2O_2 (7.8 μM) at 37 °C in 5% CO_2 for 4 h, and were stained with a mixture of Acridine Orange and ethidium bromide. The percentage of dead cells and cells with apoptotic nuclei were determined as described in the Experimental section. Values are means ± S.E.M. from *n* independent experiments. $^{**}P < 0.01$; $^{***}P < 0.001$ (compared with control by Dunn's multiple contrast hypothesis test).

	n	Dead cells (%)	Apoptotic cells (%)
Control	6	3.0 ± 1.1	0.6 ± 0.2
SNP	6	8.2 ± 1.1	2.8 ± 0.9
H_2O_2	6	3.9 ± 1.2	2.6 ± 0.8
SNP plus H_2O_2	6	49.7 ± 6.1**	33.6 ± 6.4***
SNAP	4	3.4 ± 0.3	1.4 ± 0.3
SNAP plus H_2O_2	4	39.0 ± 6.6**	26.7 ± 4.4**

chromium or [^{125}I]dUrd release. On the other hand, combining H_2O_2 with either SNP or SNAP resulted in marked increases in chromium release and DNA fragmentation (Figure 2A and Table 1). The order of addition of the NO donor and H_2O_2 did not significantly affect the degree of cytotoxicity. Chromium release exceeded DNA fragmentation at 4 h and at all other time points tested (Figure 2A). Addition of either oxyhaemoglobin (10 μM) or catalase (200 units/ml) to the cells 5 min before exposure to SNP plus H_2O_2 significantly attenuated both chromium and [^{125}I]dUrd release (Figure 2B). Percentage specific cell lysis never exceeded 2 % when YAC-1 cells were incubated with H_2O_2 (7.9 μM) plus $K_4Fe(CN)_6$ (0.78, 1.56 or 3.12 mM) for 4 h (results not shown).

Morphological and molecular evidence for apoptosis in YAC-1 lymphoma cells

A morphological assay was also performed to quantify the number of dead cells and cells with apoptotic nuclei. In this assay, cells are stained with a mixture of Acridine Orange and ethidium bromide, and fluorescence and distribution of the nuclear chromatin are examined by UV microscopy. Whereas SNP (0.78 mM), SNAP (0.18 mM) or H_2O_2 (7.8 μM) alone was unable to evoke apoptotic changes in nuclear morphology in a significant proportion of YAC-1 cells, both SNP-plus-H_2O_2-mediated and SNAP-plus-H_2O_2-mediated cytolysis was associated with apoptotic morphology in about one-third of lymphoma cells (Table 2). The results obtained in the DNA fragmentation and nuclear morphology assays were confirmed by agarose-gel electrophoresis of DNA in the 13000 ***g*** supernatant from equivalent numbers of tumour cells exposed to sublytic concentrations of SNP, H_2O_2 or their combination. Figure 3 shows the presence of low-molecular-mass DNA fragments after treatment with SNP plus H_2O_2. More than 80 % of fragmented DNA had a molecular mass below 2.0 kb. DNA fragmentation was below the level of detection in cells incubated with either SNP (0.78 mM) or H_2O_2 (7.8 μM) (Figure 3).

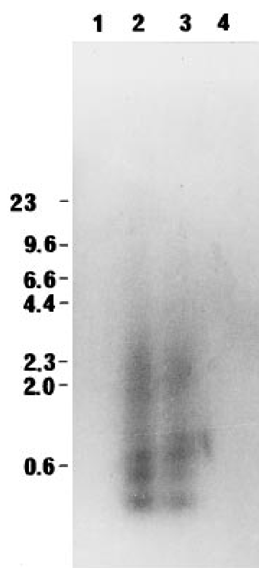

Figure 3 Induction of internucleosomal DNA fragmentation in murine lymphoma cells by SNP plus H_2O_2

Low-molecular-mass DNA, isolated from the 13 000 ***g*** supernatant of [^{125}I]dUrd-labelled tumour cells (2.5×10^6) incubated for 4 h at 37 °C with 0.78 mM SNP (lane 1), 7.8 μM H_2O_2 (lane 4) or a combination of SNP and H_2O_2 in the absence (lane 2) or presence (lane 3) of 10 mM nicotinamide was subjected to electrophoresis in 0.8% agarose and visualized by autoradiography. The molecular mass of the DNA marker ladder is indicated on the left (kb). This is a representative experiment of four.

Table 3 Attenuation of SNP-plus-H_2O_2-induced cytotoxicity by inhibitors of poly(ADP-ribose) polymerase

^{51}Cr-labelled lymphoma cells (2.5×10^3/well) were cultured with nicotinamide (Nic), 3-aminobenzamide (3-AB) or their vehicle for 30 min and then with SNP (0.78 mM) and H_2O_2 (7.8 μM) for 4 h at 37 °C in 5% CO_2. Percentage spontaneous cell lysis was 7–14%. Values are means ± S.E.M. from four to six experiments performed in triplicate. $^*P < 0.05$ (compared with SNP-plus-H_2O_2-treated cells).

	Specific lysis (%)
SNP + H_2O_2	55.8 ± 4.7
Nic (0.6 mM) + SNP + H_2O_2	49.7 ± 2.8
Nic (2.5 mM) + SNP + H_2O_2	43.0 ± 4.6
Nic (10 mM) + SNP + H_2O_2	36.1 ± 5.8*
3-AB (4 mM) + SNP + H_2O_2	38.6 ± 4.9*

Poly(ADP ribosylation) and intracellular NAD$^+$ content

To determine if poly(ADP-ribose) polymerase activation participates in the observed cytotoxicity, we studied the effects of nicotinamide and 3-aminobenzamide on lysis of YAC-1 cells. Nicotinamide produced a concentration-dependent protection with 35% attenuation of cell lysis observed with 10 mM nicotinamide (Table 3). Similar protection was observed with 3-aminobenzamide at 4 mM (Table 3). In contrast, at a concentration of 10 mM, nicotinamide did not prevent SNP-plus-H_2O_2-induced DNA fragmentation (Figure 3).

Since poly(ADP ribosylation) consumes large amounts of cellular NAD$^+$, we analysed intracellular NAD$^+$ content in YAC-1 cells. After 4 h exposure of the cells to 0.78 mM SNP plus 7.8 μM H_2O_2, intracellular NAD$^+$ content decreased to 28–35% of untreated control, whereas neither of these compounds alone had any significant effect on NAD$^+$ content (Table 4). Preincubation of lymphoma cells with nicotinamide (10 mM) or 3-aminobenzamide (4 mM) resulted in a significant increase in cellular NAD$^+$ content and completely prevented the NAD$^+$-depleting effect of SNP plus H_2O_2 (Table 4).

Table 4 Effect of poly(ADP ribosylation) inhibitors on NAD$^+$ content of YAC-1 lymphoma cells

Tumour cells (2.5×10^3/well) were cultured with nicotinamide (Nic, 10 mM) or 3-aminobenzamide (3-AB, 4 mM) for 30 min and then with SNP (0.78 mM), H_2O_2 (7.8 μM) or SNP plus H_2O_2 for 4 h at 37 °C. Intracellular NAD$^+$ content of untreated cells after 4 h culture was set as 100%. Values are means ± S.E.M. from *n* independent experiments performed in triplicate. $^*P < 0.05$; $^{**}P < 0.01$ (compared with SNP-plus-H_2O_2-treated cells).

	n	Cellular NAD$^+$ content (% of control)
SNP	6	82.2 ± 4.6
H_2O_2	7	102.2 ± 6.2
SNP + H_2O_2	6	34.5 ± 2.9
Nic	6	130.8 ± 10.3
Nic + SNP + H_2O_2	5	98.6 ± 7.9*
3-AB	4	99.2 ± 3.5
3-AB + SNP + H_2O_2	4	109.2 ± 12.3**

DISCUSSION

Our results show an interaction between sublytic concentration of chemically generated NO and H_2O_2 which leads to DNA fragmentation and lysis of tumour cells. The potent co-operative action of a combination of either SNP or SNAP with H_2O_2 was confirmed by both measuring ^{51}Cr release from labelled cells and quantifying ethidium bromide-positive cells by fluorescence microscopy. This treatment also induced DNA fragmentation. The DNA extracted from these cells showed the internucleosomal pattern characteristic of apoptosis. In addition, culture of tumour cells with SNP plus H_2O_2 or SNAP plus H_2O_2 resulted in apoptotic nuclear morphology.

The slow kinetics and less extensive DNA fragmentation compared with chromium release make it difficult to conclude whether DNA fragmentation is an early or late event in NO-plus-H_2O_2-induced cytolysis. The differences in the percentage of dead cells and apoptotic morphology would indicate that apoptosis is not the sole mechanism by which NO plus H_2O_2 treatment leads to cell death. *In vivo*, apoptotic cells are usually removed by phagocytosis. However, in culture conditions, this step does not occur because of the absence of scavenging cells. Instead, an apoptotic cell can eventually undergo secondary necrosis [20].

Although the biological activity of SNP is attributed to NO release [21], the mechanism of this release is poorly understood. SNP may release NO spontaneously or in conjunction with cyanide (up to 5 mol/mol of NO) [22]. Since the combination of H_2O_2 with SNAP, an NO donor that does not release cyanide or ferricyanide, resulted in similar synergistic toxicity to that observed with SNP plus H_2O_2, the effects of SNP can probably be solely attributed to NO. The findings that KCN even at 5 mM does not induce DNA fragmentation in murine macrophages [6] and the failure of $K_4Fe(CN)_6$ to potentiate the effect of H_2O_2 are consistent with this notion. Furthermore the cell lysis and DNA fragmentation observed with a combination of SNP plus H_2O_2 require the simultaneous presence of both agents, as removal of either from the system by oxyhaemoglobin, which binds NO [23],

or catalase, which cleaves H_2O_2, effectively prevented both cell lysis and DNA fragmentation. The molecular mechanism(s) of this interaction is not understood at present. *In vitro* studies have suggested that a reaction of NO gas and H_2O_2 produces singlet oxygen or hydroxyl radicals [24,25]. Nevertheless, this has not yet been demonstrated in cell cultures. Alternatively, a reaction of NO with H_2O_2 leading to production of peroxynitrite has also been suggested [13]. Depending on the amount generated, hydroxyl radicals and peroxynitrite can result in apoptotic or necrotic cell damage [3,26,27]. An alternative possibility is that DNA fragmentation and cell death were not a consequence of a direct chemical interaction between NO and H_2O_2, but rather occurred as a synergistic combination of independent effects of NO and H_2O_2 on different targets in YAC-1 cells. This may include accumulation of p53 [28] and/or activation of genes responsible for apoptosis [3] through an oxidative stress-responsive nuclear transcription factor such as NF-κB [29].

The ability of the poly(ADP-ribose) polymerase inhibitors, nicotinamide and 3-aminobenzamide, to attenuate NO-plus-H_2O_2-induced cytotoxicity indicates a mechanism of cell death in which DNA injury overwhelms repair mechanisms [30,31]. Activation of poly(ADP-ribose) polymerase rapidly leads to energy depletion, as each ADP-ribose unit transferred consumes one molecule of NAD^+, and an equivalent of four molecules of ATP are required to regenerate each NAD^+ from nicotinamide. Indeed, the cell death evoked by NO plus H_2O_2 was accompanied by a concomitant decrease in cellular NAD^+ pools, and attenuation of cytolysis by nicotinamide and 3-aminobenzamide parallelled their ability to prevent NAD^+ depletion. In contrast, these inhibitors did not prevent DNA strand breakage, indicating that they did not interfere with the process leading to DNA damage. These observations are consistent with previous results on H_2O_2-induced cell injury [32], and support the notion that impairment of energy supply rather than DNA breakage itself plays a role in cell death. However, whereas nicotinamide and 3-aminobenzamide completely overcame the loss of intracellular NAD^+, they only partially inhibited cell lysis. Thus cell death can be only partially attributed to activation of poly(ADP-ribose) polymerase. It is possible that part of the cell lysis and apoptosis were independent events. Indeed, NO can inhibit the function of iron-bearing enzymes in mitochondria [4,5], and hence could also lead to cell death.

In summary, we have presented evidence that an interaction between NO and H_2O_2, at concentrations at which neither by itself is sufficient to induce cell lysis, produces a potent cytotoxic effect, in part, via induction of apoptosis. The present results also suggest that activation of poly(ADP-ribose) transferase and subsequent NAD^+ depletion play a role in mediating the tumoricidal activity. This co-operative cytotoxic action of simultaneously produced low concentrations of NO and H_2O_2 represents a potential mechanism for the antineoplastic activity of macrophages.

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