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The role of hydroxyl radical as a messenger in the activation of nuclear transcription factor NF-κB

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Abstract

Although it is generally believed that reactive oxygen species activate NF-κB, a primary oxidative stress-responsive transcription factor, it is unclear which one among these species causes NF-κB activation. Our hypothesis is that hydroxyl radical (·OH) functions as a messenger for the activation of NF-κB. Jurkat cells, macrophages and JB6 cells were used to test this hypothesis. Cr(VI), silica and ZnO were used as sources of ·OH radicals. None of these ·OH generating systems involves exogenous H_2O_2. Cr(VI) expressed enhanced activity in induction of NF-κB in Jurkat cells. This activation of NF-κB was decreased by a metal chelator, diethylene triaminepentaacetic acid or a H_2O_2 scavenger, catalase, but was increased by superoxide dismutase. Mn(II), which reacts with Cr(IV) to inhibit this metal ion-mediated ·OH generation, decreased the NF-κB activation. Sodium formate, an ·OH radical scavenger, also inhibited the NF-κB activation. Electron spin resonance measurements show that Cr(VI) was reduced by Jurket cells to Cr(IV) and Cr(V). During the reduction process, molecular oxygen was reduced to O_2^- and then to H_2O_2, which reacted with Cr(IV) and Cr(V) to generate ·OH radical. The ·OH generation correlated with the Cr(VI)-induced NFκB activation. Similarly, silica caused NF-κB activation in macrophages via the ·OH radical-mediated reaction. This radical was generated via metal mediated reaction from H_2O_2, which was generated by the reduction of molecular oxygen via O_2^- as an intermediate during the silica-stimulated 'respirable burst'. Silica particles did not cause ·OH generation either in Jurket or in JB6 cells and thus did not cause any observable NF-κB activation in these cells. ZnO induced NF-κB activation in JB6 cells through the generation of ·OH resulting from light irradiation of ZnO which was measured by electron spin resonance. The results thus show that ·OH radical functions as a messenger for NF-κB activation. Antioxidants, which scavenge ·OH radical or its precursors, inhibit NF-κB activation. Metal chelators, which make metal ions incapable of generating ·OH from H_2O_2, inhibit activation of this transcription factor. (Mol Cell Biochem **194**: 63–70, 1999)

Key words: hydroxyl radical, messenger, NF-κB activation

Introduction

Nuclear transcription factor kappa B (NF-κB) is considered a primary oxidative stress-responsive transcription factor that functions to enhance the transcription of a variety of genes, including those for cytokines and growth factors, adhesion molecules, immunoreceptors, and acute response proteins [1, 2]. The major form of NF-κB in unstimulated cells is present in the cytoplasm as a heterodimer of p50 and p65 subunits complexed with the inhibitory subunit (IκB) that prevents migration of p50/p65 to the nucleus [1, 2]. Many types of stimuli trigger phosphorylation and proteolytic

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degradation of the bound IκB protein. Loss of IκB is followed by a rapid translocation of the p50/p65 heterodimer to the nucleus. As reviewed earlier [3–8], most, if not all, agents activating NF-κB tend to trigger the formation of reactive oxygen species (ROS). NF-κB activation is inhibited by a broad range of antioxidants [3–8]. These observations have led to a general agreement that NF-κB activation is at least facilitated by ROS [3–8]. Eukaryotic cells continuously produce ROS by electron transfer reactions. Exogenous sources of ROS include UV light, toxic chemicals, drugs, metals, minerals and pesticides. Generation of ROS by phagocytic cells under stimulation is a normal process of cell defense against foreign substances [9]. Biological systems are protected against oxidative injury by enzymatic and nonenzymatic antioxidants. When the balance between prooxidant and antioxidants shifts in favor of prooxidants, oxidative stress occurs.

ROS typically include, but are not limited to superoxide (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH). O_2^- is generated by one-electron reduction of molecular oxygen and is a precursor of H_2O_2. Stable overexpression of cytoplasmic Cu/Zn-dependent superoxide dismutase (SOD), which enhances the production of H_2O_2, can potentiate NF-κB activation [10]. Several studies have indicated a role of H_2O_2 in the activation of NF-κB [1, 7, 10–12]. However, several recent studies have questioned its role [13–16]. The concentrations of exogenous H_2O_2 required for NF-κB activation are in the range approaching those that cause cell injury [13]. H_2O_2 could not cause NF-κB activation in either lymphoblastoid T (J. Jhan) or monocytic (U937) cell lines [14], which responded to PMA or TNT [15]. Furthermore, transient overexpression of catalase in COS-1 cells, which should have decreased H_2O_2 levels, did not effect the TNF-α- or PMA-induced NF-κB activation [16].

As for ·OH, it is generated by reaction of metal ions with H_2O_2, the latter being produced by the dismutation of O_2^-. Thus, O_2^- and H_2O_2 function as precursors for ·OH radical generation via a metal mediated Fenton or Fenton-like reaction (Equation [a]).

$$M^{n+} + H_2O_2 \text{ ----> } M^{(n+1)+} + \cdot OH + OH^- \qquad [a]$$

Where M^{n+} represents reduced metal ions, including Fe(II), V(IV), Cr(IV), Cr(V), and Cu(I). $M^{(n+1)+}$ represent oxidized metal ions. The ·OH radical is the most reactive free radical among ROS. This radical is able to cause DNA damage, lipid peroxidation and protein modification [17]. In our earlier study [18], we have shown that Cr(VI) is able to cause NF-κB activation and ·OH radicals were implicated in the mechanism of NF-κB activation. In the present study, we hypothesize that among ROS the ·OH radical functions as a messenger in NF-κB activation. H_2O_2 would cause NF-κB activation due to ·OH radical generation via the reaction of H_2O_2 with metal ions. Any scavengers of ·OH radical or its precursors should be potential inhibitors of NF-κB activation. Any chelator, which removes metal ions and makes them incapable of generating ·OH radicals should block NF-κB activation. The major goal of the present study is to test this hypothesis. Several different sources of ·OH radicals were used. They are (a) Cr(V)-mediated Fenton-like reaction in Jurket cells, (b) silica-stimulated ·OH generation in macrophages and (c) ·OH generation by light irradiation of ZnO in JB6 cells. These ·OH generating systems are chosen in such a way that none of them involves exogenous H_2O_2. The results show that ·OH functions as a messenger in the activation of NF-κB.

Materials and Methods

Materials

Potassium dichromate ($K_2Cr_2O_7$), diethylenetriaminepentaacetic acid (DTPA), deferoxamine, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), SOD, catalase, ZnO, glutathione reductase, sodium formate, and $MnCl_2$ were purchased from Sigma (St. Louis, MO, USA). Crystalline silica (particle diameter, 2–7 g) was provided by U.S. Silica Corp., (Berkeley Springs, WV, USA). The spin trap, DMPO, was purified by charcoal decolorization and vacuum distillation and was free of ESR detectable impurities. Chelex 100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA, USA). The phosphate buffer (pH 7.4) was treated with chelex 100 to remove transition metal ion contaminants.

ESR measurements

All ESR measurements were conducted using a Varian E4 ESR spectrometer and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reference standards. Reactants were mixed in test tubes in a total final volume of 450 µl and were then transferred to a flat cell for ESR measurement. The concentrations given in the figure legends are final concentrations.

Oxygen consumption measurements

Oxygen consumption measurements were carried out using a Gilson oxygraph. Cell concentration was 1.0×10^6/ml and measurements were made over a period of 10 min.

Oligonucleotide

Oligonucleotides were synthesized by the phosphoramidite method on a DNA/RNA synthesizer (Applied Biosystems, Model 392, Foster City, CA, USA). A NF-κB binding sequence (5′GAAATTCCAAAGAGTCATCAGA 3′) from the promotor region of the human IL-2 receptor α chain gene was used to synthesize a NF-κB binding oligonucleotide. The synthesized single-stranded oligonucleotides were deprotected at 50°C overnight, dried in a speed vacuum and then resolved in the Tris-EDTA buffer. Complimentary strands were denatured at 80°C for 5 min and annealed at room temperature. The double-stranded probe was labeled with ^{32}P-dCTP (Amersham, Arlington Heights, Il, USA) using a Klenow fragment (BRL, Gaithersburg, MD, USA).

Cell culture

Jurkat cells ($CD4^+$ human lymphoblast cell line) were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 2 mM glutamine and 100 U/ml penicillin-streptomycin (complete medium). A final concentration of 2×10^6/ml cells were used.

Mouse macrophage cell line RAW 264.7 cells were obtained from American Type Culture Collection (ATCC) (Rockville, MD, USA). The cells were maintained in DMEM (Mediatech, Washington, DC, USA) supplemented with 10% fetal bovine serum, 2 mM glutamine, and 1000 units/ml penicillin-streptomycin. RAW 264.7 cells (5×10^6) were stimulated with 100 µg/ml silica and other test agents, catalase, SOD, sodium formate, and deferoxamine, for 6 h.

NF-κB luciferase reporter plasmid was described previously [19]. The stable NF-κB luciferase reporter plasmid transfected JB6 mouse epidermal cells were cultured in monolayers at 37°C, 5% CO_2 using Eagle's minimal essential medium containing 5% fetal calf serum, 2 mM L-glutamine, and 25 µg of gentamicin/ml.

Nuclear extraction

The nuclear extracts were prepared according to the method described earlier [20]. Briefly, 2×10^6 cells were treated with 500 µl lysis buffer (50 mM KCl, 0.5% NP-40, 25 mM Hepes pH 7.8, 1 mM PMSF, 10 µg/ml Leupeptin, 20 µg/ml Aprotinin, and 100 µM DTT) on ice for 4 min. After 1 min centrifugation at 14,000 rpm, the supernatant was saved as a cytoplasmic extract. The nuclei were washed once with the same volume of buffer without NP-40, then were put into a 300 µl volume of extraction buffer (500 mM KCl, 10% Glycerol with the same concentrations of Hepes, PMSF, Leupeptin, Aprotinin and DTT as the lysis buffer) and pipetted several times. After centrifugation at 14,000 rpm for 5 min, the supernatant was collected as the nuclear protein extract and stored at –70°C. The protein concentration was determined by BCA (Pierce, Rockford, IL, USA).

Electrophoretic mobility shift assay (EMSA)

The DNA-NF-κB binding reaction was conducted in a 24 µl reaction mixture containing 1 µg Poly dI.dC (Sigma), 3 µg nuclear protein extract, 3 µg BSA, 4×10^4 cpm of ^{32}P-labeled oligonucleotide probe and 12 µl of 2 × Y buffer (24% glycerol, 24 mM Hepes, pH 7.9, 8 mM Tris-HCl, pH 7.9, 2 mM EDTA, 2 mM DTT). This mixture was incubated on ice for 10 min in the absence of the radiolabelled probe, then incubated for 20 min at room temperature in the presence of radiolabelled probe. After the incubation, the DNA-protein complexes were resolved on a 5% acrylamide gel (National Diagnostics, Atlanta, GA, USA) that had been pre-run at 110 V for 1 h with 0.5 × Tris-boric acid-EDTA buffer. The loaded gel was run at 210 V for 90 min, then dried and placed on Kodak X-OMAT film (Eastman Kodak, Rochester, NY, USA) for autoradiography. The film was developed after overnight exposure at –70°C.

NF-κB transcriptional activity assay

Confluent monolayers of JB6 cells with NF-κB reporter plasmid were trypsinized and 8×10^3 viable cells suspended in 100 µl 5% FBS MEM medium were added into each well of 96-well plate. Plates were incubated at 37°C in a humidified atmosphere of 5% CO_2. Twelve to 24 h later, cells were starved by culturing them in 0.1% FBS MEM for 12 h. The cells were exposed to ZnO for NF-κB activation for 24 h at 37°C in a 5% CO_2 atmosphere and then lysed with lysis buffer. The luciferase activity was measured by a luminometer (monolight 2010). The results are expressed as the relative NF-κB-dependent luciferase activity.

Results

NF-κB activation by Cr(VI)

The reduction of Cr(VI) by Jurkat cells was studied since reduction of Cr(VI) to its low oxidation states is required for Cr(VI)-mediated free radical generation [21-23]. Figure 1 a shows an ESR spectrum (g = 1.9793) obtained from a mixture containing Cr(VI) and Jurkat cells in pH 7.4 phosphate buffer. The g = 1.9793 is typical of Cr(V) species [21]. Addition of glutathione reductase, which catalyzed the Cr(VI) reduction, enhanced the Cr(V) formation (Fig. 1b). It may

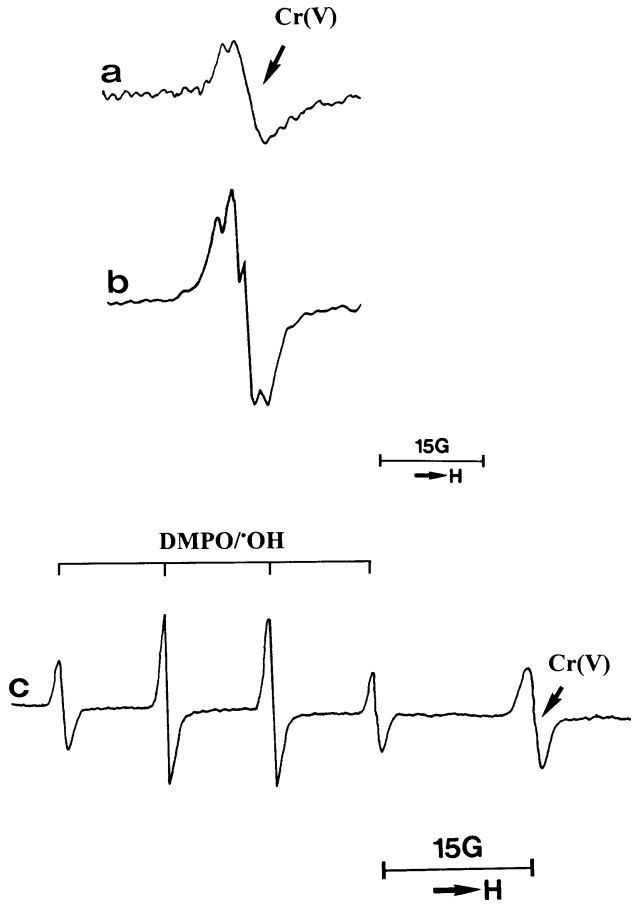

Fig. 1. (a) An ESR spectrum recorded from a phosphate buffer solution (pH 7.4) containing 2 mM $K_2Cr_2O_7$ and 2.0×1 CmI Jurkat cells. The spectrum was recorded 4 min after reaction initiation. (b) Same as (a) but with 2.5 units/ml glutathione reductase added. (c) Same as (a) but with 100 mM DMPO added. The spectrometer settings were: receiver gain, 5.0×10^4; modulation amplitude, 1.6 G; scan time, 4 min; scan width, 3 3 74 ± 100 G.

be noted from Fig. 1b that the intensity of the spectrum is strong enough to exhibit five partially resolved principal components with 0.84 G spacing. This spectrum is reminiscent of a Cr(VI)-NADPH complex reported earlier [21].

Figure 1c shows the ESR spectrum recorded from a mixture containing the Jurkat cells, Cr(VI), glutathione reductase (GSSG-R), and DMPO in a pH 7.4 phosphate buffer. The spectrum is a combination of 1:2:2:1 quartet and a peak at g = 1.9793. The computer simulation analysis of the quartet shows that it has hyperfine splittings of $a_H = a_N$ = 14.9 G, where a_H and a_N denote hyperfine splittings of hydrogen and nitroxyl nitrogen. Based on these splitting constants [21, 24], the 1:2:2:1 quartet was assigned to DMPO/·OH adduct, demonstrating the generation of ·OH radicals. The peak at g = 1.9793 was the one corresponding to the Cr(V) species. Table 1 shows the effects of various reagents on the ·OH radical generation. Cells alone or a mixture of cells and glutathione reductase did not generate any significant amount of ·OH radicals. A mixture of cells and Cr(VI) generated ·OH radicals albeit low yield. A mixture of cells, Cr(VI) and glutathione reductase generated a substantial amount of ·OH radicals. A metal chelator, DTPA, inhibited the yield. SOD enhanced the ·OH generation while catalase blocked it. Mn(II), which reacts with Cr(IV) to block Cr(IV)-mediated ·OH radical generation, decreased the ·OH radical formation. The hydroxyl radical scavenger, sodium formate, also decreased the ·OH radical generation.

Oxygen consumption measurements show that Cr(VI) stimulated cells consumed twice the amount of molecular oxygen as untreated cells (data not shown).

Jurkat cells were used to detect Cr(VI)-induced NF-κB activation. The cells were incubated in the presence of 1 μM $K_2Cr_2O_7$ for 3 h and then were harvested for extraction of the nuclear proteins. These nuclear proteins were analyzed by the electrophoretic mobility shift assay (EMSA) for the DNA binding activity of NF-κB. As shown in Fig. 2, lane 1, the untreated Jurkat cells did not exhibit any NF-κB activity. After treatment with Cr(VI), the cells showed enhanced NF-κB activity (Fig. 2, lane 2) and this activity was inhibited upon addition of DTPA (Fig. 2, lane 3).

In an effort to investigate the mechanisms by which Cr(VI) induces the activation of NF-κB, we examined the effects of several ·OH radical scavengers and enhancers on Cr(VI)-mediated NF-κB activation. SOD, which reacts with O_2^- to generate H_2O_2, enhanced the NF-κB activation (Fig. 2, lane 4). In contrast, catalase, which reacts with H_2O_2, attenuated the activation (Fig. 2, lane 5). Addition of Mn(II), which reacts with Cr(IV) to inhibit Cr(IV)-mediated ·OH generation from H_2O_2, decreased Cr(VI)-induced NF-κB activation (Fig. 2, lane 6). Sodium formate, an ·OH radical scavenger, blocked the NF-κB activation (Fig. 2, lane 7). These results indicate a role of ·OH radicals in the mechanism of Cr(VI)-induced NF-κB activation.

Table 1. DMPO/·OH formation from Cr(VI) reduction in Jurkat cells

Reaction mixture[a]	Relative DMPO/·OH intensity
Cells	0
Cells + GSSG-R	0
Cells + Cr(VI)	0.7
Cells + GSSG-R + Cr(VI)	2.0
Cells + GSSG-R + Cr(VI) + DTPA	0.6
Cells + GSSG-R + SOD	4.5
Cells + GSSG-R + Cr(VI) + catalase	0.2
Cells + GSSG-R + Cr(VI) + Mn(II)	0.8
Cells + GSSG-R + Cr(VI) + formate	0.2

[a]Concentrations of reagents in the reaction mixtures were as follows: cells, 1.0×10^6; GSSG-R, 10 units/ml; Cr(VI), 2 mM; DTPA, 2 mM; SOD, 1,000 units/ml; catalase, 10,000 units/ml; Mn(H), 2 mM; formate, 100 mM. Reatants were mixed in a pH 7.4 phosphate buffer and 100 mM DMPO at room temperature.

NF-κB activation by silica

ESR spin trapping methodology was used to study the ·OH radical generation from silica suspensions and from the mouse macrophage cell line RAW 264.7 and its possible inhibition by deferoxamine, catalase, sodium formate, and SOD. The results show that ·OH radicals are generated from both silica in suspension and from silica-stimulated macrophages (data not shown), in agreement with earlier reports [25, 26]. In both of these cases, deferoxamine, catalase and formate inhibited the ·OH generation [25, 26].

Silica-induced ·OH generation in macrophage was used to examine the role of ·OH radical as a messenger for NF-κB activation. The cells were exposed to silica for 6 h and NF-κB binding activity was analyzed by EMSA. As shown in Fig. 3, lane 1, the untreated cells did not exhibit any NF-κB activity. Upon treatment with silica, the cells showed enhanced NF-κB binding activity (Fig. 3, lane 2) in agreement with earlier reports [27, 28]. As a negative particle control, TiO_2 did not exhibit any observable enhancement of NF-κB binding activity (data not shown). The effect of catalase on silica-induced NF-κB activation was evaluated to study the role of H_2O_2 as a ·OH precursor. As shown in Fig. 3, lane 3, catalase inhibited silica-induced NF-κB activation. In contrast, SOD enhanced it (Fig. 3, lane 4). Inactivated catalase or SOD did not exhibit any observable effects (data not shown).

The ·OH radical scavenger, formate, or the metal chelator, deferoxamine, were tested for their effects on NF-κB activation induced by silica. As shown in Fig. 3, lane 5, formate significantly inhibited silica-induced NF-κB activation. Deferoxamine also inhibited silica-induced NF-κB activation (Fig. 3, lane 6).

NF-κB activation by ZnO

ESR spin trapping has been carried out to detect ·OH radical generation in a ZnO suspension under ambient light. A suspension of ZnO in DMPO in dark did not generate any significant ESR spin adduct signal (Fig. 4a). Under ambient light, this ZnO suspension generated a strong ESR signal (Fig. 4b), which is characterized as DMPO/·OH adduct, providing evidence of ·OH generation. Oxygen consumption measurements show that ZnO suspension under ambient light rapidly consumed molecular oxygen (data not shown), indicating that ·OH radicals are generated via reduction of molecular oxygen.

The stable NF-κB luciferase reporter plasmid transfected JB6 mouse epidermal cells were used to detect NF-κB activation by ZnO using luciferase assay. ZnO was used as a source of ·OH radical. As shown in Fig. 5, ZnO caused a dose dependent increase in NF-κB-mediated luciferase activity, demonstrating that ZnO is able to induce NF-κB activation.

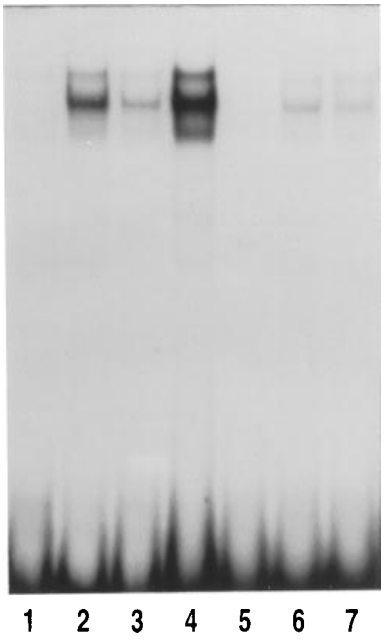

Fig. 2. NF-κB induction by Cr(VI). Jurkat cells were adjusted to a density of 2×10^6/Ml and treated with different agents for 3 h. Concentrations of each agent are DTPA, 4 μM; SOD, 500 units/ml; catalase, 10,000 units/ml; Mn^{2+}, 8 μM; sodium formate, 50 μM; In the absence or presence of the agents, the cells were stimulated with a combination of 2 μM Cr(VI) and 2.5 units/ml glutathione reductase for 3 hours. Lane 1, untreated cells; lane 2, cells + Cr(VI); lane 3, cells + Cr(VI) + DTPA; lane 4, cells + Cr(VI) + SOD; lane 5, cells + Cr(VI) + catalase; lane 6, cells + Cr(VI) + Mn^{2+}; lane 7, cells + Cr(VI) + sodium formate.

Fig. 3. Induction of DNA binding activity of NF-κB protein by silica and the effect of catalase, SOD, sodium formate, and deferoxamine. The RAW 264.7 cells were incubated with stimuli for 6 h. Lane 1, untreated cells (5×10^6/ml); lane 2, cells + 100 μg/ml silica; lane 3, cells + 100 μg/ml silica + 10,0000 units/ml catalase; lane 4, cells + 100 μg/ml silica + 500 units/ml SOD; lane 5, cells + 100 μg/ml silica + 0.625 mM sodium formate; lane 6, cells + 100 μg/ml silica + 1.5 mM deferoxamine.

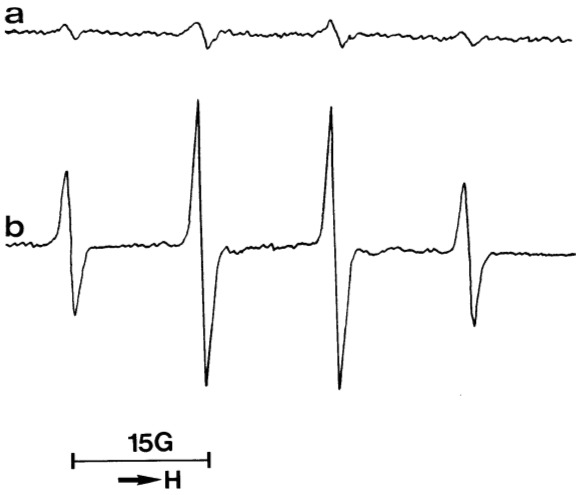

Fig. 4. (a) An ESR spectrum recorded from a phosphate buffer solution (pH 7.4) containing 1 mg ZnO and 100 mM DMPO. The experiment was carried out in the dark. (b) Same as (a) but the experiment was carried out in the ambient light. The spectra were recorded 8 min after mixing ZnO with DMPO. The spectrometer settings were the same as those described in the legend to Fig. 1.

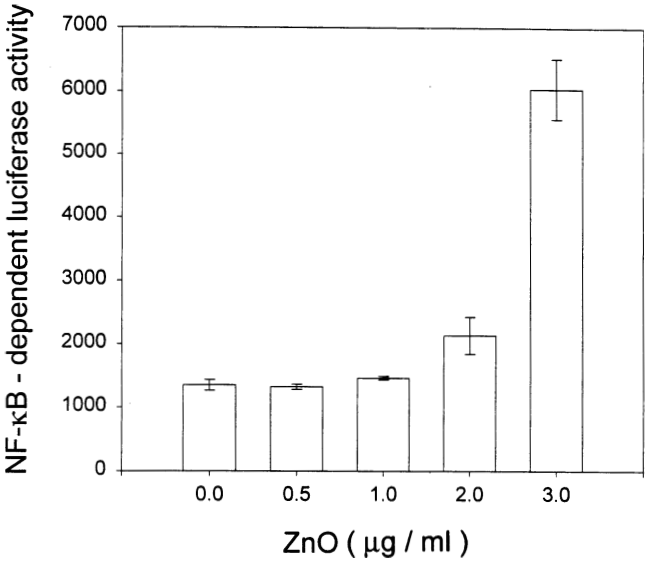

Fig. 5. Induction of transcriptional dependent activity of NF-κB by ZnO in stable NF-κB luciferase reporter plasmid transfected JB6 mouse epidermal cells. The cells were treated for 24 h with different concentrations of ZnO as indicated. The experiment was carried out in ambient light. The NF-κB activity was determined by luciferase activity assays as described in the Materials and methods.

Discussion

Using different cell lines and various ·OH generating systems, the present study shows that ·OH radical functions as a messenger for NF-κB activation. In the case of Cr(VI), the following observations support this conclusion. (a) SOD enhanced the Cr(VI)-induced NF-κB activation. This is due to the fact that SOD increases the H_2O_2 generation via O_2^- dismutation ($O_2^- + 2H^+ \text{---> } O_2 + H_2O_2$); (b) Catalase, which reacts with H_2O_2, eliminated NF-κB activation; (c) Sodium formate, an ·OH radical scavenger, decreased NF-κB activation; (d) ESR measurements show that reduction of Cr(VI) by Jurkat cells generated reactive Cr(V) intermediate. It has been reported that flavoenzymes/NAD(P)H are responsible for the Cr(VI) reduction [29]. During the reduction process, molecular oxygen is reduced to O_2^-, which generates H_2O_2 by a dismutation reaction. In this study, enhanced oxygen consumption was observed in Jurkat cells following Cr(VI) exposure. It has been demonstrated that Cr(V) is capable of generating ·OH radical from H_2O_2 via Fenton-like reaction [21]. Recently, it has also been reported that Cr(IV), an even more reactive chromium intermediate than Cr(V), can also generate ·OH radical from H_2O_2 [22]. The reaction steps of ·OH radical generation from H_2O_2 by both Cr(IV) and Cr(V) are provided in the following reactions (Equations [b] and [c]).

$$Cr(IV) + H_2O_2 \text{ ----> } Cr(V) + \cdot OH + OH^- \quad [b]$$

$$Cr(V) + H_2O_2 \text{ ----> } Cr(VI) + \cdot OH + OH^- \quad [c]$$

By adding Equations [b] and [c], the net reaction becomes (Equation [d]):

$$Cr(IV) + 2H_2O_2 \text{ ----> } Cr(VI) + 2 \cdot OH + 2OH^- \quad [d]$$

The role of Cr(IV)-mediated ·OH radical generation in Cr(VI)-induced NF-κB activation was demonstrated by the attenuation of NF-κB activation upon addition of Mn(II). Mn(II) can react with Cr(IV) and to inhibit Cr(IV)-mediated ·OH generation from H_2O_2 [22]. The Mn(II) effect further supports the conclusion that ·OH radicals play a key role in Cr(VI)-induced NF-κB activation.

Another important result obtained from this study is that a metal ion chelator, DTPA, significantly inhibited Cr(VI)-induced NF-κB activation. It is possible that DTPA inhibited ·OH radical generation via chelation of Cr(V) and Cr(IV).

It may be noted that Cr(VI) compounds are well documented carcinogens. Although their mechanism of action is not well understood, ·OH radical was considered to play an important role. Since NF-κB binding sites function as enhancer element in the c-*myc* gene and this gene is associated with formation of Burkitt's lymphoma [30], Cr(VI) could induce expression of c-*myc* proto-oncogene via NF-κB activation. It is possible that NF-κB activation and a subsequent expression of proto-oncogenes, such as c-*myc*, may serve as a mechanism for induction of neoplastic transformation by Cr(VI).

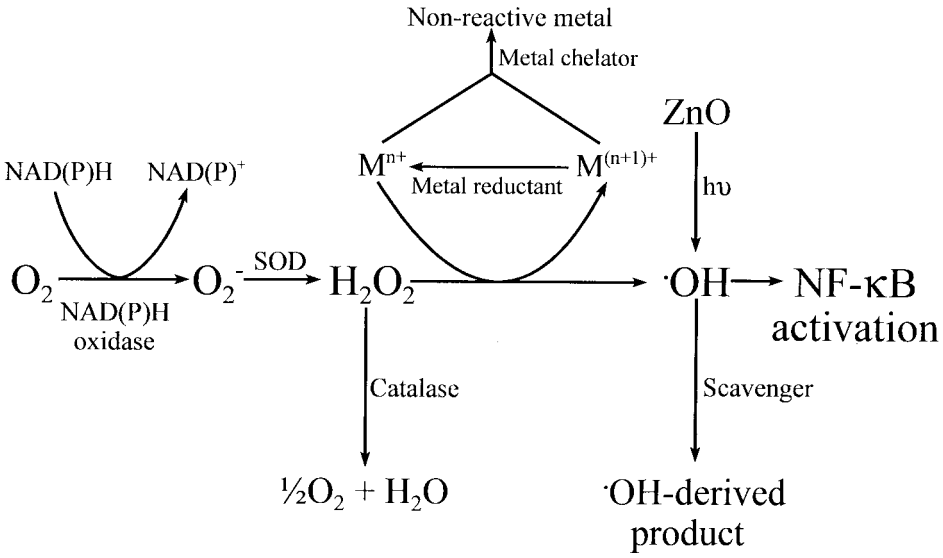

Fig. 6. Schematic representation of the hydroxyl radical-induced NF-κB activation.

The results obtained from the present study also demonstrate that silica particles are able to activate NF-κB in macrophage cells and ·OH radicals play a key role in silica-induced NF-κB activation. The following experimental observations support this conclusion. (a) Silica-stimulated macrophages caused ·OH radical generation, which can be inhibited by catalase and the ·OH radical scavenger, formate [25, 26]. In silica-stimulated macrophages, molecular oxygen is reduced to O_2^- and H_2O_2 through the so called 'respirable burst'. These reactive oxygen species are precursors of ·OH radicals. (b) Catalase blocked the NF-κB activation. (c) SOD enhanced NF-κB binding to DNA. (d) Metal ions, Fe(II) but not Fe(III), enhanced the NF-κB activation (data not shown). It is known that Fe(II) generates ·OH from H_2O_2 via the Fenton reaction (Equation [e]):

$$Fe(II) + H_2O_2 \text{ ----> } Fe(III) + OH^- + \cdot OH \qquad [e]$$

Fe(III), on the other hand, is unable to generate ·OH radical without being first reduced to Fe(II). (e) Metal chelator, deferoxamine, also reduced the NF-κB activation. Deferoxamine chelates metal ions, such as Fe(II) or Fe(III), to make them less reactive toward H_2O_2 and thus attenuates the ·OH radical generation. (f) Formate, an ·OH radical scavenger, inhibited the NF-κB activation. (g) Silica particles did not cause ·OH generation either in Jurkat or in JB6 cells and thus did not cause any observable NF-κB activation in these cell (data not shown).

Luciferase activity assay has been used to examine the role of ·OH in NF-κB activation in JB6 cells using ZnO as a source of ·OH radicals. A ZnO suspension under ambient light generates ·OH radicals as measured by ESR spin trapping and activates NF-κB. Molecular oxygen is a precursor of ·OH radical, as measured by the rapid consumption of molecular oxygen.

In summary, Jurkat cells, macrophage cells and JB6 cells were used in the present study to examine the role of ·OH radical in NF-κB activation. Cr(VI), silica and ZnO were used as sources of ·OH radicals. Molecular oxygen is reduced to O_2^- which produces H_2O_2 via a dismutation reaction. Reaction of H_2O_2 with metal ions generates ·OH radical through a Fenton or Fenton-like reaction. The ·OH radical is the species among ROS that is associated with NF-κB activation. The ·OH-induced NF-κB activation is not limited to certain cell types and ·OH radicals can be from different sources. Antioxidants that scavenge ·OH radical or their precursors inhibit NF-κB activation. Metal chelators, which bind metals and make them incapable of generating ·OH from H_2O_2, block NF-κB activation (Fig. 6).

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