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Hydrogen peroxide induces the death of astrocytes through the down-regulation of the constitutive nuclear factor-kappaB activity

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Abstract
Nuclear factor-kappaB (NF-κB) has a dual role in the promotion or attenuation of cell death. Here, we demonstrated the role of NF-κB in the H_2O_2-induced death of astrocytes. H_2O_2 evoked the release of lactate dehydrogenase (LDH), a marker of cell death, and concomitantly decreased the DNA binding and transcriptional activity of NF-κB in cultured astrocytes. H_2O_2-induced astrocyte death was markedly increased by the co-treatment with pyrrolidinedithiocarbamate, an NF-κB inhibitor. Moreover, the elevation of constitutive NF-κB activity by overexpressing p65 NF-κB subunit attenuated H_2O_2 toxicity, whereas NF-κB inhibition by overexpressing IκB potentiated the toxicity. NF-κB activity and H_2O_2 cytotoxicity was further found to be dependent on cell density. Compared with astrocytes in low cell density, those in high cell density exhibited a higher constitutive NF-κB activity and a stronger resistance to H_2O_2 cytotoxicity. These results indicate that the constitutive activity of NF-κB in astrocytes is required for their survival under oxidative stress such as H_2O_2.

Keywords: *Astrocyte, H_2O_2, NF-κB, LDH release*

Abbreviations: *NF-κB, nuclear factor-kappaB; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase; PDTC, pyrrolidine dithiocarbamate; IκB, inhibitory kappa B*

Introduction

Reactive oxygen species (ROS)-mediated oxidative stress is important in the pathogenesis of the CNS diseases, including ischemic stroke, Alzheimer's disease, Parkinson's disease, and amyotropic lateral sclerosis (for review, see Ref. [1]). Hydrogen peroxide (H_2O_2) also has been closely associated with those neurodegenerative diseases [2,3]. Like other ROS, the cytotoxicity of H_2O_2 is caused by the deterioration of cellular antioxidative systems and energy metabolism in neurons [4,5]. Compared with neurons, astrocytes are in general more resistant to the ROS-mediated oxidative stress due to their high antioxidative capacity [4,6,7]. At high concentrations (>0.2 mM), however, H_2O_2 induces the death of astrocytes [8,9]. At present, however, it is not fully understood how H_2O_2 induces the death of astrocytes.

Nuclear factor-kappaB (NF-κB) is sequestered in the cytoplasm, as bound to isoforms of inhibitory kappaB (IκB). Exposure to a number of stimuli results in the degradation of IκB and unbound NF-κB translocates into the nucleus and transcriptionally

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activates target genes associated with inflammation and cell death (for review, see Refs [10,11]). Many *in vitro* and *in vivo* data have indicated that NF-κB activity is directly or indirectly involved in neuronal death under the neuropathological conditions, including ischemia and Alzheimer's disease [12–14]. In contrast, however, it has been also reported that the inhibition of NF-κB activity exacerbates cytotoxicity by toxic stimuli, including H_2O_2, amyloid β (Aβ), tumor necrosis factor-α (TNF-α) and kainate [15–17]. Taken together with those controversial findings, the cytoprotective or cytodestructive role of NF-κB seems to be cell- and/or stimulus-dependent.

Some researchers have reported that constitutive expression of NF-κB may play a crucial role in the survival of the cells against oxidative stress [18,19]. Previously, NF-κB has been shown to be constitutively expressed in astrocytes [20,21]. Therefore, in the present study, we hypothesized that the constitutive expression of NF-κB pathway could be involved in the resistance of astrocytes to oxidative stress. In the present study, H_2O_2 was employed to study the role of NF-κB against acute oxidative stress. Our results in this study demonstrate that H_2O_2 can induce the death of astrocytes via inhibition of their constitutive NF-κB activity.

Materials and methods

Cell culture

Rat primary astrocytes were cultured from the prefrontal cortices of 2–4-day-old Sprague–Dawley rat pups, as described before [7]. Briefly, cells were dissociated for 10 min by mild trypsinization at 37°C, passed through sterile nylon sieves (80 μm pore size) into Dulbecco's modified Eagle's medium/F12 (DMEM/F12; Gibco BRL, Paisley, UK) containing 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL) and plated onto poly-D-lysine (PDL, 20 μg/ml)-coated 75-cm^2 culture flasks. One week later, cells (2×10^5 cells/ml) were replated onto PDL-coated 48-well plates or 100 mm^2 dish. Five days later, cells were used for the experiments. The cultures consisted of mainly astrocytes (>95%) and microglial cells (<5%), as identified by immunocytochemistry using antibodies against glial fibrillary acidic protein (Santa Cruz Biotech, CA, USA) for astrocytes and isolectin B_4 (Sigma, St Louis, MO, USA) labeling for microglia, respectively. Alternatively, C6 glioma cells, rat astroglial cell line, were cultured in DMEM medium containing 10% heat-inactivated FBS.

Determination of cell death

Cell injury or death was assessed by morphological examination using phase-contrast microscopy and quantified by measuring the amount of lactate dehydrogenase (LDH) released into the bathing medium [7]. Activity of LDH was measured using a diagnostic kit (Sigma Chemical Co.). Cell viability was expressed as percentage of total LDH, which was measured in sister cultures frozen and thawed after the experiments.

Electrophoretic mobility shift assay (EMSA)

Nuclear extract was prepared from astrocytes and then subjected to EMSA according to our previous method [22]. The double-stranded DNA oligonucleotide containing the NF-κB binding site that were purchased from Promega (Madison, WI, USA) were end-labeled using T4 polynucleotide kinase (New England Biolabs, Beverly, MA, USA) in the presence of [γ-^{32}P] ATP. Nuclear proteins (10 μg) were incubated with [γ-^{32}P]-labeled NF-κB probe (about 50,000 cpm) on ice for 30 min. For competition studies, prior to the addition of NF-κB labeled probe, the nuclear extracts were preincubated for 30 min on ice with 30-fold excess of unlabeled oligonucleotide for NF-κB or AP-1. For supershift analysis, the nuclear extracts were preincubated for 1 h on ice with antibodies against p50 or p65 subunit of NF-κB (Santa Cruz). Samples were resolved on a 5% non-denaturing polyacrylamide gel and visualized by autoradiography.

Transient transfection

pCMV–IκB-alpha (IκB-α)-S32AS36A (kindly donated by Dr Zantema, Leiden University, The Netherlands) was used as described elsewhere [23]. pCMV-IκB-α-S32AS36A contains mutated alanines at the serine phosphorylation sites. The cDNA for the p65 subunit of NF-κB was generated by RT-PCR from a total RNA preparation of C6 glioma cells. A set of primers, 5′-CTGGGATCCCTATGGACGAACT-GTTCCCCCTC-3′ and 5′-AGACTCGAGTTAGG-AGCTGATCTGACTCAGC-3′ were used in a PCR to generate an amplicon that consisted of the p65 coding sequence flanked by BamHI and XohI restriction sites. Correct cDNAs were verified by automated sequencing. This PCR product was then ligated with the pcDNA3.1/HisA vector (Invitrogen, Carlsbad, CA, USA) to produce pc-DNA-his-p65. Electroporation was carried out using an Electroporator II (BioRad, Richmond, CA, USA). NF-κB-reporter gene driven by four tandem copies of κ enhancer (κB4) was purchased from Clontech (Palo Alto, CA, USA). Transfection was performed using LipofectAMINE PLUS reagent (Invitrogen). C6 glioma cells were transfected with NF-κB reporter and hygromycin B resistant plasmid (pSGHyg) in the ratio of 10:1. Stably transfectant cells were selected in the presence of 400 μg/ml of hygromycin B (Gibco BRL, Grand Island, NY, USA).

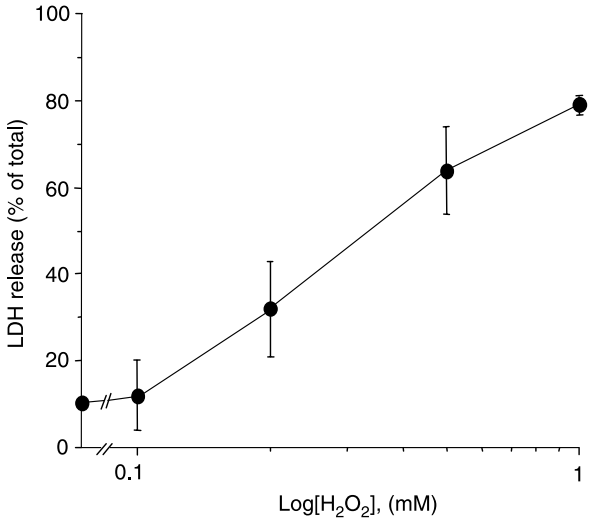

Figure 1. H_2O_2 induces the death of rat primary astrocytes. Cells were incubated for 6 h with increasing concentrations of H_2O_2 (0.1–1 mM). Cell death was determined by measuring the release of LDH, as described in Materials and methods. Data are mean ± SD ($n = 4$).

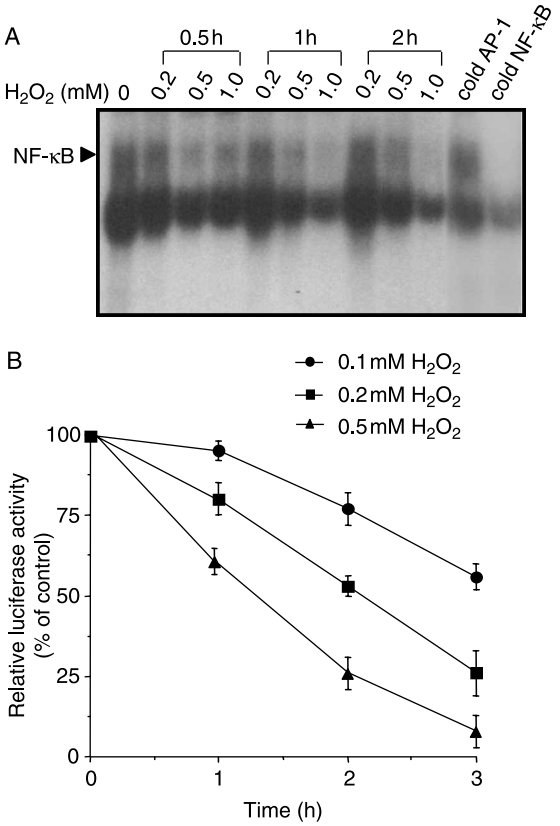

Figure 2. H_2O_2 reduces DNA binding and transcriptional activity of NF-κB in astrocytes. (A) Rat primary astrocytes were treated with various concentrations of H_2O_2 (0.2–1 mM) for the indicated times. Equal amounts of nuclear protein were incubated with the γ-^{32}P-labeled oligonucleotide harboring NF-κB and subjected to EMSA. The arrow head points the super-shifted complexes that were assessed using the antibody against p65 NF-κB subunits. (B) C6 glioma cells were transiently transfected with a plasmid containing the NF-κB-binding site-luciferase construct. Transfected-cells were incubated for 3 h with increasing concentrations of H_2O_2 (0.1–0.5 mM) and the luciferase activity was determined. The luciferase activity is expressed as a percentage of untreated cells. Data mean ± SD ($n = 4$).

Luciferase assay

The transfected cells with the NF-κB-luciferase construct were exposed to various experimental conditions. After treatments, cells were harvested and lysed with reporter lysis buffer (Promega luciferase assay kit). Luciferase assays were performed with a luminometer (LB953, Berthold Co., Wildbad, Germany) in according to the manufacturer's instructions.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) and analyzed for statistical significance by using two way analysis of variance (ANOVA) followed by Sheffe's test as a *post hoc* test and a P value < 0.05 was considered significant.

Results

H_2O_2-induced cell death and NF-κB inactivation

To investigate the effect of H_2O_2 on the viability of rat primary astrocytes, cells were exposed to the various concentrations of H_2O_2 (0.1–1 mM) for 6 h. H_2O_2 increased the release of LDH from cells at concentrations over 0.2 mM (Figure 1). To identify the role of NF-κB in the H_2O_2-induced death of rat primary astrocytes, we examined whether H_2O_2 altered the DNA-binding activity of NF-κB by a gel mobility shift assay (EMSA) using the antibody against p65 subunit. Increasing concentrations of H_2O_2 reduced the intensity of the shifted complex at all time points examined, which occurred as early as 0.5 h after H_2O_2 exposure (Figure 2A). The shifted complex was eliminated in the presence of a 30-fold molar excess of unlabeled NF-κB probe (cold NF-κB) but not an oligonucleotide containing an AP-1 consensus sequence (cold AP-1), indicating that the binding is specific to NF-κB (Figure 2A). We further investigated whether the inactivation of NF-κB by H_2O_2 would be also reflected at the transcriptional level. For this, rat C6 glioma cells were transfected with the reporter plasmid containing NF-κB-luciferase construct. H_2O_2 concentration-dependently decreased the basal luciferase activity of NF-κB (Figure 2B) prior to apparent cell injury or death (data not shown), as assessed morphologically or by measuring LDH activity.

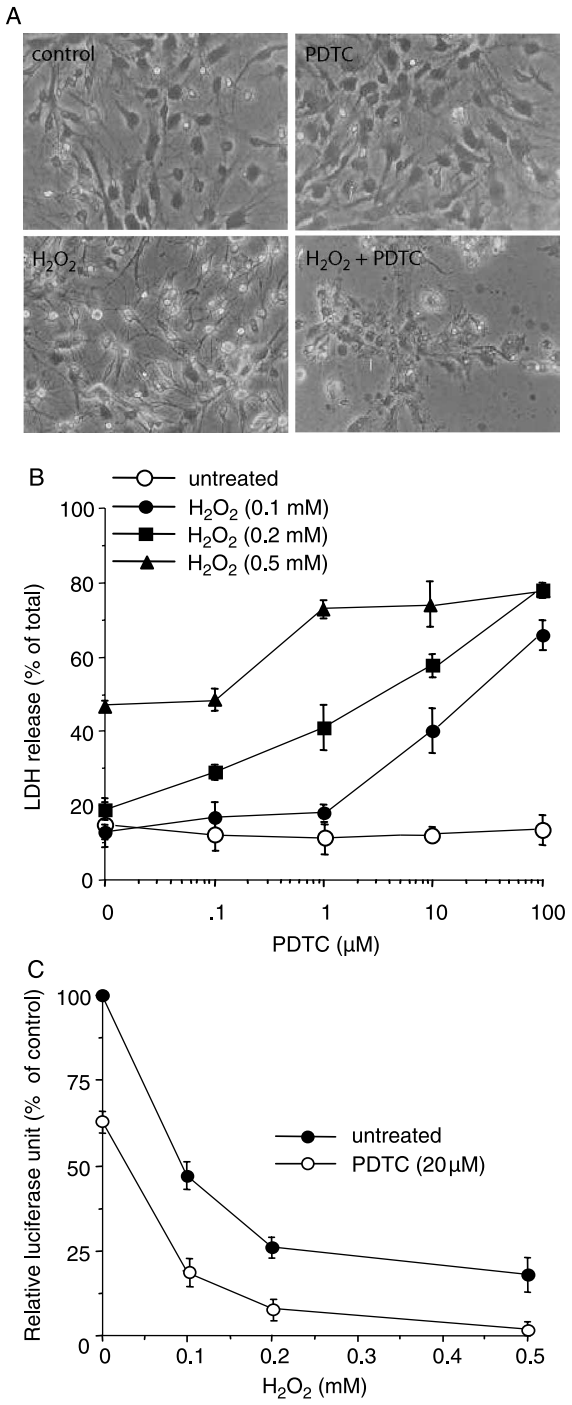

Figure 3. PDTC, a NF-κB inhibitor, potentiates the H_2O_2-induced death of astrocytes. (A) Rat primary astrocytes were exposed to H_2O_2 (0.5 mM) in the absence or presence of PDTC (20 μM). Six hours later, cell death was assessed by detecting morphological changes using a phase-contrast microscope. (B) Rat primary astrocytes were exposed to H_2O_2 (0.1–0.5 mM) in the absence or presence of various concentrations of PDTC (0.1–100 μM). Six hours later, cell death was determined by measuring the release of LDH from cells. (C) C6 glioma cells were transiently transfected with a plasmid containing the NF-κB-binding site-luciferase construct and then exposed to various concentrations of H_2O_2 (0.1–0.5 mM) in the absence or presence of PDTC (20 μM). Three hours later, the luciferase activity was determined and expressed as percentage of untreated control. Data are mean ± SD ($n = 4$).

Potentiation of H_2O_2-induced cytotoxicity by NF-κB inactivation

We further determined whether the inhibition of NF-κB modulated the degree of H_2O_2-induced cytotoxicity in astrocytes. Although it did not induce any cytotoxicity by itself, pyrrolidine dithiocarbamate (PDTC), an NF-κB inhibitor reported to inhibit IκB degradation [24], aggravated the H_2O_2-induced death of astrocytes (Figure 3A,B). PDTC reduced the transcriptional activity of NF-κB (Figure 3C). It is noteworthy that the inhibition of NF-κB by PDTC makes cells vulnerable to low concentrations of H_2O_2 (0.1–0.2 mM). MG-132, another inhibitor for the NF-κB pathway [24], also exacerbated the H_2O_2-induced death of astrocytes (data not shown). Thus, the NF-κB pathway seems to play a protective role in the H_2O_2-induced death of astrocytes.

Next, we further identified the protective role of NF-κB pathway against the H_2O_2-induced death of astrocytes using genetic tools for the activation or inhibition of NF-κB pathway. To examine whether the activation of NF-κB pathway by overexpressing p65 can prevent H_2O_2-induced death of astrocytes, C6 glioma cells were co-transfected with a p65 expressing vector and an indicator vector (4κB-Luc) and then treated with 0.5 mM H_2O_2. Overexpression of p65 partially but significantly blocked the H_2O_2-induced death (Figure 4A) and increased the transcriptional activity of NF-κB (Figure 4B). Stimulation of cells with a wide variety of agents results in the degradation of IκB-α, which allows translocation of NF-κB to the nucleus. Degradation of IκB is triggered by the phosphorylation of two serine residues, i.e. Ser32 and Ser36 and the mutation of both Ser32 and Ser36 completely inhibits ligand-induced phosphorylation of IκB [23]. Thus, we further examined whether the inhibition of NF-κB pathway by overexpressing a stable form of IκB-α (mutated-IκB-α) could exacerbate the death after exposing to H_2O_2. As expected, we observed that C6 glioma cells transfected with an IκB-α-expressing vector were more susceptible to H_2O_2 than untransfected cells (Figure 4A) and lost the transcriptional activity of NF-κB (Figure 4B).

High cell density abrogated both H_2O_2 sensitivity and NF-κB DNA binding activity

It has been reported that cell density is one of the factors for cell viability [18,19]. Thus, astrocytes were initially plated at various densities (0.4×10^5, 2×10^5 or 1×10^6 cells/ml) and cell death was determined after the treatment with 0.5 mM H_2O_2. We found that cells became more resistant to H_2O_2 (0.5 mM)-evoked cytotoxicity (Figure 5A) by increasing cell numbers. Additionally, the DNA-binding activity of NF-κB was decreased by H_2O_2 in astrocytes at lower cell density (5X of 5 days or 1X of 8 days in

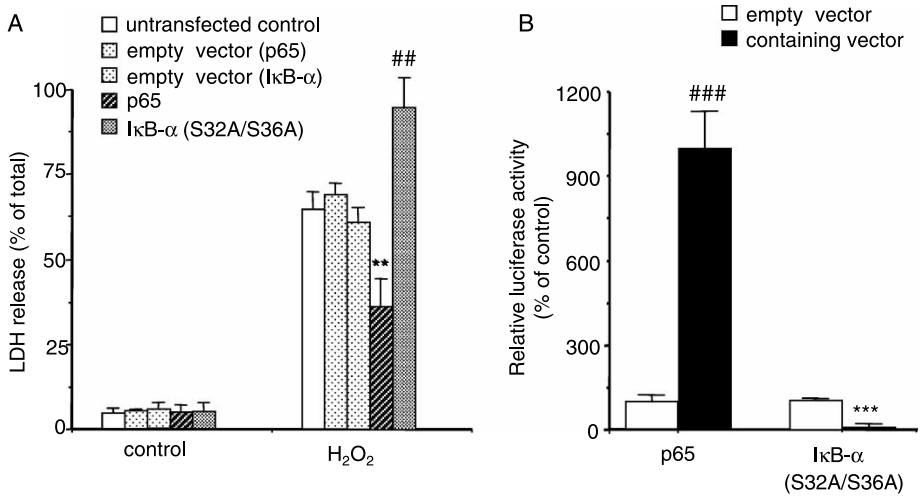

Figure 4. Overexpression of p65 inhibits the H_2O_2-induced the death of C6 glioma cells but that of IκB-α potentiates it. (A) For the increase and decrease in NF-κB activity, C6 glioma cells were transiently transfected with expression vectors for p65 and IκB-α S32A/36A, respectively, and then incubated in the absence or presence of 0.5 mM H_2O_2. Twelve hours later, cell death was determined using LDH assay method. $P < 0.01$, significantly different (**, decrease; ##, increase) from the groups transfected with each control vector. (B) C6 glioma cells were transfected with NF-κB-luciferase construct alone or in combination with each vector for p65 or IκB-α S32A/S36A. Three hours later, the luciferase activity was determined and expressed as percentage of untreated control. Data are mean ± SD ($n = 4$). $P < 0.001$, significantly different (***, decrease; ###, increase) from the groups transfected with each control vector.

culture), but it was not so much affected by H_2O_2 in high-density astrocytes (25X of 5 days or 5X of 8 days in culture) (Figure 5B).

Discussion

Astrocytes may be resistant to oxidative stress due to their high antioxidative capacity [4,6,7]. Our present study provides another evidence that the survival of astrocytes against oxidative stress may be, at least in part, due to their constitutive NF-κB activity: (1) astrocytes exhibit a constitutive activation of NF-κB; (2) H_2O_2 reduces DNA binding as well as transcriptional activity of NF-κB in astrocytes; (3) inhibition of the NF-κB pathway exacerbates, but activation attenuates H_2O_2 cytotoxicity in astrocytes; and (4) increasing cell density makes astrocytes more tolerable to H_2O_2 cytotoxicity, which is consistent with the maintenance of NF-κB activity even in the presence of H_2O_2.

H_2O_2, a major form of ROS, can induce oxidative stress and death in neuropathological conditions. In particular, the accumulated concentration of H_2O_2 in the ischemic stroke-challenged brains increases up to approximately 0.15 mM that is high enough to kill cultured neurons [2]. Even though astrocytes are more resistant to oxidative damage than neurons, they can also be eventually damaged by excessive oxidative

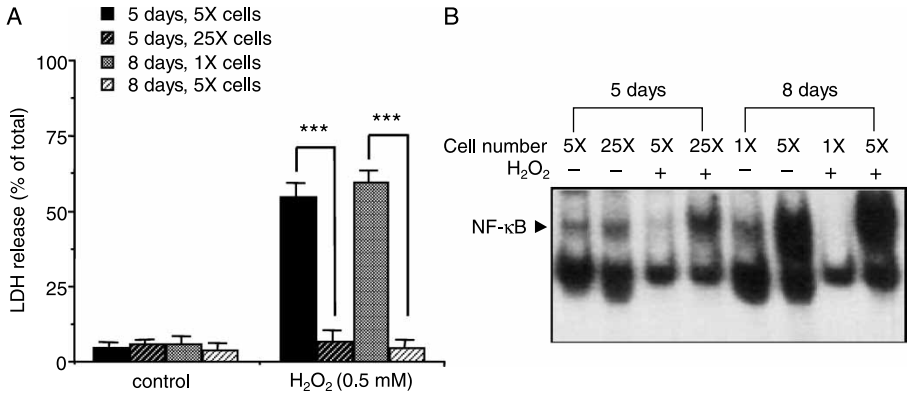

Figure 5. Effect of H_2O_2 on NF-κB activity and cell viability in low- and high-density astrocytes. Astrocytes were initially plated at different densities (4×10^4 (1×), 2×10^5 (5×) and 1×10^6 (25×) cells/ml) and grown for 5 and 8 days. (A) Cells were exposed to 0.5 mM H_2O_2 for 6 h and then cell death was determined using LDH assay method. Data are mean ± SD ($n = 5$). ***$P < 0.001$, significantly different from untreated control. (B) Cells were exposed to 0.5 mM H_2O_2 for 3 h and then the activity of NF-κB was analyzed by EMSA. Data are representative of four separate experiments. Hydrogen peroxide did not significantly change the NF-κB activities in the astrocytes with higher cell density on 5 and 8 days in culture.

stress [25,26]. In the present study, astrocytes were found to be injured or killed by the treatment of 0.2 mM H_2O_2 for several hours. Similarly, many reports have shown that excess H_2O_2 (>0.2 mM) induces the death of astrocytes [27–29].

Much evidence has been accumulated that oxidative stress activates the NF-κB pathway (for review, see Refs [30,31]). In general, however, the up- or down-regulation of NF-κB activity by oxidative stress is thought to be cell- and stimulus-dependent (for review, see Ref. [31]). H_2O_2 activates the NF-κB pathway in non-neuronal cells including Jurkat T cells [32], mouse fibroblasts [33] and neuronal cells such as cerebellar granule cells [34]. In contrast, H_2O_2 inhibits the NF-κB pathway in HeLa cells. Thus, the treatment with high (3 mM) concentration of H_2O_2 completely suppressed the NF-κB activity in unstimulated or tumor necrosis factor (TNF)-stimulated HeLa cells [35]. Similarly, the inhibition of NF-κB activity was observed in H_2O_2-treated astrocytes.

NF-κB activation has been known to mediate pro-apoptotic as well as anti-apoptotic process, depending on stimuli and its target genes (for review, see Refs [11,36]). Cells that are resistant to oxidative stress exhibit constitutive activation of NF-κB possibly as an adaptive defense mechanism [21,37]. Therefore, the inhibition of NF-κB pathway using transdominant negative IκB-α and p65 peptide potentiated Aβ-mediated apoptosis in cerebellar granule cells [38] and TNF-α-induced death in leukemic cells [17]. Overexpression of NF-κB increased cell viability by suppressing the induction of apoptosis in various cell types [39–41]. Similarly, in the present study, the inhibition of NF-κB activity by both chemical inhibitor (PDTC) and overexpressing IκB-α potentiated the H_2O_2-induced death of astrocytes. Furthermore, the elevated basal NF-κB activity by overexpressing p65 subunit reduced the H_2O_2-induced death. However, the molecular mechanisms underlying the anti-apoptotic effect of NF-κB have not been fully clarified. Recently, Jang and Surh reported that bcl-2 could protect against H_2O_2-induced apoptosis through the activation of NF-κB in cultured rat pheochromocytoma PC12 cells [16]. The relationship between bcl-2 and NF-κB activity is needed to be elucidated in astrocytes.

It is important to see the specificity of our present findings with H_2O_2. Previously, we reported that the final product of lipid membrane peroxidation 4-hydroxy-2E-nonenal (HNE) down-regulated the basal activity of NF-κB and increased cytotoxicity [42]. NF-κB inhibitors such as PDTC, *N*-acetyl cysteine, and MG-132 also enhanced the death of neuronal cells caused by oxygen glucose deprivation/re-oxygenation. Thus, inhibition of NF-κB activity might be associated with the oxidative stress-induced cell death in cells with high constitutive NF-κB activity.

In this study, the decreased NF-κB activity was not caused by cell injury or death by H_2O_2. Thus, cell death did not occur in either PDTC alone-treated (Figure 3A,B) or IκB-α-transfected (Figure 4A) cells. In accordance with our data, Takada et al. also demonstrated that the inhibition of NF-κB using p65 peptide did not itself cause any cytotoxicity but it exacerbated the TNF-α-induced cytotoxicity [17].

Some researchers have demonstrated that the density of cells in culture influences the apoptosis-associated cell death as well as the constitutive NF-κB activity [18,19]. Thus, high-density smooth muscle cells were shown to be resistant to apoptosis due to their high constitutive NF-κB activity [18,19]. In the present study, H_2O_2 induced cell death in low-density, but not high-density astrocytes. Constitutive NF-κB activity was found to be elevated in the high-density cells and the activity was not affected by the treatment with H_2O_2 even at high concentration (0.5 mM (Figure 5) and 1 or 3 mM (data not shown)). The present results imply that the high constitutive activity of NF-κB represses the susceptibility of astrocytes to H_2O_2 cytotoxicity.

In conclusion, the current study provides more insight into the protective role of constitutive NF-κB activity against H_2O_2-induced cytotoxicity. Since oxidative stress is one of main factors for the injury in the CNS degenerative diseases, our data may contribute to the better understanding of molecular basis of the diseases and the development of potential therapies to control these disorders. However, the model used in this study reflects acute condition. Thus, more studies should be done for extrapolation of our findings to chronic conditions.

Acknowledgements

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References

[1] Cui K, Luo X, Xu K, Ven Murthy MR. Role of oxidative stress in neurodegeneration: Recent development in assay methods for oxidative stress and nutraceutical antioxidants. Prog Neuropsychopharmacol Biol Psychiatry 2004;28:771–799.

[2] Hyslop PA, Zhang Z, Pearson DV, Phebus LA. Measurement of striatal H_2O_2 by microdialysis following global forebrain ischemia and reperfusion in the rat: Correlation with the cytotoxic potential of H_2O_2 *in vitro*. Brain Res 1995;671:181–186.

[3] Tabner BJ, Turnbull S, El-Agnaf OM, Allsop D. Formation of hydrogen peroxide and hydroxyl radicals from A(beta) and alpha-synuclein as a possible mechanism of cell death in Alzheimer's disease and Parkinson's disease. Free Radic Biol Med 2002;32:1076–1083.

[4] Dringen R, Kussmaul L, Gutterer JM, Hirrlinger J, Hamprecht B. The glutathione system of peroxide detoxification is less efficient in neurons than in astroglial cells. J Neurochem 1999;72:2523–2530.

[5] Vexler ZS, Wong A, Francisco C, Manabat C, Christen S, Täuber M, Ferriero DM, Gregory G. Fructose-1,6-bisphosphate preserves intracellular glutathione and protects cortical neurons against oxidative stress. Brain Res 2003;960:90–98.

[6] Desagher S, Glowinski J, Premont J. Astrocytes protect neurons from hydrogen peroxide toxicity. J Neurosci 1996;16:2553–2562.

[7] Ju C, Yoon KN, Oh YK, Kim HC, Shin CY, Ryu JR, Ko KH, Kim WK. Synergistic depletion of astrocytic glutathione by glucose deprivation and peroxynitrite: Correlation with mitochondrial dysfunction and subsequent cell death. J Neurochem 2000;74:1989–1998.

[8] Papadopoulos MC, Koumenis IL, Yuan TY, Giffard RG. Increasing vulnerability of astrocytes to oxidative injury with age despite constant antioxidant defenses. Neuroscience 1998;82:915–925.

[9] Juknat AA, del Valle M, Méndez A, Quaglino A, Fameli CI, Mena M, Kotler ML. Melatonin prevents hydrogen peroxide-induced Bax expression in cultured rat astrocytes. J Pineal Res 2005;38:84–92.

[10] May MJ, Ghosh S. Signal transduction through NF-κB. Immunol Today 1998;19:80–88.

[11] Mattson MP, Culmsee C, Yu Z, Camandola S. Roles of nuclear factor κB in neuronal survival and plasticity. J Neurochem 2000;74:443–456.

[12] Nurmi A, Lindsberg PJ, Koistinaho M, Zhang W, Juettler E, Karjalainen-Lindsberg ML, Weih F, Frank N, Schwaninger M, Koistinaho J. Nuclear factor-kappaB contributes to infarction after permanent focal ischemia. Stroke 2004;35:987–991.

[13] Chen J, Zhou Y, Mueller-Steiner S, Chen LF, Kwon H, Yi S, Mucke L, Gan L. SIRT1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF-kappaB signaling. J Biol Chem 2005;280:40364–40374.

[14] Zhang W, Potrovita I, Tarabin V, Herrmann O, Beer V, Weih F, Schneider A, Schwaninger M. Neuronal activation of NF-kappaB contributes to cell death in cerebral ischemia. J Cereb Blood Flow Metab 2005;25:30–40.

[15] Fridmacher V, Kaltschmidt B, Goudeau B, Ndiaye D, Rossi FM, Pfeiffer J, Kaltschmidt C, Israël A, Mémet S. Forebrain-specific neuronal inhibition of nuclear factor-κB activity leads to loss of neuroprotection. J Neurosci 2003;23:9403–9408.

[16] Jang JH, Surh YJ. Bcl-2 attenuation of oxidative cell death is associated with up-regulation of γ-glutamylcysteine ligase via constitutive NF-κB activation. J Biol Chem 2004;279:38779–38786.

[17] Takada Y, Singh S, Aggarwal BB. Identification of a p65 peptide that selectively inhibits NF-κB activation induced by various inflammatory stimuli and its role in down-regulation of NF-κB-mediated gene expression and up-regulation of apoptosis. J Biol Chem 2004;279:15096–15104.

[18] Erl W, Hansson GK, de Martin R, Draude G, Weber KSC, Weber C. Nuclear factor-κB regulates induction of apoptosis and inhibitor of apoptosis protein-1 expression in vascular smooth muscle cells. Circ Res 1999;84:668–677.

[19] Erl W, Weber C, Hansson GK. Pyrrolidine dithiocarbamate-induced apoptosis depends on cell type, density, and the presence of Cu^{2+} and Zn^{2+}. Am J Physiol Cell Physiol 2000;278:C1116–C1125.

[20] Bales KR, Du Y, Dodel RC, Yan GM, Hamilton-Byrd E, Paul SM. The NF-kappaB/Rel family of proteins mediates Abeta-induced neurotoxicity and glial activation. Brain Res Mol Brain Res 1998;57:63–72.

[21] Lezoualc'h F, Sagara Y, Holsboer F, Behl C. High constitutive NF-kappaB activity mediates resistance to oxidative stress in neuronal cells. J Neurosci 1998;18:3224–3232.

[22] Woo MS, Jung SH, Kim SY, Hyun JW, Ko KH, Kim W-K, Kim HS. Curcumin suppresses phorbol ester-induced matrix metalloproteinase-9 expression by inhibiting the PKC to MAPK signaling pathways in human astroglioma cells. Biochem Biophys Res Commun 2005;335:1017–1025.

[23] Schouten GJ, Vertegaal AC, Whiteside ST, Israel A, Toebes M, Dorsman JC, van der Eb AJ, Zantema A. IkappaB alpha is a target for the mitogen-activated 90 kDa ribosomal S6 kinase. EMBO J 1997;16:3133–3144.

[24] Snyder JG, Prewitt R, Campsen J, Britt LD. PDTC and MG132, inhibitors of NF-kappaB, block endotoxin induced vasodilation of isolated rat skeletal muscle arterioles. Shock 2002;17:304–307.

[25] Robb SJ, Connor JR. An *in vitro* model for analysis of oxidative death in primary mouse astrocytes. Brain Res 1998;788:125–132.

[26] Almeida A, Almeida J, Bolanos JP, Moncada S. Different responses of astrocytes and neurons to nitric oxide: The role of glycolytically generated ATP in astrocytes protection. Proc Natl Acad Sci USA 2001;98:15294–15299.

[27] Gorina R, Petegnief V, Chamorro A, Planas AM. AG490 prevents cell death after exposure of rat astrocytes to hydrogen peroxide or proinflammatory cytokines: Involvement of the Jak2/STAT pathway. J Neurochem 2005;92:505–518.

[28] Kim EJ, Park YG, Baik EJ, Jung SJ, Won R, Nahm TS, Lee BH. Dehydroascorbic acid prevents oxidative cell death through a glutathione pathway in primary astrocytes. J Neurosci Res 2005;79:670–679.

[29] Shinozaki Y, Koizumi S, Ohno Y, Nagao T, Inoue K. Extracellular ATP counteracts the ERK1/2-mediated death-promoting signaling cascades in astrocytes. Glia 2006;54:606–618.

[30] Li N, Karin M. Signaling pathways leading to nuclear factor-kappa B activation. Methods Enzymol 2000;319:273–279.

[31] Bowie A, O'Neill AJ. Oxidative stress and nuclear factor-κB activation. A reassessment of the evidence in the light of recent discoveries. Biochem Pharmacol 2000;59:13–23.

[32] Takada Y, Aggarwal BB. Flavopiridol inhibits NF-kappaB activation induced by various carcinogens and inflammatory agents through inhibition of IkappaBalpha kinase and p65 phosphorylation: Abrogation of cyclin D1, cyclooxygenase-2, and matrix metalloprotease-9. J Biol Chem 2004;279:4750–4759.

[33] Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. EMBO J 1991;10:2247–2258.

[34] Kaltschmidt B, Uherek M, Volk B, Baeuerle PA, Kaltschmidt C. Transcription factor NF-κB is activated in primary neurons by amyloid β peptides and in neurons surrounding early plaques from patients with Alzheimer disease. Proc Natl Acad Sci USA 1997;94:2642–2647.

[35] Byun MS, Jeon KI, Choi JW, Shim JY, Jue DM. Dual effect of oxidative stress on NF-κB activation in HeLa cells. Exp Mol Med 2002;34:332–339.

[36] Kaltschmidt B, Kaltschmidt C, Hofmann TG, Hehner SP, Dröge W, Schmitz ML. The pro- or anti-apoptotic function of NF-κB is determined by the nature of the apoptotic stimulus. Eur J Biochem 2000;267:3828–3835.

[37] Kim DK, Cho ES, Lee BR, Um HD. NF-kappa B mediates the adaptation of human U937 cells to hydrogen peroxide. Free Radic Biol Med 2001;30:563–571.

[38] Kaltschmidt B, Uherek M, Wellmann H, Volk B, Kaltschmidt C. Inhibition of NF-κB potentiates amyloid β-mediated neuronal apoptosis. Proc Natl Acad Sci USA 1999;96:9409–9414.

[39] Giri DK, Aggarwal BB. Constitutive activation of NF-kappaB causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. J Biol Chem 1998;273:14008–14014.

[40] Poulaki V, Mitsiades CS, Joussen AM, Lappas A, Kirchhof B, Mitsiades N. Constitutive nuclear factor-kappaB activity is crucial for human retinoblastoma cell viability. Am J Pathol 2002;161:2229–2240.

[41] Bian X, Opipari AW, Jr., Ratanaproeksa AB, Boitano AE, Lucas PC, Castle VP. Constitutively active NFkappa B is required for the survival of S-type neuroblastoma. J Biol Chem 2002;277:42144–42150.

[42] Lim JH, Lee JC, Lee YH, Choi IY, Oh YK, Kim HS, Park JS, Kim WK. Simvastatin prevents oxygen and glucose deprivation/reoxygenation-induced death of cortical neurons by reducing the production and toxicity of 4-hydroxy-2E-nonenal. J Neurochem. 2006;97:140–150.