Carbon monoxide activates NF-κB via ROS generation and Akt pathways to protect against cell death of hepatocytes

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Submitted 28 February 2007; accepted in final form 22 May 2008

Kim HS, Loughran PA, Rao J, Billiar TR, Zuckerbraun BS. Carbon monoxide activates NF-κB via ROS generation and Akt pathways to protect against cell death of hepatocytes. Am J Physiol Gastrointest Liver Physiol 295: G146–G152, 2008. First published May 22, 2008; doi:10.1152/ajpgi.00105.2007.—Heme oxygenase overexpression or exogenous carbon monoxide (CO) protects against hepatocyte apoptosis and fulminating hepatitis. The prevention of hepatocyte apoptosis by CO has been shown to require activation of NF-κB. The purpose of these investigations was to determine the mechanism of CO-induced hepatocyte NF-κB activation and protection against apoptosis. Primary rat or mouse hepatocytes and Hep3B cells were utilized. CO exposure was performed at 250 parts per million. Main outcome measures included cell viability, reactive oxygen species (ROS) generation, and changes in the levels of the intracellular antioxidants glutathione and ascorbate. Western blotting was performed for phospho-Akt, total Akt, and IκBα. NF-κB activation was determined by electrophoretic mobility shift assay and luciferase reporter assays. We found that CO treatment of hepatocytes prevents spontaneous apoptosis and leads to an increase in ROS production in association with Akt phosphorylation and IκBα degradation. CO did not increase ROS production in respiration-deficient (ρ0) Hep3B cells. Both Akt phosphorylation and IκBα degradation can be inhibited by the addition of antioxidants. Furthermore, CO-induced NF-κB activation is reversed by phosphatidylinositol 3-kinase (PI3-K) inhibitor (LY294002) or antioxidants. Additionally, prevention of spontaneous hepatocyte apoptosis by CO is reversed by PI3-K inhibition and antioxidants. In conclusion, these data implicate a survival pathway of CO-induced ROS, Akt phosphorylation, and NF-κB activation in cultured hepatocytes. This pathway may prove to be important in maintenance of hepatic function in both physiological and pathophysiological conditions.

Heme oxygenase; mitochondria; IκBα; antioxidant; PI3-kinase

Carbon monoxide (CO), a gaseous second messenger, is a byproduct in biological systems produced during the oxidative catabolism of heme by the heme oxygenase (HO) enzymes. Only recently has it become known that, at low concentrations [10–500 parts per million (ppm)], exogenous CO can regulate many physiological processes without apparent toxicity (32, 34). The exact mechanisms by which CO acts at the molecular level remain incompletely understood. CO exerts some of its physiological effects by interaction with heme moieties of certain enzymes. For example, CO activates soluble guanylyl cyclase, stimulating the production of cyclic 3′,5′-guanosine monophosphate (cGMP), which in turn regulates vasodilation, bronchodilation, neurotransmission, and the inhibition of platelet aggregation (9, 10, 31, 46). Additionally, CO has also been shown to activate p38 MAPK (7, 33, 35). One of the most remarkable consequences of CO signaling is an inhibition of apoptosis (6, 7, 23, 39–41, 47). We have shown that CO blocks hepatocyte apoptosis in vitro and in vivo through a process involving NF-κB activation (23, 47).

CO has long been known to bind to the heme of mitochondrial cytochrome c oxidase, but only recently has it been demonstrated that this inhibition can result in low-level reactive oxygen species (ROS) generation to trigger adaptive responses and cell survival (1, 16, 42). Because CO promotes cell viability via NF-κB activation, we sought to determine whether the effects of CO on NF-κB activation and cell viability are dependent on ROS signaling. The purpose of these experiments was to test the hypothesis that CO-induced ROS increases phosphorylation of protein kinase B/Akt (Akt), leading to activation and nuclear translocation of NF-κB. Furthermore, this occurs via increased protein synthesis and decreased protein levels of IκBα.

Materials and Methods

Materials. Williams medium E, penicillin, streptomycin, L-glutamate, and HEPES were purchased from Life Technologies (Rockville, MD). Insulin was obtained from Lilly (Indianapolis, IN), and calf serum was purchased from Hyclone Laboratories (Logan, UT). Mouse recombinant TNF-α was obtained from R&D Systems (Minneapolis, MN). Antibodies for Akt, IκBα (Cell Signaling Technology, Danvers, MA), and β-actin antibody (Sigma, St Louis, MO) were used. Enhanced chemiluminescence (ECL)+ Plus was obtained from Amersham Biosciences (Piscataway, NJ), and Supersignal chemiluminescence detection reagents were obtained from Pierce (Rockford, IL). 2′,7′-dichlorofluorescin diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR). Unless indicated otherwise, all other chemicals and proteins were purchased from Sigma (St. Louis, MO). Cell culture. Primary hepatocytes were isolated and purified from Sprague-Dawley rats (Harlan, Indianapolis, IN) or C57BL/6 or cyyb−/− mice (Jackson Laboratory, Bar Harbor, Maine) and cultured as described previously (24). Highly purified hepatocytes (>98% purity and >98% viability by Trypan blue exclusion) were suspended in Williams medium E supplemented with 10% calf serum, 1 μM insulin, 2 mM L-glutamate, 15 mM HEPES (pH 7.4), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were plated on collagen-coated tissue culture plates at a density of 2 × 105 cells/well in 12-well plates for cell viability analysis or 5 × 105 cells/100-mm dish for Western blot and enzyme assays. After 18-h preculture, the cells were further cultured with fresh medium containing 5% calf serum and were used for experiment. Hep3B cells (American Type Culture Center, Manassas, VA) were cultured in the same media containing 5% calf serum. Respiration-deficient Hep3B cells (ρ0 cells) were generated by incubating these cells in ethidium bromide for 2–3 wk in medium supplemented with pyruvate and uridine (12). The ρ0
cells were then selected by exposure to the mitochondrial inhibitors rotenone (1 μg/ml) and antimycin A (1 μg/ml), which are lethal to the wild-type Hep3B cells. All CO exposures were performed at 250 ppm.

**Cell viability.** Cell viability was determined by the crystal violet staining method as described previously (25). In brief, cells were stained with 0.5% crystal violet in 30% ethanol and 3% formaldehyde for 10 min at room temperature. Plates were washed four times with tap water. After being dried, cells were lysed with 1% SDS solution, and dye uptake was measured at 550 nm with the use of a 96-well plate reader. Cell viability was calculated from relative dye intensity compared with untreated samples.

**DCF fluorescence.** Intracellular ROS generation was assessed with DCF-DA (10 μM). Microscopy or fluorometry was performed. Images were acquired with an Olympus (Center Valley, PA) fluorescent microscope and camera (excitation 488 nm, emission 535 nm). Fluorometry was performed using a dual scanning microplate spectrophotometer (Molecular Devices, Sunnyvale, CA).

**CASPASE-3 ACTIVITY.** Caspase-3 activity was determined on whole cell lysates of hepatocytes on culture day 4 with a caspase-3 fluorometric assay based on the cleavage of DEVD per the manufacturer’s instructions (R&D Systems).

**HPLC analysis of GSSG and ascorbate.** HPLC analysis was carried out with a Shimadzu chromatograph equipped with ESA 5102 Coulchem detector (Milford, MA). Separations were achieved with a 4.6 mm × 25 cm C18 column (5 μm, 100 A; Microsorb, Rainin Instrument, Emeryville, CA). To assess the concentrations of ascorbic acid and GSSG, hepatocytes or mitochondria were briefly sonicated with ultrasound (30 s, 0 – 4°C), treated with CH3CN (final concentration, 50%; incubation time, 15 min at 0 – 4°C), and then centrifuged for 5 min at 10,000 g. The resulting supernatant was separated on a C18 matrix with a mobile phase consisting of 3% methanol, 1.5% CH3COOH, and 50 mM sodium phosphate (pH 3.0). Electrochemical detection of redox-sensitive analytes was performed at a holding potential of +0.8 V. The identity of the corresponding HPLC peaks was confirmed via preincubation of cell lysate with either ascorbate oxidase or GSSG. Calibration of the experimental peaks was performed via injection of standard solutions of GSSG and ascorbic acid.

**Total antioxidant power.** The antioxidant level in rat hepatocyte cell lysates was determined by the reduction of Cu²⁺ to Cu⁺ because of the combined action of all antioxidants within the lysates with the use of an assay kit according to the manufacturer’s instructions (Oxford Biomedical Research, Oxford, MI). Generated Cu⁺ was detected by the complex formation between Cu⁺ and bathocuproine, and the stable complex was detected at an absorption of 490 nm. The obtained values were compared with a standard curve using uric acid as the reagent.

**Western blot analysis.** Cells were harvested, washed twice with ice-cold PBS, and resuspended in 20 mM Tris-HCl buffer (pH 7.4) containing a protease inhibitor mixture (0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, 5 μg/ml pepstatin A, and 1 μg/ml chymostatin). Proteins (30 μg) were separated on SDS-PAGE and transferred to nitrocellulose membrane. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and then incubated with primary antibodies for 1 h at room temperature. Blots were developed by peroxidase-conjugated secondary antibody and proteins were visualized by ECL procedures according to the manufacturer’s recommendation.

**Plasmids transfection/luciferase assay.** Evaluation of NF-κB activation was performed using a luciferase reporter assay as previously described (14, 47). Briefly, hepatocytes were cotransfected with NF-κB reporter constructs [pGL3-κB luciferase (100 ng/well) and pIEP-Lac-z (0.5 μg/well)] using Lipofectin (Invitrogen, Carlsbad, CA) per manufacturers instructions. All cells were allowed to recover for 24 h after transfection. Luciferase activity (reported as relative light units) was assayed 2 h after initiation of CO exposure using a luciferase assay kit (Promega, Madison, WI) per manufacturer’s instructions and measured on a Berthold Luminometer. Results were corrected for transfection efficiency and protein concentration.

**Electrophoretic mobility shift assay.** Nuclei were extracted from hepatocytes following specified treatment. A double-stranded DNA NF-κB consensus sequence (GGGACCTTCCCGGGTCTT; Santa Cruz Biotechnology, Santa Cruz, CA) was labeled with [γ-³²P]-ATP and then incubated with 5 mg of total nuclear protein. Electrophoretic mobility shift assays (EMSAs) were performed as described previously (43).

**Statistical analysis.** Data are presented as means ± SE of at least three separate experiments. Comparisons were performed using one-
RESULTS

CO reduces spontaneous hepatocyte apoptosis. It has been previously demonstrated that the spontaneous decline in viability in primary rat cultured hepatocytes is due to apoptosis (25), and we have previously shown that CO blocks TNF-α/actinomycin D-induced apoptosis in hepatocytes in culture (23, 48). To assess whether CO would prevent spontaneous apoptosis, hepatocytes were exposed to CO (250 ppm) for 1 h twice a day, and viability was measured every 24 h for 6 days. Consistent with previous findings, viability decreased significantly by day 2 in culture. Treatment with CO significantly prevented cell loss throughout the duration of the experiment (Fig. 1A). Caspase-3 activity was determined on day 4 in culture to confirm that the hepatocytes were undergoing apoptosis and that CO was preventing this apoptotic death. CO treatment decreased relative caspase-3 activity by 83 ± 13% compared with untreated hepatocytes (Fig. 1B). Of note, treatment with the nitric oxide synthase inhibitor L-nitro-arginine methyl ester (L-NAME, 1 mM) did not influence the effects of CO on caspase-3 activity (Fig. 1B) or viability (data not shown), suggesting that the influence of CO on preventing spontaneous apoptosis was not dependent on nitric oxide. Viability studies were also performed in the presence of the pan-caspase inhibitor zVAD-fmk (100 μM; Fig. 1C). These data confirm that spontaneous hepatocyte cell death is in part attributed to apoptosis. Interestingly, the level of protection from zVAD is very similar to that seen by CO treatment. Furthermore, intermittent (1 h, twice a day) exposure to hypoxia (1, 5, 10% oxygen levels) had no significant improvement

Fig. 2. CO increases reactive oxygen species (ROS) generation. A: dichlorofluorescein (DCF) fluorescence is increased by CO. Fluorometric measurement of DCF fluorescence in cell lysates of CO and control (air) cells was determined every 10 min over the course of 1 h. DCF fluorescence peaked at 2.3 ± 0.3-fold increase over control cells (P < 0.05). B: fluorescence microscopy reveals increased DCF fluorescence after 30 min of treatment with CO. C and D: CO treatment decreases levels of the cellular antioxidant ascorbic acid (C, *P < 0.05) and decreased the total antioxidant power (D; 15 ± 4 vs. 8.1 ± 3 μM/mg protein; *P < 0.05). Results are means ± SE for 3 independent experiments, each run in duplicate.
on viability compared with controls, suggesting that the mechanism of CO is likely distinct from a "hypoxic preconditioning."

CO increases hepatocyte ROS production. CO binds to the heme of mitochondrial cytochrome c oxidase to lead to inhibition of oxidative phosphorylation (1, 16, 42). Inhibition of cytochrome c oxidase can result in the generation of ROS from the upstream electron transport chain complexes. We initially sought to determine whether CO increases ROS production in primary rat hepatocytes. ROS production was increased by 2.3-fold after 20 min in CO-treated cells compared with controls (P < 0.05; Fig. 2, A and B). Additionally, as a marker of ROS production, we determined the effects of CO on hepatocyte levels of the endogenous antioxidant ascorbic acid and on the total antioxidant power based upon the ability of hepatocyte lysates to reduce Cu²⁺ to Cu¹⁺. CO significantly decreased levels of ascorbic acid (Fig. 2C) and decreased the total antioxidant power (Fig. 2D), suggesting an increase in ROS production.

CO-induced ROS are mitochondrial in origin. To determine whether the CO-induced increase in ROS was mitochondrial in origin, we utilized wild-type Hep3B and respiration-deficient (ρ⁰) Hep3B cells (ρ⁰ cells). These cells were generated by incubating wild-type Hep3B cells with ethidium bromide in medium supplemented with pyruvate and uridine (12). Similar to primary cultured hepatocytes, CO increased DCF fluorescence in wild-type Hep3B cells (Fig. 3A); however, CO did not increase DCF fluorescence in ρ⁰ cells, suggesting that CO-induced ROS production is mitochondrial in origin. Additionally, mitochondria were freshly isolated from rat livers and were exposed to CO, and GSSG levels were determined. CO increased levels of GSSG compared with control mitochondria (Fig. 3B). Furthermore, NAD(P)H oxidase did not contribute to the generation of CO-induced ROS production in mitochondrial origin. A: Hep3B cells or respiration deficient (ρ⁰) Hep3B cells were loaded with DCF and treated with CO (250 ppm). Fluorescence was assayed by fluorometry over a 1-h time period. Similar to the effect on primary rat hepatocytes, CO increased DCF fluorescence in Hep3B cells (P < 0.05). However, CO did not increase ROS generation in ρ⁰ Hep3B cells. B: CO (250 ppm for 30 min) increased oxidized glutathione (GSSG) levels in freshly isolated rat hepatocyte mitochondria compared with controls (P < 0.05) C and D: NAD(P)H oxidase does not contribute to the generation of CO-induced ROS. The pharmacological NAD(P)H oxidase inhibitor, diphenyleneiodonium (10 μM), did not significantly influence CO-induced DCF fluorescence in rat hepatocytes (C; *P < 0.05 compared with air, control hepatocytes). Additionally, CO increased DCF fluorescence in both primary mouse hepatocytes from control C57BL/6 mice and cybb⁻/⁻ mice, which lack the necessary cytochrome b245 component of the NAD(P)H oxidase subunit gp91phox (D; *P < 0.05 compared with control C57BL/6 hepatocytes).

Fig. 3. CO-induced ROS production is mitochondrial in origin. A: Hep3B cells or respiration deficient (ρ⁰) Hep3B cells were loaded with DCF and treated with CO (250 ppm). Fluorescence was assayed by fluorometry over a 1-h time period. Similar to the effect on primary rat hepatocytes, CO increased DCF fluorescence in Hep3B cells (P < 0.05). However, CO did not increase ROS generation in ρ⁰ Hep3B cells. B: CO (250 ppm for 30 min) increased oxidized glutathione (GSSG) levels in freshly isolated rat hepatocyte mitochondria compared with controls (P < 0.05) C and D: NAD(P)H oxidase does not contribute to the generation of CO-induced ROS. The pharmacological NAD(P)H oxidase inhibitor, diphenyleneiodonium (10 μM), did not significantly influence CO-induced DCF fluorescence in rat hepatocytes (C; *P < 0.05 compared with air, control hepatocytes). Additionally, CO increased DCF fluorescence in both primary mouse hepatocytes from control C57BL/6 mice and cybb⁻/⁻ mice, which lack the necessary cytochrome b245 component of the NAD(P)H oxidase subunit gp91phox (D; *P < 0.05 compared with control C57BL/6 hepatocytes).

Fig. 4. Akt phosphorylation is increased by CO and is dependent in part on ROS generation. A: Western blot analysis shows that CO increased phosphorylation of Akt (p-Akt) but not ERK 1/2 (p-Erk). B: CO-induced Akt phosphorylation was diminished by the phosphatidylinositol 3-kinase (PI3-K) inhibitor LY294002 (LY, 10 μM). Western blot analysis shows that CO increased phosphorylation of Akt (p-Akt) but not ERK 1/2 (p-Erk).
CO-induced ROS activation via ROS production. To determine whether CO-mediated protection against apoptosis occurs via ROS, PI3-K, or NF-κB pathways, we utilized inhibitors of these pathways. Spontaneous cell death was assessed by crystal violet staining after 6 days of treatment with the inhibitors + CO (250 ppm for 1 h, twice a day). The protective effect of CO was partially reversed by these inhibitors (Fig. 6). These data suggest that both low-level ROS signaling and/or Akt phosphorylation are necessary for CO-induced NF-κB activation.

Inhibition of spontaneous hepatocyte cell death by CO is dependent on ROS, PI3-K, or NF-κB signaling. To determine whether CO-mediated protection against apoptosis occurs via ROS, PI3-K, or NF-κB pathways, we utilized inhibitors of these pathways. Spontaneous cell death was assessed by crystal violet staining after 6 days of treatment with the inhibitors + CO (250 ppm for 1 h, twice a day). The protective effect of CO was partially reversed by these inhibitors (Fig. 6). These data suggest that CO-induced protection is via a ROS, PI3-K, or NF-κB pathway.

**Fig. 6.** Protection against spontaneous hepatocyte death by CO is dependent on ROS and Akt phosphorylation. Crystal violet staining reveals that CO was completely inhibited by the PI3-K inhibitor LY294002 (10 μM). The role of ROS in inducing Akt phosphorylation was next investigated. Akt phosphorylation by CO was diminished by the antioxidants N-acetylcysteine (NAC, 5 mM) or ethylpyruvate (EP, 4.5 mM) (Fig. 4B). Exposure to H2O2 (100 μM) was used as a positive control.

CO decreases total Akt protein levels and increases NF-κB activation via ROS and PI3-K dependent pathways. Others and we have demonstrated that CO activates NF-κB in hepatocytes and other cell types (6, 23, 48). We compared the time course for CO-induced IκB degradation, one of the early steps in NF-κB activation, to Akt phosphorylation in hepatocytes. TNF-α treatment was used as a positive control for IκB degradation. CO induced a transient decrease in IκB levels, which temporally followed Akt phosphorylation (Fig. 5A).

We next sought to determine whether CO-induced ROS production or Akt phosphorylation was responsible for NF-κB activation. The activation of NF-κB was determined by EMSA as well as NF-κB reporter assays. CO consistently increases NF-κB DNA binding by EMSA or by luciferase reporter assay. This could be reversed by inhibitors of Akt phosphorylation (LY29402, 1 or 10 μM) or antioxidants (EP, 4.5 mM; NAC, 5 mM) (Fig. 5, B–D). These data suggest that both low-level ROS signaling and/or Akt phosphorylation are necessary for CO-induced NF-κB activation.

CO-induced Akt phosphorylation via ROS production. Because activation of the phosphatidylinositol 3-kinase (PI3-K) and Akt pathways is known to function as an adaptive signaling pathway triggered by ROS signaling (15, 21, 36, 45), we next sought to determine whether CO exposure leads to increased phosphorylation of Akt. CO increases Akt phosphorylation in as early as 10 min and is persistent up to 60 min as determined by Western blot analysis (Fig. 4A). This effect of CO was completely inhibited by the PI3-K inhibitor LY294002 (10 μM). The role of ROS in inducing Akt phosphorylation was next investigated. Akt phosphorylation by CO was diminished by the antioxidants N-acetylcysteine (NAC, 5 mM) or ethylpyruvate (EP, 4.5 mM) (Fig. 4B). Exposure to H2O2 (100 μM) was used as a positive control.

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DISCUSSION

This study was undertaken to characterize the mechanism by which CO activates NF-κB to protect hepatocytes. Others and we have previously shown that HO-1 upregulation overexpression or CO exposure prevent hepatocyte apoptosis in vitro and in vivo (2-4, 23, 37, 39, 40, 47). It has also been previously demonstrated that the protective effect of CO involves enhanced activation of NF-κB. We show here that CO stimulates NF-κB activation through a mechanism that involves ROS-induced Akt phosphorylation. These results provide evidence that the protective effects of CO are mediated through redox mechanisms that lead to activation of adaptive and protective pathways.

ROS encompass a variety of partially reduced metabolites of oxygen (i.e., superoxide, hydrogen peroxide, and hydroxyl radicals), all of which possess higher reactivity than molecular oxygen (44). ROS may originate from a number of intracellular redox systems such as mitochondrial respiratory chain enzymes, cytochrome P450 enzymes, xanthine oxidase, NAD(P)H oxidase, lipooxygenase, and cyclooxygenase. Even though the precise molecular targets for CO are unknown, CO is known to bind to heme moieties to affect enzymatic activity. For example, it is known that CO can bind to and increase the enzymatic activity of soluble guanylyl cyclase to generate cGMP (9, 46). Furthermore, it has long been known that CO can bind to the electron transport chain enzyme cytochrome c oxidase (1, 8, 11). The inhibition of cytochrome c oxidase results in a more reduced electron transport chain and leads to the upstream generation of ROS from complexes I and III (30). Our data suggest that the mitochondria are the source of CO-induced ROS generation on the basis of the lack of ROS generation in p60 Hep3B hepatocytes, as well as continued production of ROS with inhibition of NAD(P)H oxidase.

Although classically viewed as harmful products of oxidative phosphorylation or reperfusion injury, ROS are now known to function as second messengers to modulate intracellular signaling pathways (19, 29). Small concentrations of ROS can alter cellular or subcellular redox states that can affect the activity, as well as protein-protein and protein-DNA interaction of enzymes and transcription factors (18). Whereas transient fluctuations in ROS serve important regulatory functions, ROS when present at high or sustained levels cause severe damage to DNA, protein, and lipids. When the regulation and coordination of endogenous antioxidants is impaired or overwhelmed by the level of ROS, then oxidative stress occurs, which can be harmful. ROS signaling has been implicated in hepatocytes, and our study contributes to the growing body of literature that low levels of ROS production can influence adaptive signaling and contribute to cell viability. Previous studies have demonstrated that ROS generated by mitochondria or NAD(P)H oxidase play a physiological role in the cellular responses to hypoxia (12, 13, 17, 20). In hepatoma cell lines, elevated basal ROS production has been demonstrated to induce proliferation and render cells resistant to apoptosis (26). By inducing low-level ROS production, CO appears to induce a similar resistance to spontaneous apoptosis in cultured primary hepatocytes. Both endogenous and exogenous CO have previously been shown to increase cellular ROS generation (5, 42). The data presented in this manuscript integrate previous findings linking CO-induced ROS to Akt phosphorylation and NF-κB activation. Although it is known that ROS can activate the PI3K/Akt pathways and NF-κB, these mechanisms remain somewhat elusive (29).

NF-κB activation requires the degradation of IκB proteins, which allows NF-κB to translocate to the nucleus to influence transcription. It has been suggested that the prosurvival actions of Akt are through the activation of NF-κB (22, 27, 28, 36, 38). Akt has been linked to NF-κB activation in response to TNF-α exposure, and NF-κB activation can be diminished by PI3K inhibitors. This relationship prompted us to explore the relationship between Akt and NF-κB activation in hepatocytes. Exposure of hepatocytes to TNF-α or CO resulted in phosphorylation of Akt and degradation of IκB. Furthermore, CO-induced NF-κB activation was significantly decreased by PI3K inhibitors or antioxidants. Akt activation of NF-κB has been shown to promote cell survival. Interestingly, the CO-induced increase in hepatocyte viability was diminished by antioxidants of PI3K inhibition. Taken together, our data suggest that CO induces ROS generation, which in turn leads to NF-κB activation via Akt.

Heme oxygenase/CO signaling is becoming increasingly more recognized as an important mediator in hepatic and intestinal physiological and pathophysiological states. Further investigation is warranted to determine the role(s) of CO in the liver, including hepatocyte aging, liver regeneration, and cirrhosis.

ACKNOWLEDGMENTS

The authors thank Detcho Stoyanova, Ph.D., for assistance with HPLC measurement of ascorbate and glutathione.

GRANTS

B. S. Zuckerbraun is the recipient of the American College of Surgeons C. James Carrico M.D. Faculty Research Fellowship.

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