Protective Effects of Curcumin on Manganese-Induced BV-2 Microglial Cell Death

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Curcumin, a bioactive component in turmeric, has been shown to exert antioxidant, anti-inflammatory, anticarcinogenic, hepatoprotective, and neuroprotective effects, but the effects of curcumin against manganese (Mn)-mediated neurotoxicity have not been studied. This study examined the protective effects of curcumin on Mn-induced cytotoxicity in BV-2 microglial cells. Curcumin (0.1–10\(\mu M\)) dose-dependently prevented Mn (250\(\mu M\))-induced cell death. Mn-induced mitochondria-related apoptotic characteristics, such as caspase-3 and -9 activation, cytochrome c release, Bax increase, and Bel-2 decrease, were significantly suppressed by curcumin. In addition, curcumin significantly increased intracellular glutathione (GSH) and moderately potentiated superoxide dismutase (SOD), both of which were diminished by Mn treatment. Curcumin pretreatment effectively suppressed Mn-induced upregulation of malondialdehyde (MDA), total reactive oxygen species (ROS). Moreover, curcumin markedly inhibited the Mn-induced mitochondrial membrane potential (MMP) loss. Furthermore, curcumin was able to induce heme oxygenase (HO)-1 expression. Curcumin-mediated inhibition of ROS, down-regulation of caspases, restoration of MMP, and recovery of cell viability were partially reversed by HO-1 inhibitor (SnPP). These results suggest the first evidence that curcumin can prevent Mn-induced microglial cell death through the induction of HO-1 and regulation of oxidative stress, mitochondrial dysfunction, and apoptotic events.

Key words curcumin; heme oxygenase-1; manganese; mitochondrial dysfunction; oxidative stress

MATERIALS AND METHODS

Chemicals and Reagents Curcumin, manganese chloride and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Tin protoporphyrin IX dichloride (SnPP) was purchased from Tocris Bioscience (Minneapolis, MN, U.S.A.). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), other culture supplements and antibody against cytochrome c oxidase subunit 4 (COX-4) were obtained from Invitrogen (Carlsbad, CA, U.S.A.). Antibodies against actin, Bax, Bel-2, cytochrome c and heme oxygenase (HO)-1 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.). Antibody against cleaved Caspase-3 was from Cell Signaling Technology Inc. (Danvers, MA, U.S.A.).

Cell Culture and Chemical Treatments The murine microglial cell line, BV2, was maintained in DMEM supplemented with 10% FBS and antibiotics at 37°C in 5% CO\(_2\). BV2 cells were cultured at a seeding density of 1.5\(\times\)10^5 cells/mL and the culture medium was changed to low-serum medium (0.5% FBS) before any chemical treatment to reduce the serum effect and to prevent the direct interaction between the treated chemicals. When indicated, BV-2 cells were exposed to MnCl\(_2\) for maximum 24 h with or without pretreatment with curcumin for 1 h.

Cell Viability Assay Cell viability was assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay. After cells were treated and the culture medium was removed, 50\(\mu L\) of MTT solution (1 mg/mL in phosphate buffered saline; PBS) was added to each well in 96-well culture plate and incubated at 37°C for 4 h. After-

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wards, the medium was carefully removed and the formazan crystals were dissolved in 100 µL of 100% dimethyl sulfoxide (DMSO) for 15 min on orbital shaker. The cell viability was determined by measuring the optical density (OD) at 540 nm using a microplate reader (VersaMax; Molecular Devices, Sunnyvale, CA, U.S.A.). In a given experiment, each treatment was performed in triplicate and result was expressed as a percentage of untreated control.

**Immunoblot Analysis** Total cell proteins were prepared from the BV-2 cells grown under various experimental conditions for immunoblot analysis. Briefly, cells were washed twice with PBS and then lysed on ice with RIPA buffer (1% Nonidet P-40, 0.5% Na deoxycholate, 0.1% sodium deoxycholate (SDS), 0.1 mg/mL phenylmethylosulfonlfyl fluoride, 30 mg/mL aprotinin, and 1 mM sodium orthovanadate in PBS). Resulting lysates were centrifuged at 14000×g for 10 min at 4°C and the supernatants were collected as the total protein lysates. To detect cytochrome c in the cytosol and mitochondria, BV-2 cells were lysed with mitochondrial/cytosolic fraction kit (Biovision Inc., Mountain View, CA, U.S.A.) according to the manufacturer’s protocol. The protein samples (each 30 µg) were separated on SDS-polyacrylamide gel electrophoresis (PAGE) and electrotransferred to polyvinylidene difluoride (PVDF) membrane. The membranes were blocked in TBST blocking buffer (5% nonfat dry milk in Tris-buffered saline, pH 7.4, containing 0.1% Tween 20) for 2 h at room temperature and incubated with primary antibodies for cleaved Caspase-3 (1:1000 dilution), cytochrome c (1:1000 dilution), Bax (1:1000 dilution), Bcl-2 (1:2000 dilution), HO-1 (1:4000 dilution), COX-4 (1:4000 dilution), or actin (1:4000 dilution) overnight at 4°C. The membranes were washed three times with TBST buffer (0.1% Tween 20 in Tris-buffered saline, pH 7.4) and then reacted with horse radish peroxidase (HRP)-conjugated secondary antibodies (1:2000 dilution) for 2 h at room temperature. After washing again with TBST buffer, membranes were reacted with enhanced chemiluminescence (ECL) reagent (GE Healthcare, Pittsburgh, PA, U.S.A.) and exposed on X-ray film. The immunoreactive bands were quantified by densitometric analysis.

**Measurement of Reduced Glutathione (GSH)** The level of intracellular GSH was measured using the fluorescent dye monochlorobimane (MCB) as previously described. Briefly, BV-2 cells cultured in black 96-well culture plates were incubated with MCB (40 µM) over 20 min at room temperature in the dark. After twice washing with HBSS, fluorescence intensity was determined at 355 nm (excitation) and 460 nm (emission) in a fluorescence microplate reader (SpectraMax M2, Molecular Devices). GSH level was calculated from a standard curve constructed using known amounts of GSH and the values were expressed in micromoles per mg of protein.

**Measurement of Cellular Superoxide Dismutase (SOD) Activity** SOD activity was measured by a previously described method with slight modifications. BV-2 cells grown under various experimental conditions were washed twice with PBS, scraped off and collected into Eppendorf tube. Cells were lysed in ice-cold PBS by sonication and centrifuged at 15000×g for 5 min at 4°C. The supernatant was used immediately for the measurement. Each 25 µL of supernatant was mixed with 200 µL of reaction buffer (50 mM potassium phosphate buffer, pH 7.8, 1.33 mM diethylenetriaminepenta-acetic acid, 1.0 µM catalase, 70 µM nitroblue tetrazo-

**Measurement of Lipid Peroxidation** The lipid peroxidation was estimated by measuring malondialdehyde (MDA) levels. Cell homogenate was prepared by sonication and centrifuged at 3000×g for 10 min at 4°C. The resulting 100 µL of supernatant was mixed with 900 µL of reaction buffer (150 mM Tris–HCl buffer, pH 7.1, 1 mM FeSO₄, 1.5 mM ascorbic acid) and incubated for 15 min at 37°C, and then stopped the reaction by adding 1 mL of trichloroacetic acid (10%). After addition of 2 mL of thioarbitratic acid (0.375%), sealed and heated for 15 min in a boiling water bath to release MDA (the product of lipid peroxidation) from proteins. Then the reactant was cooled off to 4°C and centrifuged at 4000×g for 10 min. The amount of MDA formed in supernatant was measured at 532 nm. The levels of MDA were expressed micromoles per mg of protein.

**Measurement of Intracellular Reactive Oxygen Species (ROS) and Peroxynitrite** Intracellular ROS generation was measured using fluorescent dichlorofluorescein (DCF) from nonfluorescent 2′,7′-dichlorodihydrofluorescein diacetate (H₂DCFDA) and the production of peroxynitrite (ONOO⁻) was examined by using dihydroxorodamine 123 (DHR 123). Briefly, BV-2 cells were treated with various experimental conditions and washed with HBSS. After 30 min of incubations with 20 µM H₂DCFDA or 50 µM DHR 123, cells were rinsed twice with HBSS and then the fluorescence intensity was measured at 485 nm/535 nm (excitation/emission) in a fluorescence microplate reader (SpectraMax M2, Molecular Devices).

**Measurement of Caspase Activity** Caspase-3 and -9 activities were measured by colorimetric assay as previously described. Briefly, Cells were lysed with ice-cold lysis buffer (50 mM N-2-hydroxethylpiperazine-N'2-ethanesulfonic acid (HEPES), pH 7.4, 1 mM dithiothreitol (DTT), 0.1 mM ethylene-diaminetetraacetic acid (EDTA), 0.1% CHAPS), incubated for 5 min on ice, and centrifuged at 10000×g for 10 min at 4°C. Subsequent protein samples (10 µg) were incubated with 200 µM of substrates (Ac-DEVD-p-nitroanilide (pNA) for Caspase-3 and Ac-LEHD-pNA for Caspase-9, respectively) at 25°C. Formation of pNA from the reaction was measured at 405 nm wavelength over 1 h.

**Measurement of Mitochondrial Membrane Potential (MMP)** MMP was determined using the fluorescent dye JC-1. Mitochondria-specific lipophilic cationic fluorescent dye, JC-1 can enter the mitochondria selectively and accumulates as red aggregates in healthy cells, but it exists as green monomers in the cytosol when the mitochondrial membrane collapsed during apoptosis. Thus, the red-to-green fluorescence ratio indicates mitochondrial membrane damage. BV-2 cells were pretreated with curcumin (10 µM) and/or SnPP (3 µM) for 1 h and then treated with Mn (250 µM) for 16 h. Cells were incubated with JC-1 (5 µg/mL) for 15 min at 37°C in the dark, and then washed with PBS. The fluorescence intensity was measured at 535 nm/590 nm (excitation/emission) for red and green channels of fluorescent microscope.
485 nm/535 nm (excitation/emission) for green using a fluorescence multimode microplate reader (Infinite 200; Tecan, Grodig, Austria). The result was calculated as the ratio of red/green fluorescence and the value was expressed as a relative percentage over non-treated control sample.

**Statistical Analysis** Results were expressed as the mean±standard error of the mean (S.E.M.) The data were analyzed using by SPSS 12.0 software package (SPSS Inc., Chicago, IL, U.S.A.). A one-sample t-test was used for comparisons between two groups. Comparisons between multiple groups were analyzed using one-way factorial ANOVA and the Duncan’s post hoc test. Statistics was evaluated at the significance level $2\alpha=0.05$.

**RESULTS**

**Protective Effect of Curcumin against Mn-Induced Toxicity in BV-2 Microglia** BV-2 cells are derived from C57BL/6 newborn mice immortalized with v-raf/v-myc retrovirus and widely used to study microglial activation and pathogenesis because this cell line expresses CD40 and reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, some representative microglial markers. In this study, BV-2 cells were adopted as a suitable model system to investigate the role of curcumin against Mn-induced microglial cell death.

Initial studies were performed to examine the cytotoxic response of BV-2 cells to Mn at various concentrations. As shown in Fig. 1A, incubation of BV-2 cells with 10–500 µM Mn for 24 h resulted in significant cell death in a dose-dependent manner, and 250 µM Mn induced approximately 60% cell death. Hence we did subsequent experiments using 250 µM Mn. Curcumin itself at concentrations of 0.1–10 µM showed no cytotoxicity in BV-2 cells (Fig. 1A). To investigate the effect of curcumin on Mn-induced microglial cell death, BV-2 cells were pretreated with 0.1–10 µM curcumin for 1 h, followed by 250 µM Mn treatment for 24 h. Mn-induced cytotoxicity was attenuated by curcumin dose-dependently, and especially pretreatment with 10 µM curcumin significantly increased cell survival to 90% after 24 h (Fig. 1B).

**Curcumin Inhibited Mn-Induced Apoptotic Proteins Expression** Caspase-3 and several mitochondria-associated proteins, such as cytochrome $c$, Bax, and Bcl-2 play critical roles in mitochondrial oxidative damage and apoptosis. We performed immunoblot analysis to reveal the expression of...
In our experiment, Mn induced a significant release of cytochrome c from mitochondria to the cytosol. 22) It has been known that mitochondrial dysfunction is accompanied by Bcl-2 down-regulation, Bax up-regulation, and the release of cytochrome c from mitochondria to the cytosol. 23) In our experiment, Mn induced a significant release of cytochrome c to the cytosol (3.01-fold of control). However, the Mn-induced cytochrome c release was attenuated by curcumin (2.01-fold of control). In addition, Mn treatment induced an increase in Bax expression (2.23-fold of control) but a decrease in Bcl-2 expression (0.77-fold of control). However, pretreatment with curcumin prior to Mn treatment significantly attenuated the changes of these proteins. Interestingly, curcumin per se 1.5-fold up-regulated the anti-apoptotic Bcl-2 compared to the control.

Effects of Mn Treatment and/or Curcumin Pretreatment on GSH, SOD, and MDA Levels

To investigate the involvement of oxidative stress damage in Mn-induced BV-2 cell cytotoxicity, we measured GSH, SOD, and MDA levels. As shown in Table 1, the levels of intracellular GSH and SOD (anti-oxidative enzymes) were decreased after treatment with Mn (250 μM) for 16 h to 68.4% of control and 88.9% of control, respectively. On the other hand, curcumin (10 μM) single treatment significantly elevated GSH (4.1-fold of control) and caused small enhancement of SOD levels (1.1-fold of control). Moreover, curcumin pretreatment resulted in significant increase in both GSH and SOD levels in a dose-dependent manner.

Mn treatment led to a remarkable increase of MDA (oxidative damage marker) levels (1.5-fold of control). However, pretreatment with curcumin inhibited Mn-induced MDA increase in BV-2 cells dose-dependently (1 μM curcumin, 87.4%; 10 μM curcumin, 75.2% of Mn-treated cells). Moreover, curcumin pretreatment significantly elevated GSH (4.1-fold of control) and decreased MDA (oxidative damage marker) levels (1.5-fold of control). However, pretreatment with SnPP (3 μM) significantly suppressed the curcumin-induced HO-1 expression. In addition, Mn (250 μM) did not modulate HO-1 expression in BV-2 microglial cells.

Caspase-3 was markedly activated and expressed as cleaved forms by Mn treatment. However, curcumin pretreatment effectively reduced the cleaved Caspase-3 expression. It has been known that mitochondrial dysfunction is accompanied by Bcl-2 down-regulation, Bax up-regulation, and the release of cytochrome c from mitochondria to the cytosol. 23) In our experiment, Mn induced a significant release of cytochrome c to the cytosol (3.01-fold of control). However, the Mn-induced cytochrome c release was attenuated by curcumin (2.01-fold of control). In addition, Mn treatment induced an increase in Bax expression (2.23-fold of control) but a decrease in Bcl-2 expression (0.77-fold of control). However, pretreatment with curcumin prior to Mn treatment significantly attenuated the changes of these proteins. Interestingly, curcumin per se 1.5-fold up-regulated the anti-apoptotic Bcl-2 compared to the control.

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BV2 cells were pretreated with various concentration of curcumin (0.1–10 μM) with or without SnPP (HO-1 inhibitor, 3 μM) for 1 h, followed by MnCl2 (250 μM) treatment for 16 h. The intracellular ROS production and peroxynitrite (ONOO−) generation were respectively determined by using fluorescent H2DCFDA and DHR 123. All values were expressed as a percentage of fluorescence intensity to the untreated control. Data are the mean±S.E.M. of three independent experiments in triplicate. *p<0.01 as compared with untreated control. **p<0.01 as compared with MnCl2 alone. ***p<0.01 as compared with curcumin+MnCl2-treated group.

Table 1. Effects of Curcumin Pretreatment on GSH, SOD, and MDA Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (μmol/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>MDA (μmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.23±0.55</td>
<td>27.15±4.12</td>
<td>4.96±0.22</td>
</tr>
<tr>
<td>MnCl2 (250 μM)</td>
<td>2.21±0.43</td>
<td>24.15±2.25</td>
<td>7.41±1.01</td>
</tr>
<tr>
<td>Curcumin (10 μM)</td>
<td>13.22±1.41*</td>
<td>30.13±5.18</td>
<td>4.72±0.15</td>
</tr>
<tr>
<td>MnCl2+Curcumin (0.1 μM)</td>
<td>2.54±0.38</td>
<td>24.91±5.63</td>
<td>7.59±1.34</td>
</tr>
<tr>
<td>MnCl2+Curcumin (1 μM)</td>
<td>4.58±0.59*</td>
<td>26.04±3.72</td>
<td>6.48±0.82</td>
</tr>
<tr>
<td>MnCl2+Curcumin (10 μM)</td>
<td>7.67±0.97*</td>
<td>28.84±3.89</td>
<td>5.57±0.71</td>
</tr>
</tbody>
</table>

The values represent the mean±S.E.M., n=6 each. *p<0.01, statistical significance compared with control group. **p<0.05, ***p<0.01, statistical significance compared with MnCl2 treated cells.
of control) and more significantly increased total ROS (4.5-fold of control), whereas pretreatment with curcumin (0.1–10 \( \mu \text{M} \)) dose-dependently lowered the ROS and ONOO\(^{-} \) generation. Of interest, the presence of HO-1 inhibitor (SnPP, 3 \( \mu \text{M} \)) significantly blocked the effect of curcumin on the production of ROS. SnPP alone did not influence ROS generation in microglial cells (data not shown).

**Role of HO-1 in the Protective Effects of Curcumin** To evaluate the importance of HO-1 induction by curcumin in microglia, a pharmacological approach was used with specific HO-1 inhibitor, SnPP. As shown in Fig. 5A, SnPP augmented the Mn-induced Caspase-9 and -3 activities. Additionally, SnPP partly abolished the curcumin-mediated suppressive effect on these caspases (Fig. 5A).

The changes of MMP were evaluated by using a specific mitochondria fluorescent dye JC-1. As shown in Fig. 5B, Mn treatment significantly decreased MMP to 62.6% of control, and SnPP augmented the loss of MMP to 33.6% of control group. Although the Mn-induced MMP loss was relieved by curcumin (91.4% of control), SnPP reduced the curcumin effect on the MMP maintenance. Furthermore, loss of cell viability was significantly increased by SnPP (45% cell viability of control) when compared with Mn alone treatment condition (60% cell viability of control) when compare with Mn alone treatment condition. SnPP pretreatment partly abolished the effect of curcumin on cell viability (Fig. 5B). SnPP alone did not significantly affect Caspase-9 and -3 activities, MMP maintenance, or BV-2 cell viability (Fig. 5). These results indicate that HO-1 mediates the protective effects of curcumin against Mn toxicity in BV-2 microglia.

**DISCUSSION**

Curcumin, a naturally occurring polyphenol found in turmeric, has been reported to exhibit therapeutic potential for various diseases including cancers, psoriasis, and Alzheimer’s disease. \(^{24}\) Recent studies have also revealed that curcumin has protective effects against PD-related neurotoxicants, such as MPP\(^{+}\), rotenone, salsolinol, and 6-hydroxyp Parkinsonism (6-OHDA) in neurons and/or glial cells. \(^{14,15,25}\) However, the effects of curcumin against Mn toxicity in microglia have not been reported. Therefore, this study investigated that curcumin can suppress Mn-induced cytotoxicity in BV-2 microglial cells.

Several studies using Mn reported the alteration of iron homeostasis in the brain and the induction of neuroinflammation and oxidative stress. \(^{26,27}\) Mn can induce neuronal damage directly and also indirectly enhance neuronal cell death through activation of microglia. \(^{26}\) Microglia easily respond to Mn and release ROS and inflammatory mediators, such as proinflammatory cytokines, nitric oxide (NO), and prostaglandins, inducing detrimental toxicity to neighboring neurons. \(^{27}\) Although there are limited reports on the effects of Mn-induced toxicity on glial cells, some studies suggested that self-produced ROS can stimulate degradation of ferritin in microglia and subsequent microglial cell death. \(^{29,30}\) However, little is known about the molecular mechanisms responsible for Mn toxicity in microglia.

Previous studies demonstrated that Mn caused mitochondrial complex I and/or complex II activity in various cell types. \(^{5,31}\) In agreement with previous studies, our results revealed that Mn (250 \( \mu \text{M} \)) induced typical mitochondrial function changes, such as pro-apoptotic cytochrome c release into the cytosol, Bax increase, anti-apoptotic Bcl-2 decrease, and Caspase-3 activation in BV-2 cells (Fig. 2). However, curcumin pretreatment significantly suppressed those changes. Previous study reported that curcumin effectively protected mitochondria from oxidative damage and attenuated apoptosis in cortical neurons. \(^{32}\) Moreover, curcumin prevented H\(_2\)O\(_2\)-induced apoptotic cell death in microglia. \(^{33}\) However, as far as we are aware, the protective effect of curcumin against Mn-induced cytotoxicity in microglia has not been studied until now. Our results clearly demonstrated for the first time that curcumin can prevent mitochondria-related apoptotic damage from Mn toxicity in microglial cells.

Because the nervous system is vulnerable to oxidative stress, there is a complex antioxidant defense system. GSH, an endogenous cysteine-containing tripeptide ((L-\( \gamma \)-glutamyl-L-cysteinyl-glycine), is the ubiquitously existing antioxidant thiol in most cells. SOD also acts as primary antioxidative defense enzyme to prevent further generation of ROS. \(^{34,35}\) Adversely, increased oxidative stress accumulates product of lipid peroxidation, such as MDA. Recent study suggested that excessive Mn caused depletion of cellular GSH and over production of MDA in the striatum of mouse. \(^{36}\) However, curcumin has been shown to decrease the elevated level of MDA and prevent decline of antioxidant enzymes in experimental animal.
models. In accordance with previous studies, our results revealed that the reduction of GSH and SOD, but the elevation of MDA by Mn treatment in BV-2 cells. Of interest, these changes were significantly reversed by curcumin pretreatment (Table 1). Thus, we propose that curcumin protects microglial cells by enhancing antioxidant enzymes and by inhibiting lipid peroxidation.

Curcumin is an electrophilic compound triggering activation of transcription factor nuclear factor-E2-related factor 2 (Nrf2), which normally sequestered by cytoskeleton-associated protein Keap1. Therefore, curcumin enables Nrf2 to translocate into the nucleus, which results in bind to the antioxidant-response element (ARE) and subsequent induction of detoxifying enzymes and cytoprotective proteins, such as HO-1 and γ-glutamyl cysteine synthetase.

Cells lacking HO-1 are vulnerable to oxidatively induced cytotoxicity, and HO-1 exerts potent antioxidant defense activity in various cell types including glial cells. Previous studies suggested that natural antioxidants, such as baicalein and quercetin, protect glial cells via induction of HO-1. Recent study suggests that curcumin can also protect microglial cells via up-regulation of HO-1 under an lipopolysaccharide (LPS)-mediated inflammatory environment. However, the role of HO-1 in curcumin against Mn-induced microglial cytotoxicity was not studied. Our results clearly demonstrated that curcumin induced HO-1 up-regulation in BV-2 cells (Fig. 3). On the other hand, attenuation of HO-1 expression by specific inhibitor SnPP significantly diminished curcumin-mediated ROS suppression (Fig. 4). In addition, pretreatment with HO-1 inhibitor SnPP aggravated the Mn-induced cytotoxicity and decreased the protective effects induced by curcumin (Fig. 5). These results supporting the essential involvement of HO-1 in the curcumin-mediated microglial cell protection against Mn-induced cytotoxicity.

Accumulating evidences suggested that various signaling pathways have been implicated in HO-1 expression and regulation. Therefore, further studies are required to fully explain the action of curcumin and the mechanism of HO-1 induction in microglial cells.

CONCLUSION

This study demonstrated for the first time that curcumin can protect microglial cells from cytotoxic effects of Mn. Curcumin significantly suppressed Mn-induced apoptotic events, such as Caspase-9 and -3 enzyme activation, MMP decrease, Bax increase, Bcl-2 decrease, and cytochrome c release. In addition, curcumin elevated antioxidant enzymes, GSH and SOD, but decreased the production of MDA and ROS caused by Mn. Importantly, induction of HO-1 is a critical event in the protective effect of curcumin in microglial cells.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

19. Hennek A, Land S, Hedjarn M, Schrattenholz A, Porzgen P, Lest M. The suitability of BV2 cells as alternative model system for primary...
microglia cultures or for animal experiments examining brain inflammation. ALTEX, 26, 83–94 (2009).


