ELSEVIER

NeuroToxicology 24 (2003) 261–268

NeuroToxicology

Brief communication

Aluminum Disrupts the Pro-Inflammatory Cytokine/Neurotrophin Balance in Primary Brain Rotation-Mediated Aggregate Cultures: Possible Role in Neurodegeneration

Victor J. Johnson[†], Raghubir P. Sharma[*]

Department of Physiology and Pharmacology, College of Veterinary Medicine, The University of Georgia, Athens, GA 30602-7389, USA

Received 15 August 2002; accepted 21 October 2002

Abstract

The etiology of human neurodegenerative diseases including Alzheimer's disease (AD) is exceedingly complex and our understanding of the mechanisms involved is far from complete. The experimental neurotoxicology of aluminum has been shown to recapitulate many of the pathophysiological features of AD and therefore represents a useful model to study the mechanisms involved in neurodegeneration. The present study investigated the effects of aluminum maltolate (Al-maltol) on the delicate balance that exists between pro-inflammatory cytokines and neurotrophins using primary brain rotation-mediated aggregate cultures. Aggregates were treated with Al-maltol (5–150 μM) on day 15 in vitro for 72 h. Cell death increased in a time- and concentration-dependent manner reaching significance in aggregates treated with 150 μM Al-maltol in 48 h and 50 μM by 72 h. Analysis of gene expression at 72 h revealed a concentration-dependent increase in tumor necrosis factor α (TNFα) and macrophage inflammatory protein-1α (MIP-1α) suggestive of a state of inflammation. In contrast, a dramatic concentration-dependent decrease in the expression of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) was observed. In fact, NGF expression could not be detected in aggregates treated with 50 and 150 μM Al-maltol. These changes in gene expression correlated with a decrease in aggregate size and an increase in neurodegeneration as indicated by Fluoro-Jade B staining. The results indicated a differential regulation of pro-inflammatory cytokines and neurotrophins in brain tissue following treatment with Al-maltol. Such findings provide insight into the possible involvement of deregulation of the cytokine/neurotrophin balance in the etiology of neurodegeneration.

Keywords: Aluminum; Aggregate culture; TNFα; MIP-1α; NGF; BDNF; Cytokine/neurotrophin balance; Neurodegeneration

INTRODUCTION

Current understanding of the mechanisms involved in the development and progression of neurodegeneration is far from complete. Even less is known about the role environmental factors play in the initiation and progression of human neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease. Numerous studies have indicated a role for oxidative stress and pro-inflammatory cytokine production in neurodegeneration (Akiyama et al., 2000; McGeer et al., 2001). Disruption of normal levels of neurotrophic factors has also been linked to neurodegeneration (Connor and Dragunow, 1998). Recent evidence strongly implicates disruption of the pro-

[*] Corresponding author. Tel.: +1-706-542-2788; fax: +1-706-542-3015.
E-mail address: rpsharma@vet.uga.edu (R.P. Sharma).
[†] Present address: Toxicology and Molecular Biology Branch, National Institute of Occupational Safety and Management, Morgantown, WV 26505, USA.

0161-813X/02/$ – see front matter
PII: S0161-813X(02)00194-8

inflammatory cytokine/neurotrophin balance as a causative factor in neurodegeneration seen in Parkinson's disease (Nagatsu et al., 2000), central nervous system (CNS) *Schistosoma mansoni* infection (Fiore and Aloe, 2001) and autoimmune encephalitis (Muhallab et al., 2002). Additionally, Alzheimer's-like neurodegeneration was demonstrated in anti-nerve growth factor (NGF) transgenic mice (Capsoni et al., 2000). The interaction between pro-inflammatory cytokines and neurotrophins is further supported by the observations that overexpression of tumor necrosis factor α (TNFα) in the brain decreases NGF levels in the hippocampus (Aloe et al., 1999) and low non-cytotoxic levels of TNFα markedly reduced signaling from neurotrophic factors (Venters et al., 1999, 2000).

Direct intracisternal administration of aluminum maltolate (Al-maltol) has been shown to induce neurodegeneration similar to that observed in human AD and has been proposed as a model to study the mechanisms of neurodegeneration (Ghribi et al., 2002). Studies indicate that Al can also induce the production of TNFα in human T98G glioblastoma cells (Campbell et al., 2002). Alterations in the production of NGF have also been reported in mice treated with Al (Alleva et al., 1998). The purpose of the present study was to determine the effect of Al-maltol on the pro-inflammatory cytokine/neurotrophin balance in the CNS. We employed the rotation-mediated neural cell aggregate culture system and found that Al-maltol concentration-dependently increased the expression of TNFα and macrophage inflammatory protein-1α (MIP-1α) concomitant with marked decreases in the expression of NGF and brain derived neurotrophic factor (BDNF). These alterations in gene expression correlated with a concentration-dependent increase in cell death. Results of the present study implicate a shift towards increased inflammatory mediators and decrease trophic factors in the etiopathogenesis of Al-maltol-induced neurodegeneration and extend current knowledge of the mechanisms involved.

MATERIALS AND METHODS

Materials

Aluminum chloride was purchased from J.T. Baker (Mallinckrodt-Baker Inc., Phillipsburg, NJ). 3-Hydroxy-2-methyl-4-pyrone (maltol) was obtained from TCI America (Portland, OR) and the aluminum maltolate (Al-maltol) complex was made as previously described in detail (Bertholf et al., 1989). Delbecco's Modified Eagle's Medium (DMEM) and Albumax II were purchased from Gibco Life Technologies (Invitrogen, Carlsbad, CA). All other cell culture reagents were procured from Sigma. The primers used for expression analysis were synthesized by Integrated DNA Technologies (Coralville, IA). Fluoro-Jade B was purchased from Histo-Chem Inc. (Jefferson, AR). All other reagents were obtained from Sigma and were of cell culture grade when available.

Cell Culture System

The rotation-mediated aggregate neural culture system has been described in detail by Honegger and Monnet-Tschudi (1997). This culture system has been used previously to investigate the neurotoxicity of Al (Atterwill et al., 1992). Briefly, the telencephalon was aseptically removed from gestation day 15 Swiss Webster mice (Harlan, Indianapolis, IN). The tissue was mechanically dissociated in Puck's D (Honegger and Monnet-Tschudi, 1997) by gently teasing through a 200 μM nylon mesh (Sefar America Inc., Depew, NY) followed by filtration through a 120 μM nylon mesh to remove debris. The single cell suspension was counted using a hemocytometer and the viable cell concentration was adjusted to 10×10^6 cells/ml in serum-free media (Honegger and Monnet-Tschudi, 1997). Immediately, a total of 40×10^6 cells were added to each 25 ml Delong flask, 4 ml of media was added and the flasks were placed on an orbital shaker at 68 revolutions per minute (rpm) in a humidified incubator at 37 °C with 10% CO_2. The speed of rotation was progressively increased to 74 rpm by day 1 and then the aggregates were transferred to 50 ml Delong flasks. Rotation speed was increased by 1 rpm per day until a final speed of 80 rpm for the rest of the culture period. Aggregates were treated on day 15 in vitro by removing 4 ml of media and adding 4 ml of fresh media containing 2× concentrations of Al-maltol (5–150 μM). Since each molecule of Al is bound by three molecules of maltol, a control group was treated with 450 μM maltol. Media samples were taken every 24 h until 72 h when the aggregates were harvested for gene expression analysis.

Cytotoxicity Assay

Cell death induced by Al-maltol was determined using the lactate dehydrogenase (LDH) release assay as previously described (He et al., 2002). Briefly, 40 μl of culture media was added to a 96-well plate and then 100 μl of 4.6 mM pyruvic acid in 0.1 M potassium

phosphate buffer (pH 7.5) was added using a repeater pipette. One hundred microliters of 0.4 mg/ml reduced β-nicotinamide adenine dinucleotide in 0.1 M potassium phosphate buffer (pH 7.5) was added to the wells and the kinetic change in absorbance at 340 nm was read for a duration of 1 min using a PowerWaveX™ absorbance microplate reader (Bio-Tek Instruments Inc., Winooski, VT). Data are presented as percentage of control (no treatment) LDH release.

Analysis of Gene Expression

Cell aggregates were washed with ice-cold phosphate buffered saline (PBS) and then disrupted in TRI® reagent (Molecular Research Center, Cincinnati, OH) according to manufacturer's protocol. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to analyze the expression of mRNA for TNFα, MIP-1α, NGF, BDNF and β-actin (internal control) as previously described (Johnson and Sharma, 2001). The sense and antisense primers used were 5′-CTCTTCAAgggACAAggCTg-3′ and 5′-CggACTCCgCAAAgTCTAAg-3′ for TNFα, 5′-CTgCCTgCTgCTTCTCCTAC-3′ and 5′-CTgCCTCCAAgACTCTCAgg-3′ for MIP-1α, 5′-TCAgCATTCCCTTgACACAg-3′ and 5′-CACTgAgAACTCCCCCATgT-3′ for NGF, 5′-gCggCAgATAAAAAgACTgC-3′ and 5′-CTTATgAATCgCCAgCCAAT-3′ for BDNF and 5′-ATggATgACgATATCgCT-3′ and 5′-ATgAggTAgTCTgTCAggT-3′ for β-actin. The thermal cycles consisted of denaturation at 94 °C for 15 s, annealing at 54 °C for 15 s and extension at 72 °C for 30 s followed by a final extension at 72 °C for 5 min. The numbers of cycles optimized within the linear range of amplification for each primer set were 25 cycles for TNFα and MIP-1α, 30 cycles for NGF and BDNF and 35 cycles for β-actin.

The amplification products were fractionated on 2% agarose gel and documented using a Kodak DC290 digital camera. The resulting images were digitized and quantified using UN-SCAN-IT software (Silk Scientific Inc., Orem, UT) and the pixel values for each cytokine were normalized to that of β-actin.

Aggregate Size and Pathology

Aggregates were treated with Al-maltol (5–150 μM) or maltol (450 μM) for 72 h and then collected and washed once in ice-cold PBS. The aggregates were then fixed in 4% paraformaldehyde for 1 h. Aggregate size was determined using an Olympus IX71 inverted microscope (Olympus America Inc., Melville, NY). Digital images were captured using a MagnaFire SP® digital camera and size analysis was performed using Image-Pro® Express software (Olympus).

Pathological damage was examined using Fluoro-Jade B, a specific marker for degenerating neurons, as previously described (Schmued et al., 2000). Paraformaldehyde-fixed frozen sections (25 μM) were dried at 50 °C and rehydrated in 80% ethanol with 1% NaOH for 5 min followed by 70% ethanol for 2 min and distilled water for 2 min. Sections were then immersed in 0.6% potassium permanganate for 10 min while shaking to evenly suppress background staining and then rinsed in distilled water for 2 min. The sections were transferred to Fluoro-Jade B staining solution (0.0004%) in 0.1% acetic acid for 20 min followed by three changes of distilled water 2 min each. Slides were dried at 50 °C, cleared in CitriSolv™ (Fischer Chemical, Fairlawn, NJ) and coverslipped using Flo-Texx® mounting media (Lerner Laboratories, Pittsburg, PA). Digital images were captured using an Olympus IX71 inverted microscope equipped with a MagnaFire SP® digital camera.

Statistical Analysis

All statistical analysis was performed using Minitab statistical software (Minitab Inc., State College, PA). Treatment effects were determined using analysis of variance followed by Fisher's PLSD post-hoc test. Effects were considered significant at $P < 0.05$.

RESULTS

Treatment of fetal aggregate cultures with Al-maltol resulted in a concentration- and time-dependent increase in LDH release indicating cell death (Fig. 1). At 24 h increased cell death was observed in aggregates treated with 150 μM Al-maltol although statistical significance was not reached. LDH release in this group was significantly increased over control by 48 h and remained significantly elevated at 72 h. At 72 h a concentration-dependent increase in cell death was evident in aggregates treated with Al-maltol. Unexpectedly, aggregates treated with a 450 μM maltol (3× molar concentration of highest Al-maltol group) showed increased cell death but only at 72 h (Fig. 1).

Analysis of gene expression showed that Al-maltol induced a concentration-dependent increase in the expression of TNFα, except at the highest (150 μM) concentration (Fig. 2). A concomitant increase in MIP-1α was observed suggesting a chemotactic response in

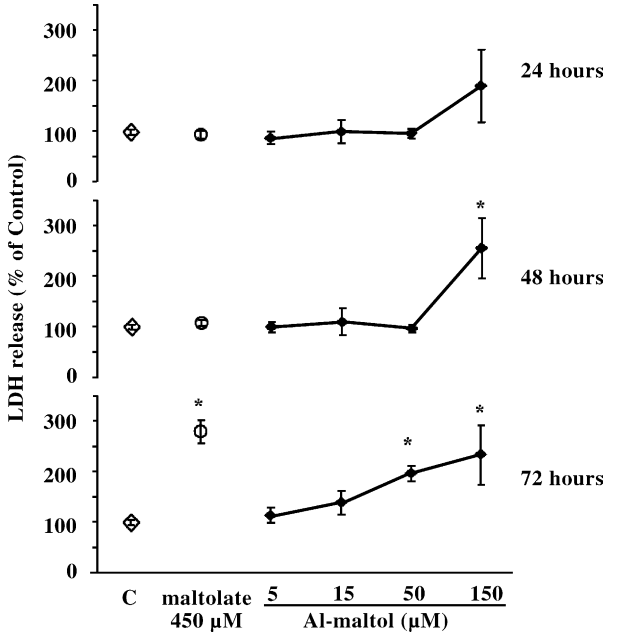

Fig. 1. Treatment of aggregating neural cell cultures with Al-maltol induces cell death. Aggregate cultures were treated on day 15 in vitro with Al-maltol (5–150 μM) or maltol (450 μM) for 72 h. Samples of the culture media were taken every 24 h for the determination of LDH release. Data are expressed as percentage of control LDH release at the respective time point. C: control. Mean ± S.E.M. ($n = 3$ flasks per group). Data are representative of three independent experiments with similar trends. (∗) Significantly different from control group at $P < 0.05$.

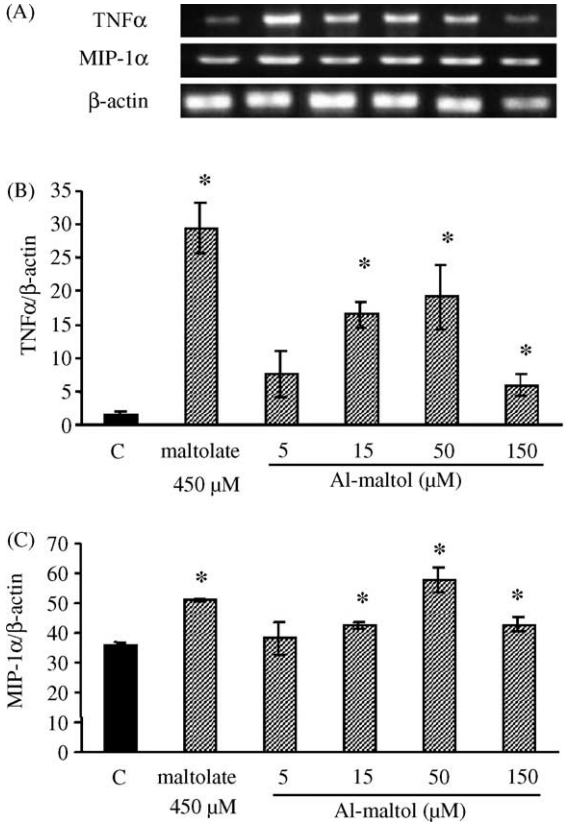

Fig. 2. Al-maltol increases pro-inflammatory gene expression in rotation-mediated neural aggregate cultures. Aggregate cultures were treated on day 15 in vitro with Al-maltol (5–150 μM) or maltol (450 μM) for 72 h. Following treatment, aggregates were washed in ice-cold PBS and disrupted in TRI® reagent and total RNA was extracted. RT-PCR was used to examine the expression of TNFα and MIP-1α. The order of the bands in the representative gels (A) is the same as for the bars. Densiometric analysis of the gels for TNFα (B) and MIP-1α (C) was performed. Mean ± S.E.M. ($n = 3$). Data are representative of three independent experiments with similar trends. (∗) Significantly different from control group at $P < 0.05$.

microglial cells to sites of cell death. The expression of pro-inflammatory genes in aggregates treated with 150 μM Al-maltol was also significantly increased over control. The expression of β-actin was also decreased (Fig. 2) in this group over the control indicating a deficit in total gene expression at this dose likely due to the high toxicity at this concentration of Al-maltol.

The increase in pro-inflammatory gene expression was accompanied by a dramatic concentration-dependent loss in the expression of neurotrophic factors (Fig. 3). NGF mRNA was not detectable in aggregates treated with 50 and 150 μM Al-maltol. Treatment with 450 μM maltol also increased TNFα and MIP-1α gene expression (Fig. 2) correlating with the cell death (LDH release) seen at 72 h. In contrast to the effect of Al-maltol, no changes in the expression of NGF or BDNF were evident in aggregates treated with maltol (Fig. 3).

Morphometric analysis using digital imaging software revealed that aggregates treated with maltol (450 μM) alone showed an increased size when compared to untreated aggregates (Fig. 4) suggesting a proliferative effect of maltol on the stem cells in the aggregate culture. Al-maltol decreased the size of aggregates (Fig. 4). The decrease in size may reflect an inhibition of stem cell proliferation concomitant with the loss of cells due to toxicity. Pathological examination using Fluoro-Jade B showed minimal neurodegeneration in the center of control aggregates (Fig. 4). Neurodegeneration was increased in maltol-treated aggregates but the damage was confined to the center of the aggregate. In contrast, neurodegeneration was evident throughout aggregates treated with 150 μM Al-maltol (Fig. 4) suggesting different mechanisms of toxicity for maltol versus Al-maltol.

DISCUSSION

The term neurodegeneration describes the cell death that is observed in many diseases of the CNS including

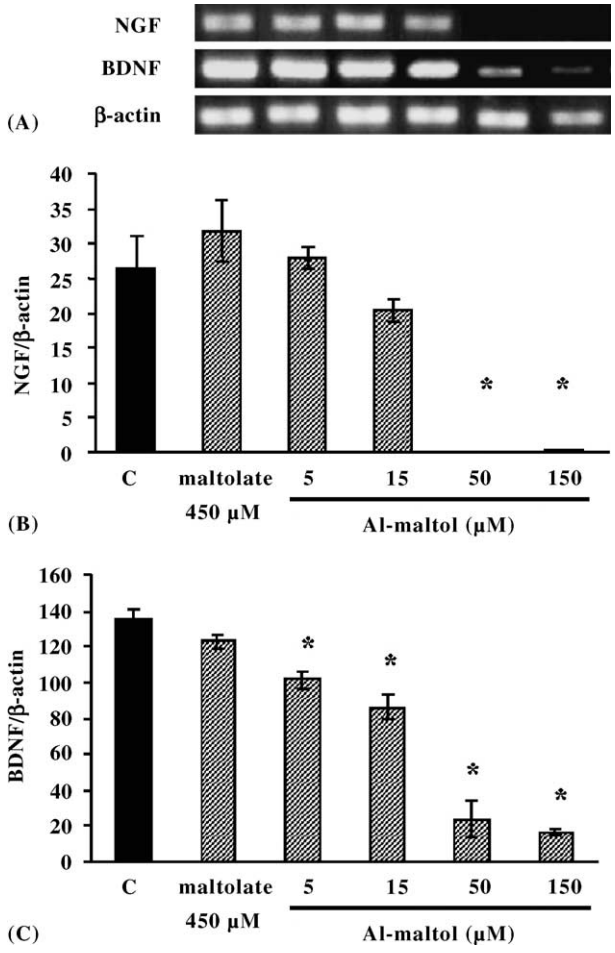

Fig. 3. Al-maltol decreases neurotrophin gene expression in rotation-mediated neural aggregate cultures. Aggregate cultures were treated on day 15 in vitro with Al-maltol (5–150 μM) or maltol (450 μM) for 72 h. Following treatment, aggregates were washed in ice-cold PBS and disrupted in TRI® reagent and total RNA was extracted. RT-PCR was used to examine the expression of NGF and BDNF. The order of the bands in the representative gels (A) is the same as for the bars. Densiometric analysis of the gels for NGF (B) and BDNF (C) was performed. Mean ± S.E.M. ($n = 3$). Data are representative of three independent experiments with similar trends. (∗) Significantly different from control group at $P < 0.05$.

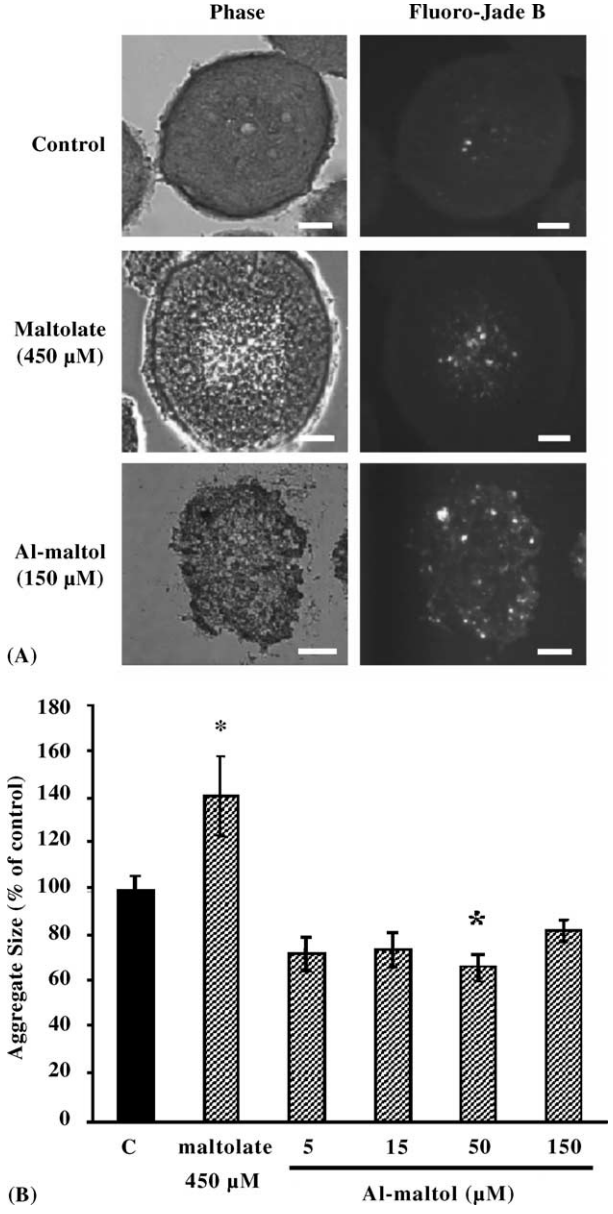

Fig. 4. Al-maltol decreased the size of aggregates and caused neurodegeneration whereas maltol increased aggregate size and neurodegeneration. Aggregate cultures were treated on day 15 in vitro with Al-maltol (5–150 μM) or maltol (450 μM) for 72 h following which aggregates were fixed in 4% paraformaldehyde for 1 h. (A) Frozen sections (25 μM) were stained with Fluoro-Jade B specific for degenerating neurons and examined under phase contrast or fluorescence using a fluorescein filter cube (Olympus) and digital microscopy. (B) Fixed aggregates were allowed to settle in wells of a 96-well plate and digital micrographs were captured and used to determine aggregate size. Data are presented as mean ± S.E.M. of 25 randomly selected aggregates. (∗) Significantly different from the control group at $P < 0.05$.

AD and Parkinson's disease. The series of pathophysiological events that culminate in neurodegeneration are poorly understood. A promising hypothesis is that oxidative stress and inflammation play a central role in the cell death observed (Akiyama et al., 2000). Studies demonstrate that oxidative stress and inflammation are key factors in the neurotoxicity of Al (Campbell et al., 2002) and this metal has been considered a risk factor for the development of AD (Perl and Brody, 1980) and other human neurodegenerative syndromes (Perl et al., 1982). Therefore, we tested the hypothesis that Al causes a shift towards a state of inflammation in the CNS that is involved in cell loss and neurodegeneration. We found that pro-inflammatory gene expression was increased in neural tissue treated with Al-maltol. This inflammation correlated in time and concentration

with Al-maltol-induced cell death. Interestingly, a decrease in trophic support appears to play a role in the neurodegeneration observed since the expression of NGF and BDNF was markedly reduced. Decreased trophic factor expression was observed at doses that did not produce cell death or inflammation. This suggests that decreased growth factor production may be the initiating signals leading to cell death and the inflammation may be a response to cell death.

Previous studies have demonstrated a reciprocal relationship between pro-inflammatory cytokines and neurotrophic factors in the CNS. It has been proposed that there is a cytokine/neurotrophin balance that is central to the maintenance of homeostasis in the brain and disruption of this balance can lead to detrimental changes in the CNS (Aloe et al., 1999). Such a balance plays an important role in neurodegeneration associated with disease (Nagatsu et al., 2000) and with normal aging (Macdonald et al., 2000). In the present investigation, Al, an environmental factor possibly involved in neurodegeneration, caused a shift in this balance towards increased pro-inflammatory cytokine production. In addition to the increase in TNFα and MIP-1α, a dramatic decrease in NGF and BDNF was evident. These changes in gene expression were accompanied by neurodegeneration and a decrease in overall aggregates size. These findings indicate that Al-maltol may cause neurodegeneration by two potentially interrelated mechanisms, immune-mediated neuronal death and neuronal death due to diminished trophic support. The increase in inflammatory gene expression may also be caused by Al-maltol-induced necrosis since necrotic neurons have been shown to increase TNFα production in primary glial cells (Viviani et al., 2000).

Strong evidence supports an interaction between cytokines and neurotrophins. Aloe et al. (1999) showed that the overexpression of TNFα in Tg6074 transgenic mice resulted in a significant decrease in NGF in the hippocampus. The physiological consequence of this decrease was a loss of septal cholinergic neurons that depend on NGF for survival. Cholinergic neurons are intimately involved in learning and memory and Tg6074 transgenic mice show neurobehavioral alterations including decreased learning ability (Fiore et al., 1996, 2000). Therefore, the increase in TNFα observed in the present study may be responsible for the down regulation of NGF and BDNF expression both of which may cause neuronal loss. Since the removal of NGF from PC-12 cells and primary neurons in culture has been shown to induce apoptosis (Chang and Johnson, 2002), the observed decrease in neurotrophin gene expression may play an important role in the apoptosis seen in Al-maltol-treated rabbits (Savory et al., 1999; Ghribi et al., 2001a). Interestingly, co-administration of glial-derived neurotrophic factor (GDNF) prevented Al-maltol-induced neurodegeneration in the rabbit brain (Ghribi et al., 2001b) suggesting a protective role for neurotrophic factors in Al-maltol neurotoxicity. The results of the present investigation offer the possibility that Al-maltol decreased neurotrophic factor expression in the CNS and that supplementation with GDNF may be counteracting the decrease, thus protecting against Al-maltol neurotoxicity in the rabbit.

Paradoxically, we observed a similar increase in TNFα and MIP-1α in aggregates treated with maltol alone and this correlated with increased LDH release at 72 h. The observation of an increase in the size of the aggregates following treatment with maltol may be a critical factor. The aggregates proliferate in the initial stages of the culture until they reach a terminal diameter that is dictated by the limit of diffusion of nutrients, oxygen and waste (Honegger and Monnet-Tschudi, 1997). An increase in diameter as observe in the present study resulted in an increased core of necrosis in the center of the aggregate due to the lack of nutrients and oxygen delivery and waste removal. This conclusion is supported by our findings that the neurodegeneration in maltol-treated aggregates was confined to the central area. Therefore, it is possible that the increase in TNFα is secondary to maltol-induced proliferation and aggregate growth. We recently found that maltol (>250 μM) stimulated proliferation in Neuro-2a cells (Johnson and Sharma, unpublished data) thus further supporting this hypothesis. Another possible explanation for this effect is the direct induction of cell death as previously observed in neurons treated with >150 μM maltol for 72 h (Hironishi et al., 1996). This could lead to secondary inflammation and an increase in TNFα expression. The lack of effect of maltol on NGF and BDNF expression indicates that the mechanisms responsible for the cell death are different than for the cell death caused by Al-maltol.

The observed increase in pro-inflammatory gene expression in maltol-treated aggregates was greater in magnitude than aggregates treated with Al-maltol. Cell death (LDH release) was also higher in maltol-treated cultures at 72 h. Cultures treated with Al-maltol showed a large increase in LDH release by 48 h, which decreased by 72 h. It is likely that Al-maltol increased pro-inflammatory gene expression maximally at 48 h although this was not tested in the present study. Pathological analysis at 72 h showed damage throughout

the aggregates treated with high concentrations of Al-maltol, an observation not seen following maltol treatment. This suggests a greatly reduced viability in the aggregates treated with 150 μM Al-maltol possibly accounting for the lesser increase in pro-inflammatory gene expression. This is further supported by the observed decrease in β-actin expression in this group indicating a reduction in total gene expression.

In summary, Al-maltol caused cell death in a three-dimensional model of brain tissue. A disruption in the pro-inflammatory cytokine/neurotrophin balance was observed and is likely responsible for the cell death. These findings provide insight into etiology of Al neurotoxicity and afford support for a role of Al in neurodegeneration. The current in vitro neural model will be useful for investigating the mechanisms involved in neurodegeneration. Differential regulation of cytokine and neurotrophin genes may play a prominent role in neurodegenerative diseases. Pharmacological alterations of these pathways may provide promise for possible prevention and therapeutic intervention in neurodegenerative disease states as has been demonstrated for neurotrophic factors and Al neurotoxicity (Ghribi et al., 2001b; Ohyashiki et al., 2002).

REFERENCES

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.

Alleva E, Rankin J, Santucci D. Neurobehavioral alteration in rodents following developmental exposure to aluminum. Toxicol Ind Health 1998;14:209–21.

Aloe L, Fiore M, Probert L, Turrini P, Tirassa P. Overexpression of tumour necrosis factor alpha in the brain of transgenic mice differentially alters nerve growth factor levels and choline acetyltransferase activity. Cytokine 1999;11:45–54.

Atterwill CK, Johnston H, Thomas SM. Models for the in vitro assessment of neurotoxicity in the nervous system in relation to xenobiotic and neurotrophic factor-mediated events. NeuroToxicology 1992;13:39–53.

Bertholf RL, Herman MM, Savory J, Carpenter RM, Sturgill BC, Katsetos CD, et al. A long-term intravenous model of aluminum maltol toxicity in rabbits: tissue distribution, hepatic, renal, and neuronal cytoskeletal changes associated with systemic exposure. Toxicol Appl Pharmacol 1989;98:58–74.

Campbell A, Yang EY, Tsai-Turton M, Bondy SC. Pro-inflammatory effects of aluminum in human glioblastoma cells. Brain Res 2002;933:60–5.

Capsoni S, Ugolini G, Comparini A, Ruberti F, Bernard N, Catatonia A. Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. Proc Natl Acad Sci USA 2000;97:6826–31.

Chang LK, Johnson Jr, EM. Cyclosporin A inhibits caspase-independent death of NGF-deprived sympathetic neurons: a potential role for mitochondrial permeability transition. J Cell Biol 2002;157:771–81.

Connor B, Dragunow M. The role of neuronal growth factors in neurodegenerative disorders of the human brain. Brain Res Brain Res Rev 1998;27:1–39.

Fiore M, Aloe L. Neuroinflammatory implication of *Schistosoma mansoni* infection in the mouse. Arch Physiol Biochem 2001; 109:361–4.

Fiore M, Probert L, Kollias G, Akassoglou K, Alleva E, Aloe L. Neurobehavioral alterations in developing transgenic mice expressing TNF-alpha in the brain. Brain Behav Immun 1996;10:126–38.

Fiore M, Angelucci F, Alleva E, Branchi I, Probert L, Aloe L. Learning performances. Behav Brain Res 2000;112:165–75.

Ghribi O, DeWitt DA, Forbes MS, Herman MM, Savory J. Co-involvement of mitochondria and endoplasmic reticulum in regulation of apoptosis: changes in cytochrome *c*. Brain Res 2001a;903:66–73.

Ghribi O, Herman MM, Forbes MS, DeWitt DA, Savory J. GDNF protects against aluminum-induced apoptosis in rabbits by upregulating Bcl-2 and Bcl-XL and inhibiting mitochondrial Bax translocation. Neurobiol Dis 2001b;8:764–73.

Ghribi O, Herman MM, Spaulding NK, Savory J. Lithium inhibits aluminum-induced apoptosis in rabbit hippocampus, by preventing cytochrome *c* translocation, Bcl-2 decrease, Bax elevation and caspase-3 activation. J Neurochem 2002;82: 137–45.

He Q, Riley RT, Sharma RP. Pharmacological antagonism of fumonisin B1 cytotoxicity in porcine renal epithelial cells (LLC-PK1): a model for reducing fumonisin-induced nephrotoxicity in vivo. Pharmacol Toxicol 2002;90:268–77.

Hironishi M, Kordek R, Yanagihara R, Garruto RM. Maltol (3-hydroxy-2-methyl-4-pyrone) toxicity in neuroblastoma cell lines and primary murine fetal hippocampal neuronal cultures. Neurodegeneration 1996;5:325–9.

Honegger P, Monnet-Tschudi F. Aggregating neural cell cultures. In: Fedoroff S, Richardson A, editors. Protocols for neural cell culture. 2nd ed. Totowa: Humana Press; 1997. p. 25–49.

Johnson VJ, Sharma RP. Gender-dependent immunosuppression following subacute exposure to fumonisin B1. Int Immunopharmacol 2001;1:2023–34.

Macdonald NJ, Decorti F, Pappas TC, Taglialatela G. Cytokine/neurotrophin interaction in the aged central nervous system. J Anat 2000;197(Pt 4):43–551.

McGeer PL, Yasojima K, McGeer EG. Inflammation in Parkinson's disease. Adv Neurol 2001;86:83–9.

Muhallab S, Lundberg C, Gielen AW, Lidman O, Svenningsson A, Piehl F, et al. Differential expression of neurotrophic factors and inflammatory cytokines by myelin basic protein-specific and other recruited T cells infiltrating the central nervous system during experimental autoimmune encephalomyelitis. Scand J Immunol 2002;55:264–73.

Nagatsu T, Mogi M, Ichinose H, Togari A. Changes in cytokines and neurotrophins in Parkinson's disease. J Neural Transm Suppl 2000;2000:277–90.

Ohyashiki T, Satoh E, Okada M, Takadera T, Sahara M. Nerve growth factor protects against aluminum-mediated cell death. Toxicology 2002;176:195–207.

Perl DP, Brody AR. Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. Science 1980;208:297–9.

Perl DP, Gajdusek DC, Garruto RM, Yanagihara RT, Gibbs CJ. Intraneuronal aluminum accumulation in amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam. Science 1982;217:1053–5.

Savory J, Rao JK, Huang Y, Letada PR, Herman MM. Age-related hippocampal changes in Bcl-2:Bax ratio, oxidative stress, redox-active iron and apoptosis associated with aluminum-induced neurodegeneration: increased susceptibility with aging. NeuroToxicology 1999;20:805–17.

Schmued LC, Hopkins KJ, Fluoro-Jade B. a high affinity fluorescent marker for the localization of neuronal degeneration. Brain Res 2000;874:123–30.

Venters HD, Tang Q, Liu Q, VanHoy RW, Dantzer R, Kelley KW. A new mechanism of neurodegeneration: a proinflammatory cytokine inhibits receptor signaling by a survival peptide. Proc Natl Acad Sci USA 1999;96:9879–84.

Venters HD, Dantzer R, Kelley KW. Tumor necrosis factor-alpha induces neuronal death by silencing survival signals generated by the type I insulin-like growth factor receptor. Ann N Y Acad Sci 2000;917:210–20.

Viviani B, Corsini E, Galli CL, Padovani A, Ciusani E, Marinovich M. Dying neural cells activate glia through the release of a protease product. Glia 2000;32:84–90.