

# THERAPEUTIC POTENTIAL OF METALLOTHIONEINS AS ANTIINFLAMMATORY AGENTS IN POLYSUBSTANCE ABUSE

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## ABSTRACT

*The incidence of multiple drug abuse is becoming more prevalent particularly in underdeveloped countries. In addition to caffeine and nicotine; ethanol, cocaine, met-amphetamine (METH) are the most common recreational drugs of abuse and induce early morbidity and mortality particularly in developing embryos and among teen-age population. We used human dopaminergic (SK-N-SH and SHS-Y5Y) neurons and metallothionein (MTs) gene manipulated mice to determine whether MTs-induced Coenzyme Q10 synthesis provides neuroprotection in multiple drug abuse. MTs were over-expressed by cell transfection and by using metallothionein transgenic (MTtrans) mesencephalic fetal stem cells. We performed in-vivo longitudinal analysis with microPET neuroimaging using 18F-dG and 18F-DOPA as specific biomarkers of brain regional mitochondrial bioenergetics and dopaminergic neurotransmission respectively. Alcohol accentuated cocaine and METH neurotoxicity by increasing the bio-availability of these drugs in the CNS. We used weaver mutant (wv/wv) mice because these genotypes exhibit neurodegeneration in the hippocampus, striatum, and cerebellar regions, and neurobehavioral abnormalities, body tremors, postural irregularities, and walking difficulties as seen in poly-substance abuse. Brain regional pro-inflammatory cytokines IL-1 $\beta$ , TNF- $\alpha$ , and NF- $\kappa$  $\beta$  were significantly increased, whereas anti-inflammatory MTs, melatonin, CoQ10, and mitochondrial complex-1 were significantly reduced in these genotypes. Cross-breeding wv/wv mice with MTtrans mice provided a colony resistant to poly-substance abuse with significantly reduced striatal dopaminergic degeneration as compared to wv/wv mice suggesting that MTs provide neuroprotection by augmenting brain regional CoQ10 and melatonin synthesis and acting as anti-inflammatory and free radical scavenging agents. MTs may scavenge free radicals and trap iron which participates in Fenton reaction to generate hydroxyl radicals and is significantly increased in the CNS of subjects addicted to poly-substance abuse. Furthermore, MTs may prevent neurotoxicity by inhibiting IL-1 $\beta$ , TNF- $\alpha$  and NF- $\kappa$  $\beta$  and by preserving CoQ10 involved in mitochondrial complex-1 replenishment and oxidative phosphorylation. Hence therapeutic interventions involving brain regional MTs induction may provide neuroprotection in polysubstance abuse.*

**Keywords:** Metallothioneins; poly-substance abuse; coenzyme q10; dopamine; cocaine; methamphetamine; alcohol; nicotine; free radicals; zinc; detoxification

## [1] INTRODUCTION

Chronic abuse of cocaine, METH, and ethanol is quite prevalent among Native Americans of North America and worldwide. These substances induce microglial immunocompromise, neuroinflammation, increased susceptibility to HIV/AIDS, and premature neurodegeneration, resulting in early morbidity and mortality. Moreover treatment of poly-substance abuse and drug-related HIV/AIDS is extremely costly all over the world.

Hence, there is a dire need to establish the therapeutic strategies of poly-substance abuse.

In recent years we have explored the therapeutic potential of metallothioneins (MTs) as anti-inflammatory agents in cocaine, METH, and ethanol models of multiple drug abuse using cultured human dopaminergic (SK-N-SH and SH-S-Y5Y) neurons and metallothioneins (MTs) gene-manipulated weaver mutant (wv/wv) mice.

Although it is known that cocaine [1-3], METH [1, 2], morphine [4], ethanol [5-13] and nicotine [14] induce microglial activation through induction of pro-inflammatory cytokine, NF $\kappa$ B, its exact clinical significance in the CNS pathogenesis is yet to be established. It has been reported that NF $\kappa$ B and API-1 in conjunction with calcium-calmodulin-dependent protein kinase (MAPK-38) are induced in activated microglia as a pro-inflammatory response to drug-induced neurotoxic insult [15].

By employing high-resolution magic angle spinning nuclear magnetic resonance (NMR) spectroscopy, we have discovered that morphine addiction induces neuro-adaptation by inhibiting inositol trisphosphate (IP<sub>3</sub>/Ca<sub>2+</sub>)<sup>+</sup>-mediated signal transduction in the rat peri-aqueductal grey and locus coeruleus neurons and neurodegeneration in the spinal lumbar dorsal horn neurons [16]. These original findings encouraged us to propose the hypothesis that drugs of abuse induce early neuroadaptation followed by delayed neurodegeneration associated with induction of pro-inflammatory cytokine genes such as NF $\kappa$ B, as we have recently discovered in wv/wv mice. Hence we have used these genotypes as experimental model of multiple drug abuse in our studies [17-19].

We have discovered that weaver (wv/wv) mice exhibit progressive neurodegeneration in the striatum, hippocampal CA-3 and dentate gyrus, and cerebellar Purkinje neurons as seen in cocaine, METH, and ethanol addiction. However the exact molecular mechanism of neurodegeneration in poly-substance abuse and its prevention and/or treatment remains enigmatic [20-25]. One of the several possible mechanisms of neurodegeneration could be through NF $\kappa$ B mediated microglial activation and MTs down-regulation which may provide better insight in learning the precise molecular mechanism of neurodegeneration in poly-substance abuse and its prevention or treatment. Immunoreactivity and mRNA expression of macrophage colony stimulating factor (M-CSF), which triggers microglial activation and neurodegeneration in the cerebellar Purkinje neurons and olfactory lobe mitral cell has been discovered [26], whereas microglial activation during progressive neurodegeneration in wv/wv mice suggests the clinical significance of neuro-inflammation in poly-substance abuse [27].

To establish the therapeutic potential of MTs in poly-substance abuse, we developed novel  $\alpha$ -synuclein-metallothioneins triple knockout ( $\alpha$ -Syn-MTtko) mice and MTs over-expressing weaver (wv/wv-MTs) mice [28, 29]. We have discovered that the striatal CoQ10 is significantly reduced in  $\alpha$ -Syn-MTtko mice and is increased in wv/wv-MTs mice supporting our original hypothesis that MTs provide COQ10-mediated neuroprotection in neurodegenerative disorders such as Parkinson's disease (PD) and drug addiction. Indeed COQ10 inhibited NF $\kappa$ B and accentuated mitochondrial ubiquinone-NADH Oxidoreductase (complex-1; a rate limiting enzyme e complex involved in oxidative phosphorylation and ATP synthesis during TCA cycle) in wv/wv mice, whereas MTtrans mice were resistant to 1-methyl, 4-phenyl, 1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity and Parkinsonism due

to significantly increased COQ10 as compared to wv/wv and MTdco mice [30]. Based on these findings, we proposed that since mesencephalic MTtrans fetal stem cells are resistant to 3-morpholinolinosydnonimine (SIN-1) and dihydroxy phenyl acetaldehyde (DOPAL) apoptosis implicated in progressive neurodegeneration and neural graft rejection [31, 32]; these robust cells could be used for the successful transplantation in wv/wv mice and the graft outcome could be assessed in-vivo by performing microPET neuroimaging and in-vitro by microarray analysis of pro-inflammatory cytokines genes [33].

Recently the outcome of intrastriatal grafts of embryonic mesencephalic tissue in PD patients has been evaluated with 18F-DOPA and 11C-raclopride-PET neuroimaging [34, 35]. Withdrawal of immune suppression 2.5 years after transplantation caused no reduction of 18F-DOPA uptake. However, the patients developed dyskinesia due to inflammation, indicating that poor graft outcome was associated with dopaminergic denervation. However dyskinesia was not associated with dopamine release suggesting that long-term immunosuppressive treatment can be withdrawn without interfering with graft survival. Although this therapeutic approach is promising and has direct clinical significance, it requires considerably large number of fetal stem cells and has some ethical issues. Therefore we developed a novel colony of MTs over-expressing weaver (wv/wv-MTs) mice which exhibit attenuated nigrostriatal degeneration without any overt clinical symptom of poly-substance abuse, hence could be used to establish MT-mediated inhibition of pro-inflammatory cytokines involved in neurodegeneration, early morbidity, and mortality.

By performing high-resolution microPET imaging, we have demonstrated that the distribution kinetics of 18F-DOPA is impaired [18, 36] with significant reduction in the striatal dopamine, COQ10, complex-1 activity, and increase in NF $\kappa$ B as a consequence of peroxynitrite ion (ONOO<sup>-</sup>) stress in aging wv/wv mice [17,33]. Indeed wv/wv mice exhibit age-dependent ONOO<sup>-</sup> stress and down-regulation of MTs, whereas MTs attenuate MPTP-induced  $\alpha$ -Synuclein nitration implicated in Lewy body formation [33]. However, significantly increased striatal 18F-DOPA uptake and COQ10 in wv/wv-MTs mice suggests the therapeutic potential of MTs in poly-substance abuse. Furthermore, we have discovered that ethanol augments cocaine and METH-induced reduction in the striatal 18F-DOPA uptake in C57/BL6J mice [19], whereas MTs provide zinc-mediated neuroprotection via transcriptional regulation of genes involved in growth and survival and by inhibiting pro-inflammatory cytokine genes including NF $\kappa$ B [21-25, 33, 37,38].

### 1.1. Microglial activation

Microglial activation participates in neuro-inflammation in response to environmental stress, aging, diet, drugs, and diseases that regulate protein acylation. Upon injury microglia, express macrophage colony stimulating factor (M-CSF) and release cytokines which induce activation, proliferation, or migration as a pro-inflammatory response [39, 40]. Activated microglia release nitric oxide (NO), increase in number, accumulate towards the damaged area, and perform both neuroprotective as well as neurotoxic functions depending on the state of activation

and release of mediators implicated in the pathogenesis of neurodegenerative disorders [41-43]. We have reported that glutathione and MTs synthesis are increased as an attempt to combat iron-induced NF- $\kappa$ B induction and oxidative stress, whereas MTs or COQ10 provide neuroprotection by inhibiting NF- $\kappa$ B-mediated microglial activation in SK-N-SH neurons [44,45].

We have discovered that CoQ10 provides neuroprotection by inhibiting NF- $\kappa$ B and by augmenting complex-1 activity in wv/wv mice and in rotenone-exposed SK-N-SH neurons [17]. Kainite-induced seizures also induce microglial activation, astrogliosis, cathapsin-S induction, and neurodegeneration in mice [46]. Giunta et al. [47] have shown that cholinergic pathway regulates anti-inflammatory response by acting at the  $\alpha$ -7nACh receptor and p44/42 (MAPK) system on macrophages. Hence inflammatory mechanism is the central component of HIV-associated dementia (HAD). Microglial activation is attenuated by nicotine and by choline esterase inhibitor, galantamine in IFN- $\gamma$ -HIV-1 gp120 model of HAD. Prostaglandin E2 also modulates macrophages and lymphocytes during inflammation [48]. Acetylcholine and nicotine inhibit LPS-induced TNF- $\alpha$  release in murine microglia, which is attenuated by  $\alpha$ -7nAChR antagonist,  $\alpha$ -Bungarotoxin through inhibition of p44/p42 and p38 MAPK phosphorylation, suggesting that cholinergic pathways regulate microglial activation through  $\alpha$ -7nACh receptors. Hence inhibition of microglial activation may represent mechanism underlying nicotine's neuroprotective potential in PD [49, 50]. However, chronic abuse of nicotine induces hypersensitivity following peripheral nerve injury that may increase inflammatory response via release of cytokines [51].

### 1.2. In-Vivo assessment of microglial activation

Activated microglia expresses specific binding sites for ligands that recognize the 18-KDa translocator protein (TP-18) in the diseased brain. Hence 1-(2-Chlorophenyl)-N-methyl-N (1methylpropyl) 3-isoquinoline-carboximide [PK-11195] is now used for the functional characterization of TP-18 in neurodegenerative disorders. Its localization in the activated microglia has been established by autoradiography with [3H] (R)-PK11195, whereas [11C] (R)-PK-11195 is used in-vivo to evaluate neuro-inflammatory diseases by PET imaging [52-64]. Recently [11C] (R)-PK11195-PET has been used to establish that intrauterine exposure of LPS to pregnant female rabbits leads to microglial activation that may induce periventricular leukomalacia and cerebral palsy in the progeny [65]. Microglial activation also regulates CNS immune response in multiple sclerosis (MS) and in experimental autoimmune encephalitis (EAE). Autoradiography and immunohistochemical studies have established a correlation between [3H]-PK-11195 binding and microglial marker, Mac-1 [CD11 $\beta$ ] and CD68 immunoreactivity at the site of inflammatory lesion. PET imaging with [11C]-PK11195 has identified uptake only at sites of active lesions as confirmed by MRI criteria [66]. Furthermore microglial

response in dopaminergic degeneration in a rat model of PD has been investigated by intra-striatal microinjection of 6-OH-DA using 2 $\beta$ -carbomethoxy-3 $\beta$ -(4-fluorophenyl) tropane (11C-CFT) binding, which was significantly reduced in the striatum, whereas 11C-PK-11195 binding was increased, confirming microglial activation in neurodegeneration. Immunohistochemical analysis using antibodies against CR3 for microglial activation, exhibited initially focal, then wide-spread response in the nigrostriatal region within 4 weeks, authenticating inflammation as the primary component of dopaminergic degeneration [67].

### 1.3. MTs neuroprotection

Although the exact molecular mechanism remains enigmatic, experimental evidence from our labs suggests that MTs provide neuroprotection by attenuating peroxynitrite (ONOO-) ion apoptosis in SK-N-SH neurons by inhibiting SIN-1 and MPTP-induced  $\alpha$ -Synuclein nitration, and by augmenting COQ10 synthesis in MTtrans mice [31-33]. Peroxynitrite ions induce pro-inflammatory cytokine, NF- $\kappa$ B and inhibit complex-1 which leads to progressive dopaminergic neurodegeneration in wv/wv mice [33, 36]. We have discovered that Selegiline inhibits MPP+ apoptosis and provides neuroprotection by augmenting MTs-mediated COQ10 synthesis [29, 68]. Transfection of SK-N-SH neurons with MTsense oligonucleotides inhibited whereas with MT1antisense oligonucleotides accentuated MPP+ and SIN-1 apoptosis, indicating oxidative and nitrative stress in the etiopathogenesis of dopaminergic degeneration and neural graft rejection [69] and the neuroprotective role of MTs [29,36]. These findings suggest that it would be extremely important to evaluate the therapeutic potential of MTtrans fetal stem cells in aging wv/wv mice exhibiting progressive neurodegeneration and establish the clinical significance of neuronal replacement therapy in poly-substance abuse-induced neuropathies.

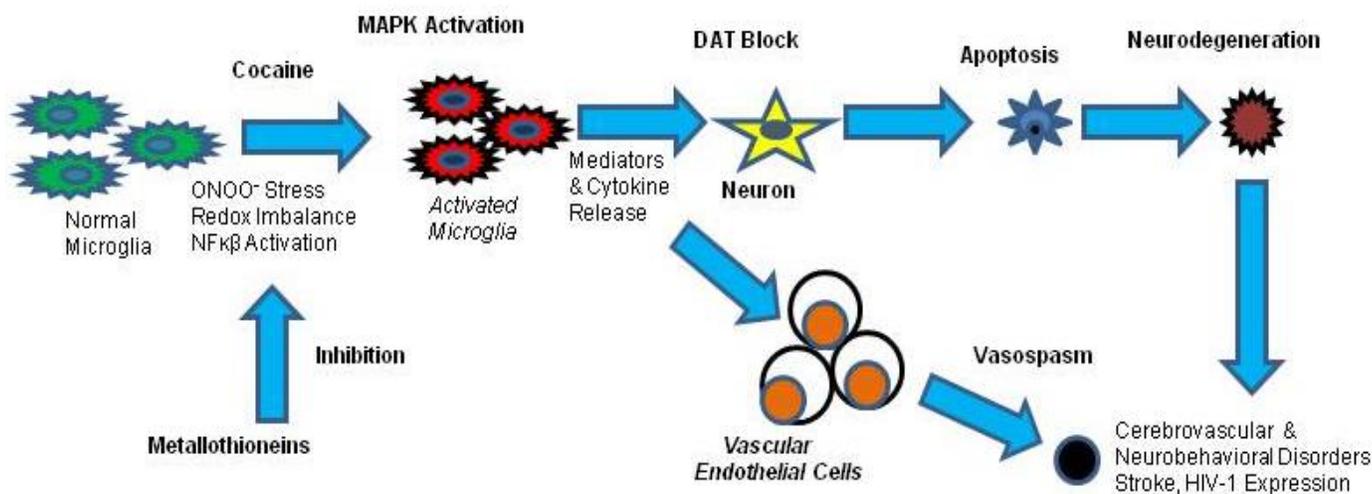
We have discovered that cocaine, METH, and ethanol-induced oxidative and nitrative stress, causes neurodegeneration in C57BL.6J mice and in SK-N-SH neurons [19, 70], whereas Selegiline and MTs inhibit ONOO- stress by inhibiting SIN-1, METH, and MPTP-induced  $\alpha$ -Synuclein nitration, involved in Lewy body formation and PD pathogenesis [31-33,36-38]. Direct exposure to MPP+ caused neurodegeneration in PC-12 cells and down-regulated synaptosomal dopamine transporter (sDAT) by releasing dopamine in DAT-over-expressing HEK-293 cells [71,72]. Furthermore MDMA-induced neurotoxicity in dopaminergic neurons was associated with increased MT1 and MT2 gene transcription as a neuroprotective mechanism, which might have therapeutic potential in dopaminergic neuropathies [73]. Cadmium (Cd) exposure to microglial cultures was also associated with NF- $\kappa$ B and AP-1 activation, and increased expression of MTs, heme oxygenase (HO-1), glutathione S-transferase, and metal transport protein-1, indicating primary involvement of oxidative stress in neurodegeneration [74]. We have also discovered that MTs regulate cytokines and NF- $\kappa$ B in cultured fibroblasts [75, 76] and inhibit salsolinol-induced neurodegeneration in SH-SY5Y cells

through zinc-mediated transcriptional regulation of NF $\kappa$ B [77, 78]. Zinc deficiency and chronic inflammation were observed in aging individuals, whereas induction of cytokine genes was associated with atherosclerosis and type-2 diabetes. Therefore, zinc turnover via MTs homeostasis, in individuals genetically predisposed to impaired inflammatory/immune response may augment age-related diseases [79].

It has been shown that MT-1 mRNA expression is increased 18 hrs after ethanol intoxication in mouse cerebral cortex, whereas MT-3 expression is increased at higher doses suggesting the neuroprotective role of MT1 as an antioxidant, whereas MT-3 may provide protection in critical neuronal injury [80]. Recently Penkowa et al [81] have reviewed the therapeutic potential of MTs in various neuro-inflammatory and neurodegenerative disorders. In this report, we have specifically highlighted NF $\kappa$ B-mediated microglial activation as a common neuro-inflammatory mechanism in cocaine, METH, morphine, and ethanol addiction to establish the therapeutic potential of MTs in poly-substance abuse.

#### 1.4. Cocaine

It is now well established that chronic abuse of cocaine induces oxidative and nitritative stress via ONOO<sup>-</sup> generation, ROS synthesis, enhanced lipid peroxidation, and severe depletion of glutathione [82- 84]. Furthermore cocaine triggers activation of transcription factors, NF $\kappa$ B and AP-1 and inflammatory cytokine IL-1 $\beta$ , which may augment inflammatory response to cause various cerebro-vascular disorders such as stroke and subarachnoid hemorrhage [1]. Caspases were induced when NGF-differentiated PC12 cells were exposed to cocaine for 24 hrs, suggesting the clinical significance of NF $\kappa$ B-mediated microglial activation in cocaine addiction [85]. Furthermore, NF $\kappa$ B induction in mice over-expressing  $\Delta$ FosB, and mice treated with cocaine have suggested NF $\kappa$ B as a primary target in the long-term adaptation of nucleus accumbens neurons [86]. Cocaine induced NF $\kappa$ B reporter gene via free radical overproduction, whereas I $\kappa$ B inhibited NF $\kappa$ B in H9C2 cells [87]. These deleterious changes were blocked by N-acetyl cysteine, glutathione, and lipoic acid, suggesting that cocaine-induced free radical generation triggers NF $\kappa$ B and pro-inflammatory response. At low concentrations cocaine induced c-fos, c-jun, AP-1, and NF $\kappa$ B, whereas at higher concentrations induced down-regulation of these genes.



**Fig.1. MTS-Mediated Neuroprotection in Cocaine Abuse.** Chronic abuse of cocaine causes dopaminergic degeneration by blocking dopamine transporter (DAT) in the nucleus accumbens and cerebrovascular damage, leading to stroke. MTs may provide neuroprotection by inhibiting cocaine-induced ONOO<sup>-</sup> stress and pro-inflammatory changes in the CNS.

Recently, Arango et al [88] have evaluated cocaine-abusing patients who survived with HIV/AIDS in relation to premature neurodegeneration. Cocaine altered cytokine production and HIV-1 expression and increased viral load as assessed by p24 antigen in the microglial supernatants. Cocaine-induced HIV-1 expression was blocked by inhibitors of  $\gamma$ -1 receptors (BD1047), TGF- $\beta$ 1 antibodies (SB-1431442), and Anti-TGF- $\beta$ 1), suggesting involvement of microglial  $\gamma$ -1 receptors and TGF- $\beta$ 1 in HIV expression [89, 90].

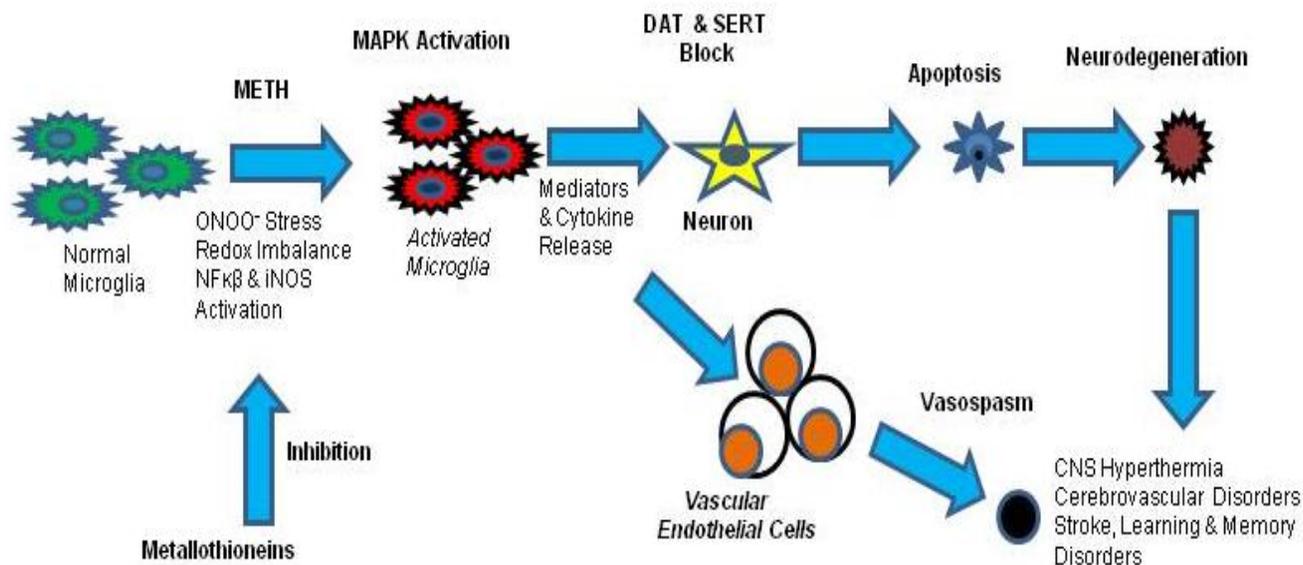
It is now known that one of the active metabolite of cocaine, cocaethylene (CE) increases the permeability of cerebrovascular endothelial cells through calcium-mediated p38-MAPK and NF $\kappa$ B activation. Treatment with lipo-polysaccharides (LPS) had similar effects on p38 MAPK phosphorylation and NF $\kappa$ B DNA binding. Coaethylene decreased DNA binding of RelA/p50 and p50/p50 dimers, increased NF $\kappa$ B and p-38 MAPK

activity, suggesting that CE may also induce inflammatory response in cocaine addicts [3].

### 1.5. Methamphetamine

Chronic abuse of METH causes long-lasting damage to striatal dopaminergic neurons via ONOO<sup>-</sup> stress, redox imbalance, and depletion of glutathione [91, 92], resulting in induction of inflammatory genes, and increase in DNA binding of AP-1 and NFκβ in cerebrovascular endothelial cells [1]. TNFα promoter constructs with mutated AP-1 or NFκβ sites have suggested that METH-induced redox imbalance and transcription factor

activation play a crucial role in the inflammatory response. A significant induction in AP-1 and cAMP-response element-DNA binding protein in the striatum, frontal cortex, hippocampus, and cerebellum, has also suggested induction of pro-inflammatory genes in METH addiction [2, 3]. MDMA-induced serotonin depletion in the rat brain was also induced via ONOO<sup>-</sup> stress, suggesting the involvement of oxidative and nitrative stress in METH addiction [93] [For details please refer 94-96].



**Fig: 2. MTS-Mediated Neuroprotection in METH Abuse.** Chronic abuse of METH causes dopaminergic degeneration by blocking dopamine transporter (DAT) and serotonin transporter (SERT) in the nucleus accumbens, cerebrovascular damage leading to stroke, hyperthermia, seizures, and learning and memory impairments. MTs may provide neuroprotection by inhibiting METH-induced ONOO<sup>-</sup> stress and pro-inflammatory changes in the CNS.

METH can also impair blood brain barrier, resulting in hippocampal and amygdallar damage, which may induce seizures and compromise learning and memory following stroke, suggesting the clinical significance of microglial activation in METH addiction [97]. METH neurotoxicity was attenuated by maintaining mice at low ambient temperature [98]. We have established that MTs attenuate ONOO<sup>-</sup> stress in SK-N-SH neurons and MTs gene manipulated mice to provide neuroprotection [31-33].

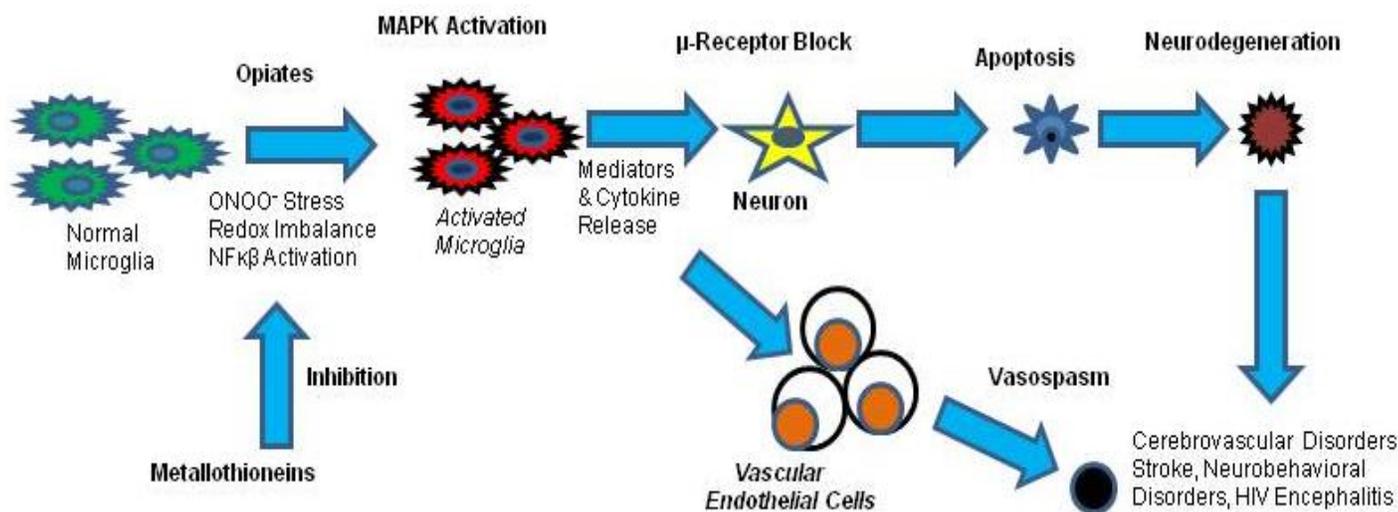
### 1.6. Opiates

Chronic abuse of opiates activates microglia and causes inflammation and disruption of neuron-glia relationship, resulting in neuronal dysfunction and susceptibility to HIV

encephalitis. The neurotoxic effects of opiates are primarily mediated through μ-receptors. Protein kinase C and transcription factor AP-1 plays a significant role in μ-opioid receptor gene induction. Protein kinase C activator, phorbol ester 12-o-tetradecanoylphorbol-13-acetate (TPA), activates NFκβ and AP-1 in SH-S-Y5Y cells. By excluding the effects of TPA on NFκβ with NFκβ inhibitor sulphasalazine, AP-1 regulatory elements have identified two positions-2388 and 1434 in the thymidine kinase promoter. These findings suggest that pro-inflammatory cytokines may exacerbate the pathogenesis of HIV-1 by disturbing glial homeostasis, increasing inflammation, and decreasing the threshold of apoptotic events in opioid addiction [4].

Recent studies have emphasized that opiate-induced HIV inflammation through PI3-K/Akt and MAPK signaling can be further explored as therapeutic targets for neuro-AIDS [99]. Opiates modulate inflammation and disrupt normal interactions among macrophages and lymphocytes, which promote neurodegeneration. Spinal glial cell are also activated by chronic morphine abuse leading to physical tolerance and dependence.

Intra-theal injections of morphine for 7 days increased phospho-p-38-MAPK immunoreactivity in the activated microglia, whereas a specific p-38-MAPK inhibitor, 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridyl)-1H-imidazole (SB-203580), attenuated physical tolerance and dependence as assessed by tail flick test, suggesting NF $\kappa$ B-mediated p-38-MAPK activation in morphine analgesia [100].



**Fig. 3. MTS-Mediated Neuroprotection in Opiates Abuse.** Chronic abuse of opiates causes dopaminergic degeneration by blocking  $\mu$ -activity in the nucleus accumbens and cerebrovascular cell death leading to stroke and susceptibility to HIV-encephalitis. MTs may provide neuroprotection by inhibiting opiate-induced ONOO<sup>-</sup> stress and pro-inflammatory response.

### 1.7. Ethanol

It is now well established that ethanol enhances lipid peroxidation and DNA-binding of proteins (p50, p65, and c-Rel) and down-regulates I $\kappa$ B phosphorylation, which is blocked by polyphosphatidylcholine (PDTC) in cerebro-vascular smooth muscle cells [6]. Ethanol-induced proteolysis of I $\kappa$ B $\alpha$ , vasoconstriction, leukocyte-endothelial wall interaction and capillary damage in the rat brain were attenuated by PDTC [101]. Transfection of annexin-V-DNA to C-6 glioma cells and SH-S-Y5Y cells enhanced ethanol-induced lesion via NF $\kappa$ B activation, suggesting that annexin-IV facilitates pro-inflammatory response [7, 8]. In cultured astrocytes, ethanol enhanced both COX-2 and iNOS expression via NF $\kappa$ B as confirmed by PDTC or BAY 11-7082 [9]. Cytokines (Interleukin-1 $\beta$ +interferon- $\gamma$ + TNF $\alpha$ ) and ethanol-induced nuclear translocation of NF $\kappa$ B occurred within 30 min in human A-172 astrocytes. N ( $\alpha$ )-L-tosyl-L-phenylalanine chloromethyl ketone (TPCK), a specific inhibitor of I $\kappa$ B proteolysis attenuated these deleterious changes, suggesting that inhibition of I $\kappa$ B prevents microvascular changes of alcohol-intoxicated subjects and stroke victims [9]. Microglial hypertrophy and hyperalgesia were noticed in rats intoxicated with ethanol-diet

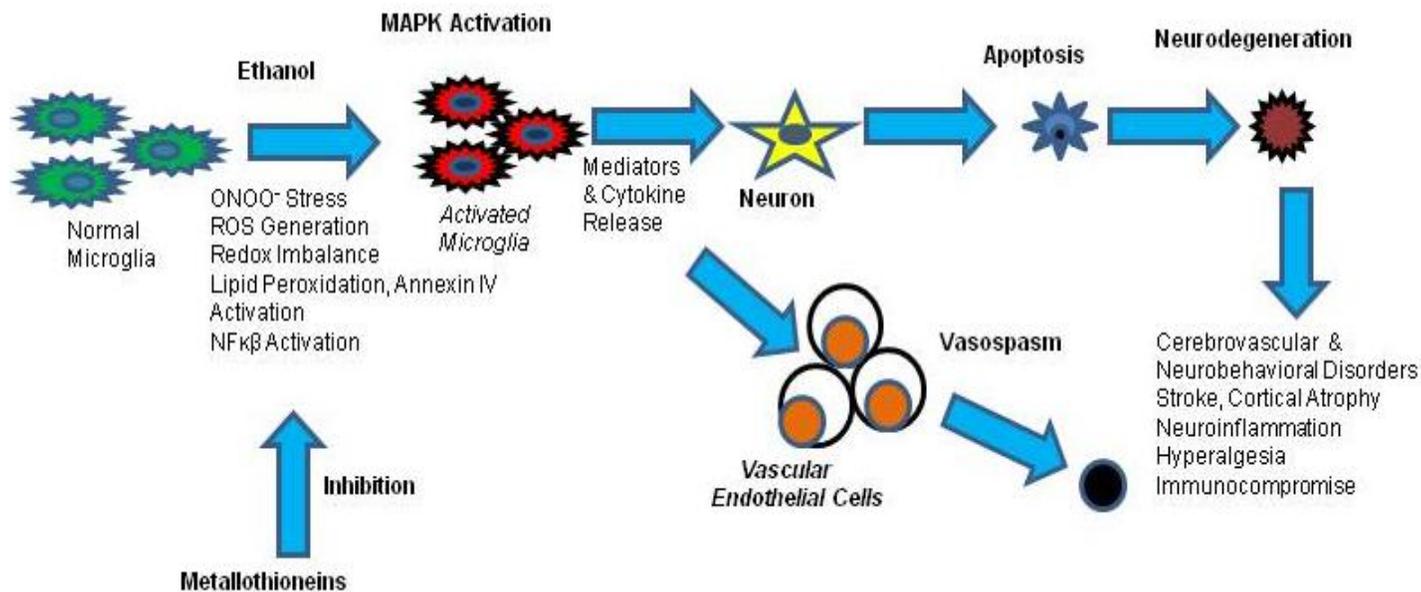
for 72 hrs even after ethanol withdrawal [102]. Furthermore binge ethanol-induced microglial activation, NF $\kappa$ B binding, and COX-2 expression, were inhibited by butylated hydroxytoluene (BHT)-mediated reduction of NF $\kappa$ B binding and COX-2 expression, supporting primary involvement of pro-inflammatory mechanisms in ethanol-induced neurodegeneration [103].

It has been reported that ethanol alters CNS immunocompetence to augment HIV/AIDS through ROS production, NF $\kappa$ B activation, via inhibition of p300 protein which may impair CNS immune-inflammatory response [104, 105]. Recently microarray analysis has been performed to detect ethanol-regulated genes and discover how transcriptional changes may alter behavior [12]. Ethanol-induced change in gene expression correlated with strain-specific differences and activation of Sp1 and NF $\kappa$ B pathways. The regulator of NF $\kappa$ B and NF $\kappa$ B-binding partner (RelA) were induced whereas SP1 and NF $\kappa$ B $\alpha$  were down-regulated suggesting their role in ethanol-induced neurobehavioral adaptations.

It has been shown that histone deacetylase inhibitors, trichostatin A (TSA) and suberoylanilide hydroxamic acid

potentiate the LPS-induced inflammatory response in murine N9 microglia and hippocampal slice cultures [106]. TSA potentiated the LPS-induced IL-6 and iNOS mRNA expression and secretion of IL-6, TNF $\alpha$ , NO, and macrophage inflammatory protein (MIP-2). These pro-inflammatory changes were attenuated by NF $\kappa$ B inhibitors caffeic acid, phenethyl ester, and

helenalin. Further studies have shown that upon P2X (7) receptor stimulation, microglia release small amounts of TNF $\alpha$ , leading to neuro-inflammation. Hence brain regional MTs induction or agents, inhibiting NF $\kappa$ B, AP-1 and p-38-MAPK signal transduction might have clinical significance in the treatment of ethanol abuse [107].



**Fig. 4. MTS-Mediated Neuroprotection in Ethanol Abuse.** Chronic abuse of ethanol causes neurodegeneration by NF $\kappa$ B, AP-1, and MAPK activation, involved in the deterioration of cells and organs. Ethanol may also induce CNS immunoinflammatory response and cerebrovascular cell death leading to stroke, cortical atrophy, hyperalgesia, and immunocompromise. MTs may provide neuroprotection by inhibiting ethanol-induced ONOO<sup>-</sup> stress and pro-inflammatory changes in the CNS.

### 1.8. Original discoveries

Recently we have made several original discoveries to understand the basic molecular mechanism of MTs-mediated neuroprotection in multiple drug abuse. We selected homozygous wv/wv mice in our studies as an experimental animal model of poly-substance abuse and discovered that progressive neurodegeneration in wv/wv mice is associated with NF $\kappa$ B induction, down-regulation of MTs, reductions of tyrosine hydroxylase, dopamine, 18F-DPA uptake, complex-1 activity, and CoQ10, and CNS swelling, whereas COQ10 attenuated these deleterious changes [17-25]. Direct exposure of rotenone to SK-N-SH neurons also reduced CoQ10 and complex-1 activity which was averted by COQ10 treatment, suggesting its anti-inflammatory role [19, 21] and MTs provide COQ10-mediated neuroprotection in PD and multiple drug abuse [29-33]. Furthermore we have discovered that METH-induced  $\alpha$ -Synuclein nitration and ROS synthesis are attenuated by zinc, suggesting ONOO<sup>-</sup> stress and the therapeutic potential of MTs in METH addiction [37, 38]. We also discovered that MPP<sup>+</sup> neurotoxicity is attenuated in cultured SK-N-SH neurons by Selegiline via MTs induction [68] and in SH-SY5Y cells by

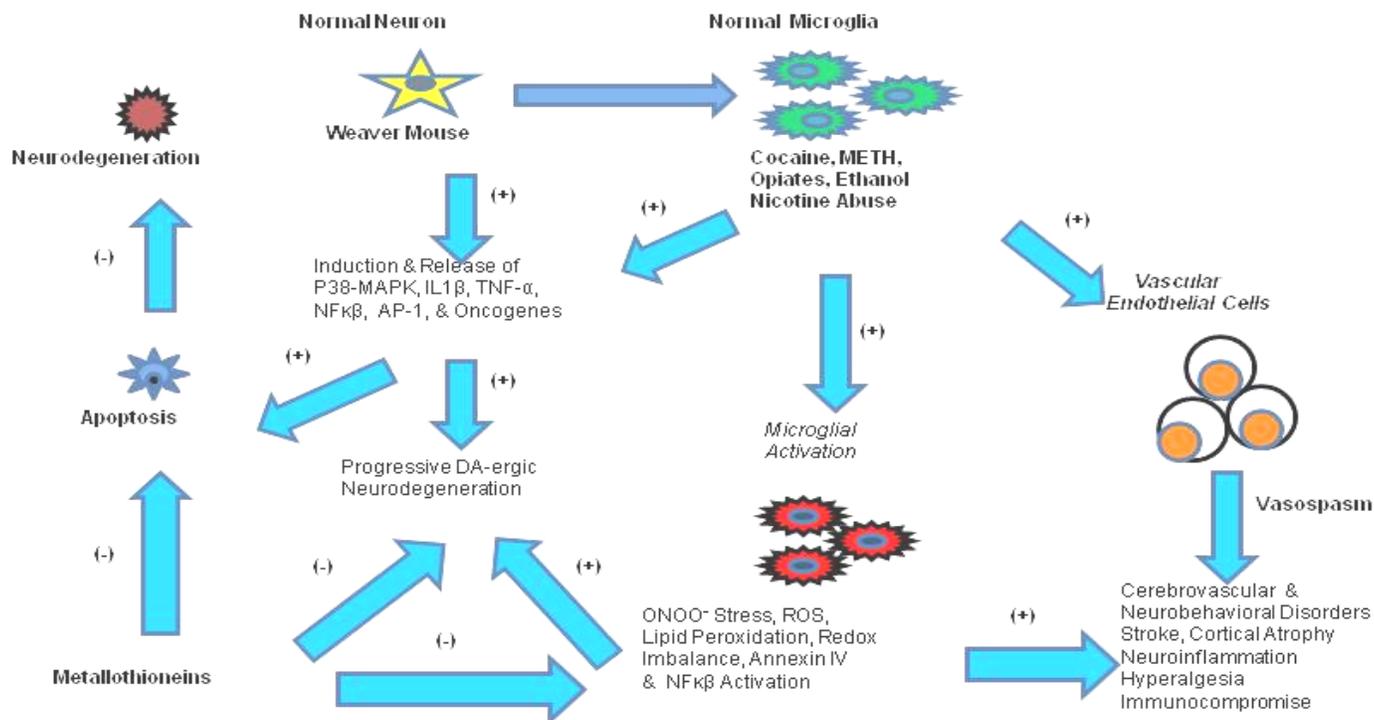
Ca<sup>2+</sup> regulatory protein, TRPC-1 [108]. Furthermore iron-induced NF $\kappa$ B activation,  $\alpha$ -Synuclein aggregation and BCL-2 down-regulation are inhibited by COQ10 in SK-N-SH neurons [44, 45]. Chronic abuse of METH and cocaine to C57/BL/6J mice caused significant reduction in the striatal TH, dopamine, complex-1, and 18F-DOPA uptake as seen in wv/wv mice, suggesting a common molecular mechanism of neurodegeneration in poly-substance abuse [70].

As circadian rhythm is usually disturbed among multiple drug abusers, we explored the therapeutic potential of melatonin in experimental model of multiple drug abuse and determined whether melatonin treatment could attenuate the neurotoxic effects of METH and cocaine in mice and in cultured neurons. Chronic abuse of cocaine, METH, and ethanol induced early morbidity and mortality in C57BL/6J mice, whereas MTs and melatonin provided neuroprotection in cocaine and METH-exposed C57BL/6J mice and SK-N-SH neurons [37, 38, 70, 109]. Furthermore, d-amphetamine-induced  $\alpha$ -Synuclein expression in SK-N-SH neurons was attenuated by melatonin suggesting primary involvement of oxidative stress and ROS synthesis in drug addiction [109]. Microglial activation by LPS or Salsolinol also induced NF $\kappa$ B-mediated pro-inflammatory

response which was attenuated by melatonin [110-112]. However brain regional melatonin was significantly reduced in *wv/wv* mice and was increased in *wv/wv*-MTs mice. These findings suggest that therapeutic interventions targeting brain regional MTs induction might have clinical significance in the prevention and/or treatment of poly-substance abuse.

By performing microPET imaging we have established that cocaine and METH cause reduction in the striatal 18F-DOPA

uptake in C57BL/6J mice. *Weaver* (*wv/wv*) mice also exhibited progressive neurodegeneration and reduction in the striatal 18F-DOPA uptake as function of aging as seen in individuals with multiple drug abuse. The distribution kinetics of 18F-DOPA was also impaired in *wv/wv* mice [18]. 18F-DOPA uptake was further reduced when cocaine and METH were co-administered along with ethanol suggesting that ethanol augments cocaine and METH neurotoxicity [19]. [For details please refer to our recent reports [19, 113].



**Fig. 5 Therapeutic Potential of MTS in Poly-substance Abuse.** A simplified diagram illustrating cocaine, METH, opiate, ethanol & nicotine-induced ONOO<sup>-</sup> stress through increased iNOS activation and NO synthesis which is associated with microglial ROS generation, lipid peroxidation, and p-38MAPK activation. Activated microglia, proliferate and release pro-inflammatory cytokines such as IL-1 $\beta$ , TNF- $\alpha$ , NF $\kappa$ B, and transcription factor AP-1, causing progressive neurodegeneration as seen in *wv/wv* mice and poly-substance abuse. MTs provide neuroprotection by attenuating above deleterious changes.

## [II] CONCLUSION

Although the information provided in this review is far from complete we have highlighted some of our recent research as well as from other labs on poly-substance abuse. Based on our experimental findings, we have furnished evidence that MTs provide neuroprotection by acting as free radical scavengers and by inhibiting various transcriptional factors such as AP-1 and pro-inflammatory cytokines such as NF $\kappa$ B-mediated microglial activation which is implicated in neuro-inflammation, and attenuate neurodegeneration through zinc-mediated transcriptional regulation of pro-inflammatory cytokine genes and by inhibiting ONOO<sup>-</sup> stress in poly-substance abuse. Hence specific brain regional MTs induction (via diet, exercise, and

chemotherapeutic agents etc) may prevent progressive neurodegenerative changes in multiple drug abuse. Further studies in this direction would go a long way in the effective prevention and/or treatment of poly-substance abuse and other neurodegenerative disorders of unknown etiopathogenesis.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest in this manuscript.

## REFERENCES

- [1] Lee YW, Hennig B, Fiala M, Kim KS, Toiborek M. [2001] Cocaine activates redox-regulated transcription factors and induces TNF-alpha expression in human brain endothelial cells. *Brain Res* 920: 125–133.
- [2] Lee YW, Son KW, Flora G, Hennig B, Nath A, Toborek M. [2002] Methamphetamine activates DBA binding of specific redox-responsive transcriptional factors I mouse brain. *J Neurosci Res* 70: 82–89.
- [3] Tacker DH, Herzog NK, Okorodudu AO. [2006] Cocaethylene affects human microvascular endothelial cell p38 mitogen-activated protein kinase activation and nuclear factor kappa B DNA binding activity. *Clin Chem* 52: 1926–1933.
- [4] Borner C, Holtt V, Kraus J. [2002] Involvement of activator protein-1 in transcriptional regulation of the human mu-opioid receptor gene. *Mol Pharmacol* 61:800–805.
- [5] Li Y, Kang J, Friedman J, Tarassishin L, Ye J, Kovalenko A, Wallach D, Horwitz MS. [1999] Identification of a cell protein (FIP-3) as a modulator of NF-kappa-B activity and as a target of an adenovirus inhibitor of tumor necrosis factor alpha-induced apoptosis. *Proc Natl Acad Sci USA* 96: 1042–1047.
- [6] Altura BM, Gebrewold A. [2002] Inhibitor of nuclear kappa-B activation attenuates venular constriction, leukocyte rolling-adhesion and microvessel rupture induced by ethanol in intact rat brain microcirculation: Relation to ethanol-induced brain injury. *Neurosci Lett* 334:21–24.
- [7] Ohkawa H, Sohma H, Sakai R, Kuroki Y, Hashimoto E, Murakami S, Saito T. [2002] Ethanol-induced augmentation of annexin IV in cultured cells and the enhancement of cytotoxicity by overexpression of annexinIV by ethanol. *Biochim Biophys Acta* 1588: 217–225.
- [8] Sohma H, Ohkawa H, Sakai R, Hashimoto E, Ukai E, Saito T. [2003] Augmentation of ethanol-induced cell damage and activation of nuclear factor-kappa B by annexin IV in cultured cells. *Alcohol Clin Exp Res* 27: 64S–67S.
- [9] Blanco AM, Pascual M, Valles SL, Guerri C. [2004] Ethanol-induced iNOS and COX-2 expression in cultured astrocytes via NF-kappa B. *Neuroreport* 22:681–685.
- [10] Zima T, Kalousova M. [2005] Oxidative stress and signal transduction pathways in alcoholic liver disease. *Alcoholism: Clin & Exp Res* 29: 110S–115S.
- [11] Crews F, Nixon K, Kim D, Joseph J, Shukitt-hale B, Qin L, Zou J. (2006) BHT blocks NFkappa B activation and ethanol-induced brain damage. *Alcohol Clin Exp Res* 30:1938–1949.
- [12] Rulten SL, Ripley TL, Hunt CL, Stephens DN, Mayne LV. [2006] Sp1 and NFkappaB pathways are regulated in brain in response to acute and chronic ethanol. *Gene Brain Behave* 5: 257–273.
- [13] Peng Z, Peng L, Fan Y, Zandi E, Shertzer HG, Xia Y. [2007] A critical role for ikappB kinase beta in metallothionein-1 expression and protection against toxicity. *J Biol Chem* 282: 21487–21496.
- [14] Brett K, Parker R, Wittenauer S, Hayashida K, Young T, Vincler M. [2007] Impact of chronic nicotine on sciatic nerve injury in the rat. *J Neuroimmunol* 186:37–44.
- [15] Noda M, Kariura Y, Amano T, Manago Y, Nishikawa K, Aoki S, Wada K. [2003] Expression and function of bradykinin receptors in microglia. *Life Sci* 72: 1573–1581.
- [16] Sharma SK, Yashpal K, Fundytus ME, Sauriol F, Henry JL, Coderre TJ. [2003] Alterations in brain metabolism by chronic morphine treatment: NMR study in rat CNS. *Neurochem Res* 28: 1369–1373.
- [17] Ebadi M, Sharma S, Wanpen S, Amornpan A. [2004] Coenzyme Q10 inhibits mitochondrial compex-1 down-regulation and nuclear factor kappa B activation. *J Cell Mol Med* 8:213–222.
- [18] Sharma S and Ebadi M. [2005] Distribution kinetics of 18F-DOPA in weaver mutant mice. *Brain Res Mol Brain Res* 139:23–30.
- [19] Sharma S and Ebadi M. [2008] SPECT Neuroimaging in Translational Research of CNS Disorders. *Neurochem Int* 52:352–362.
- [20] Maharajan P, Maharajan V, Ravagnan G, Paino G. [2001] The wv/wv mouse: a model to study the ontogeny of dopaminergic transmission system as their role in drug addiction. *Prog Neurobiol* 64: 269–276.
- [21] Ebadi M, Brown-Borg H, Garrett S, Singh BB, Shavali S, Sharma SK. [2005] Metallothionein-mediated neuroprotection in genetically-engineered mouse models of Parkinson's disease. *Brain Res Mol Brain Res* 134:67–75.
- [22] Ebadi M, Sharma S, Ajjimaporn A, Maanum S. [2005] Weaver mutant mouse in progression of neurodegeneration in Parkinson's disease. In Parkinson's Disease P537, *CRC Press*, Boca Rota FL.
- [23] Ebadi M, Sharma S, Ghafourifar P, Brown-Borg H, Refaey HEI. [2005] Peroxynitrite in the pathogenesis of Parkinson's disease and the neuroprotective role of metallothioneins. *Methods in Enzymol* 396: 276–298.
- [24] Ebadi M, Sharma S, Wanpen S, Shavali S. [2005] Metallothionein isoforms attenuate n Parkinson's Disease M Ebadi and R. Pfeiffer, *CRC press* New York pp 479–499.
- [25] Ebadi M, Wanpen S, Shavali S, Sharma S. [2005] Coenzyme Q10 stabilizes mitochondria in Parkinson's disease. *Molecular Interventions in Life-Style-Related Diseases*. Ed. *Hiramatsu* pp 127–153.
- [26] Murase S, Hayashi Y. [2002] Neuronal expression of macrophage colony stimulating factor in Purkinje cells and olfactory mitral cells of wild-type and cerebellar-mutant mice. *Histochem J* 34:84–95.
- [27] Douhou A, Debeir T, Michel PP, Stankoviski L, Oueghlani-Bousslama L, et al. [2003] Differential activation of astrocytes and microglia during post natal development of dopaminergic neuronal death in the weaver mouse. *Brain Res. Dev Brain Res* 145:9–17.
- [28] Sharma S and Ebadi M. [2004] An improved method for analyzing coenzyme Q homologues and multiple detection of rare biological samples. *J Neurosci Methods* 137: 1–8.
- [29] Sharma S, Kheradpezhou M, Shavali S, Refaey HEI, Eken J, Hagen C, Ebadi M. [2004] Neuroprotective actions of coenzyme Q10 in Parkinson's disease. *Method in Enzymol* 382:488–509.
- [30] Ebadi M, Brown-Borg H, Ren J, Sharma S, Shavali S, Rafey EHI, Carlson EC. [2006] Therapeutic efficacy of Selegiline in Neurodegenerative Disorders and Neurological Diseases. *Curr Drug Targets* 7: 1513–1529.
- [31] Ebadi M and Sharma S. [2003] Peroxynitrite and mitochondrial dysfunction in the pathogenesis of Parkinson's disease. *Antiox & Redox Signal* 5: 319–335.
- [32] Sharma S and Ebadi M. [2003] Metallothionein attenuates 3-morpholinodnonimine (SIN-1)-induced oxidative stress in dopaminergic neurons. *Antioxid Redox Signal* 5: 251–264.
- [33] Ebadi M and Sharma S. [2006] Metallothioneins 1 and 2 attenuate peroxynitrite-induced oxidative stress in Parkinson's Disease. *J Exp Biol and Med* 231:1576–1583.
- [34] Cochen V, Ribeiro MJ, Nguyen JP, Gurruchaga JM, Villafane G, et al. [2003] Transplantation in Parkinson's disease: PET changes

- correlate with the amount of grafted tissue. *Mov Disord* 18: 928–932.
- [35] Piccini P, Pavese N, Hagell P, Reimer J, Bjorklund A, et al. [2005] Factors affecting the clinical outcome after neural transplantation in Parkinson's disease. *Brain* 128: 2977–2986.
- [36] Sharma S, Refaey EHI, Ebadi M. [2006] Complex-1 activity and 18F-DOPA uptake in genetically-engineered mouse model of Parkinson's disease and the neuroprotective effect of coenzyme Q10. *Brain Res Bull* 70: 22–32.
- [37] Ajjimaporn A, Phansuwan-Pujito P, Ebadi M, Govitrapong P. [2007] Zinc protects SK-N-SH cells from methamphetamine-induced  $\alpha$ -Synuclein expression. *Neurosci Lett* 419:59–63.
- [38] Ajjimaporn A, Swinscoe J, Shavali S, and Ebadi M. [2005] Metallothionein provides zinc-mediated protective effects against Methamphetamine toxicity in SK-N-SH cells. *Brain Res Bull* 67:466–475.
- [39] Takeuchi A, Miyaishi PD, Kiuchi K, Isobe K. [2001] Macrophage colony stimulating factor is expressed in neuron and microglia after focal brain injury. *J Neurosci Res* 65: 38–44.
- [40] Suhara T. [2007] Phase-dependent roles of reactive microglia and astrocytes in nervous system injury as delineated by imaging of peripheral benzodiazepine receptor. *Brain Res* 1157: 100–111.
- [41] Banati RB. [2003] Neuropathological imaging: In-vivo detection of glial activation as a measure of disease and adaptive change in the brain. *Br Med Bull* 65:121–131.
- [42] Banati RB, Egensperger R, Maassen A, Hager G, Kreutzberg GW, Graeber MB. [2004] Mitochondria in activated microglia in vitro. *J Neurocytol* 33:535–541.
- [43] Zhang Y, Fong CC, Wong MS, Tzang CH, Lai WP, et al. [2005] Molecular mechanisms of survival and apoptosis in RAW-2647 macrophages under oxidative stress. *Apoptosis* 10: 545–556.
- [44] Sangchot P, Sharma S, Chetsawang B, Porter J, Govitrapong P, Ebadi M. [2002] Deferoxamine attenuates iron-induced oxidative stress and prevents mitochondrial aggregation and alpha-synuclein translocation in SK-N-SH cells in culture. *Dev Neurosci* 24: 143–153.
- [45] Koocumchoo P, Govitrapong P, Sharma S, Ebadi M. [2006] Coenzyme Q10 provides neuroprotection in iron-induced apoptosis in dopaminergic neurons. *Mol Neurosci* 28: 125–142.
- [46] Akahoshi N, Marushima YL, Himi T, Ishizaki Y, Ishii Y. [2007] Increased expression of the lysosomal protease cathepsin S in hippocampal microglia following kainate-induced seizures. *Neurosci Lett* 429:136–141.
- [47] Guinta B, Ehrhart J, Townsend K, Sun N, Vendrame M, et al. [2004] Galantamine and nicotine have a synergistic effect on inhibition of microglial activation induced by HIV-1 gp120. *Brain Res. Bull* 64:165–170.
- [48] de Simone R, Ajimone-cat MA, Carnevale D, Minghetti L. [2005] Activation of alpha 7-nicotinic acetylcholine receptor by nicotine selectively up regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. *J Neuroinflammation* 25:2(1) 4.
- [49] Shytle RD, Mori T, Townsend K, vendrame K, Sun N, et al. [2004] Cholinergic modulation of microglial activation by alpha 7-nicotinic receptors. *J Neurochem* 89: 337–343.
- [50] Park HJ, Lee PH, Ahn YW, Choi YJ, Lee G, et al. [2007] Neuroprotective effects of nicotine on dopaminergic neurons by anti-inflammatory action. *Eur J Neurosci* 26: 79–89.
- [51] Brett K, Parker R, Wittenauer S, Hayashida K, Young T, Vincler M. [2007] Impact of chronic nicotine on sciatic nerve injury in the rat. *J Neuroimmunol* 188:37–44.
- [52] Cagnin A, Gerhard A, Banati RB. [2002] In-Vivo Imaging of Neuroinflammation. *Eur Neuropharmacol* 12:581–586.
- [53] Cagnin A, Kassiou M, Meikle SR, Banati RB. [2007] Positron emission tomography imaging of neuroinflammation. *Neurotherapeutics* 4: 443–452.
- [54] Gerhard G, Banati RB, Goerres GB, Cagnin A, Myers R, et al. [2003] [11C](R)-PK11195 in progressive supranuclear palsy. *Mov Disord* 21: 89–93.
- [55] Gerhard A, watts J, Trender-Gerhard I, Turkheimer F, Banati RB, et al. [2004] In-vivo imaging of microglial activation with [11C] (R) PK-11195 PET in corticobasal degeneration. *Mov Disord* 19: 1221–1226.
- [56] Gerhard A, Pavrse N, Hottan G, Turkheimer F, ESM, hammers A, et al. [2006] In-vivo imaging of microglial activation with [11C] (R) –PK-11195 PET in idiopathic Parkinson's disease. *Neurobiology of Disease* 21: 404–412.
- [57] Gerhard A, Trender-Gerhard I, Turkheimer F, Quinn NP, Bhatia KP, Brooks DJ. [2006] In vivo imaging of microglial activation with [11C](R)-PK-11195 in progressive supranuclear palsy. *Mov Disord* 21: 89–93.
- [58] Wiley CA, Lopresti BJ, Becker JT, Boada F, Lopez OL, et al. [2006] Positron emission tomography imaging of peripheral benzodiazepine receptor binding in human immunodeficiency virus-infected subjects with and without cognitive impairment. *J Neurovirol* 12: 262–271.
- [59] Hammoud DA, Endres CJ, Chander AR, Guilarte TR, Wong DF, et al. [2005] Imaging glial cells activation with [11C] (R)-PK-11195 in patients with AIDS. *J Neurovirol* 11: 346–355.
- [60] Kropfeller MA, Boelaard R, Schuitemaker A, Folkersma H, vanBerckel BN, Lammertsma AA. [2006] Evaluation of reference tissue models for the analysis of [11C] ( R) PK-11195 studies. *J Cerebral Blood Flow and Metab* 26: 1431–1441.
- [61] Kropfeller MA, Boelaard R, vanBerckel BN, Schuitemaker A, Kloet RW, et al. [2007] Evaluation of reference regions for (R) [11C] PK-11195 studies in Alzheimer's disease and mild cognitive impairment. *J Cereb Blood Flow and Metab* 26: 1431–1441.
- [62] Price CJS, Wang D, Menon DK, Guadagno JV, Cleij M, et al. [2006] Intrinsic activated microglia map to the peri-infarct zone in the subacute phase of ischemic stroke. *Stroke* 37:1749–1753.
- [63] Tai YF, Pavese N, Gehard A, Tabrizi AJ, Barker RA, Brooks DJ, Piccini P. [2007] Microglial activation in presymptomatic Huntington's disease gene carriers. *Brain* 130: 1759–1766.
- [64] Turkhemier FE, Edison P, Pavese N, Roncaroli F, Anderson AN, et al. [2007] Reference and target region modeling of [11C] –(R) –PK-11195 brain studies. *J Nucl Med* 48: 156–167.
- [65] Kannan S, Saadani-Makki F, Muzik O, Chakraborty P, Mangner TJ, et al. [2007] Microglial activation in perinatal rabbit brain induced by intrauterine inflammation: detection with [11C] ( R ) PK11195 and small animal PET. *J Nucl Med* 48: 946–954.
- [66] Vowinckel E, Reutens D, Becher B, verge G, Evans J, Qwens T, Antel JP. [1997] PK-11195 binding to the peripheral benzodiazepine receptor as a marker of microglia activation in multiple sclerosis and experimental immune encephalomyelitis. *J Neurosci Res* 50: 345–353.
- [67] Cicchetti F, Brownell AI, Williams K, Chen YI, Livni E, Isacson O. [2002] Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. *European J Neurosci* 15:991–998.
- [68] Sharma S, Carlson E, Ebadi M. [2003] Neuroprotective actions of selegiline in inhibiting 1-methyl, 4-phenyl pyridinium ion (MPP+)-induced apoptosis in SK-N-SH neurons. *J Neurocytol* 76:563–571.

- [69] McGuire SO, Ling ZD, Lipton JW, Sortwell CI, Collier TJ, Carvey PM. [2001] Tumor necrosis factor alpha is toxic to embryonic mesencephalic dopaminergic neurons. *Exp Neurol* 169: 219–230.
- [70] Klongpanichapak S, Govitrapong P, Sharma S, Ebadi M. [2006] Attenuation of cocaine and methamphetamine neurotoxicity by coenzyme Q10. *Neurochem Res* 31: 303–311.
- [71] Chagkutip J, Vaughan RA, Govitrapong P, Enadi M. [2003] 1-Methyl-4-phenylpyridinium-induced down-regulation of dopamine transporter function correlates with reduction in dopamine transporter cell surface expression. *Biochem Biophys Res Commun* 311:49–54.
- [72] Chagkutip J, Govitrapong P, Klongpanichapak S, Ebadi M. [2005] Mechanism of 1-methyl-4-phenylpyridinium-induced dopamine release from PC12 cells. *Neurochem Res* 30:633–639.
- [73] Xie T, Tong L, McCann UD, Yuan J, Becker KG, Mehan AO, et al. [2004] Identification and characterization of metallothioneins-1 and 2 gene expression in the context of (+/-) 3,4-methylene dioxy methamphetamine-induced toxicity to brain dopaminergic neurons. *J Neurosci* 24: 7043–7050.
- [74] Yang Z, Yang S, Qian SY, Hong JS, Kadiiska MB, et al. [2007] Cadmium-induced toxicity in rat primary mid-brain neuroglia cultures: Role of oxidative stress from microglia. *Toxicol Sci* 98: 488–494.
- [75] Butcher HL, Kennette WA, Collins O, Zalups RK, Koroparnick J. [2004] Metallothionein mediates the level and activity of nuclear factor kappa B in murine fibroblasts. *J Pharmacol Exp Ther* 310:589–598.
- [76] Itoh N, Kimura T. [2007] Cytokine-induced metallothionein expression and modulation of cytokine expression by metallothionein. *Yakgaku Zasshi* 127: 685–694.
- [77] Wanpen S, Govitrapong P, Shavali S, Sangchot P, Ebadi M. [2004] Salsolinol, a dopamine-derived tetrahydroisoquinoline, induces cell death by causing oxidative stress in dopaminergic SH-SY5Y cells, and the said effect is attenuated by metallothionein. *Brain Res* 1005: 67–76.
- [78] Wanpen S, Kooncumchoo P, Shavali S, Govitrapong P, Ebadi M. [2007] Salsolinol, an endogenous neurotoxin, activates JNK and NFkappaB signaling pathways in human neuroblastoma cells. *Neurochem Res* 32: 443–450.
- [79] Vasto S, Mocchegiani E, Malavolta M, Cuppari I, Listi F, et al. [2007] Zinc and inflammatory/immune response in aging. *Ann N Y Acad Sci* 1100:111–122.
- [80] Ono S, Ishizaki Y, Tokuda E, Tabata K, Asami S, Suzuki T. [2007] Different patterns in the induction of metallothionein mRNA synthesis among isoforms after acute ethanol administration. *Biol Tracer Element Res* 115: 147–156.
- [81] Penkowa M. [2006] Metallothioneins are multipurpose neuroprotectants during brain pathology. *FEBS Journal* 273: 1857–1870.
- [82] Boess F, Ndikum-Moffor FM, Boeslste UA, Roberts SM. [2000] Effects of cocaine and its oxidative metabolites on mitochondrial respiration and generation of reactive oxygen species. *Biochem Biopharmacol* 60:615–623.
- [83] Dietrich JB, Poirrier R, Aunis D, zwiller J. [2004] Cocaine down-regulates the expression of mitochondrial genome in rat brain. *Ann N Y Acad Sci* 1025:345–350.
- [84] Dietrich JB, Mangeol A, Revel MO, Burgun C, Aunis D, Zwiller J. [2005] Acute or repeated Cocaine administration generates reactive oxygen species and induces antioxidant enzyme activity in dopaminergic rat brain structures. *Neuropharmacol* 48:965–974.
- [85] Imam SZ, Duhart HM, Skinner JT, Ali SF. [2005] Cocaine induces a differential dose-dependent alteration in the expression profile of immediate early genes, transcriptional factors, and caspases in PC12 cells. A possible mechanism of neurotoxic damage in cocaine addiction. *Ann N Y Acad Sci* 1053: 482–490.
- [86] Ang E, Chen Z, Zagouras P, Magna H, Holland J, Schaeffer E, Nestlet EJ [2001] Induction of nuclear factor kappa B in nucleus accumbens by chronic cocaine administration. *J Neurochem* 79:221–224.
- [87] Hargrave BY, Tiangco DA, Lattanzio FA, Beebe SJ. [2003] Cocaine, not morphine, causes the generation of reactive oxygen species and activation of NF-kappa B in transiently cotransfected heart cells. *Cardiovasc Toxicol* 3: 141–151.
- [88] Arango JC, Simonds P, Brettle RP, Bell JE. [2004] Does drug abuse influence the microglial response in AIDS and HIV encephalitis? *AIDS* 18:S69–74.
- [89] Gekker G, Hu S, Sheng WS, Rock RB, Lokensgard JR, Peterson PK. [2004] kappa-opioid receptor ligands inhibit cocaine-induced HIV-1 expression in microglial cells. *J Pharmacol Exp Ther* 309:600–606.
- [90] Gekker G, Hu S, Sheng WS, Rock RB, Lokensgard JR, Petersen PK. [2006] Cocaine-induced HIV-1 expression in microglia involves sigma-1 receptors and transforming growth factor-beta-1. *Int Immunopharmacol* 6: 1029–1033.
- [91] Imam SZ, el Yazal J, Newport DJ, Itzhak Y, Cadet JL et al. [2001] Methamphetamine-induced dopaminergic neurotoxicity. Role of peroxynitrite and neuroprotective role of antioxidants and peroxynitrite decomposition catalysts. *Ann N Y Acad Sci* 939:366–380.
- [92] Jayanthi S, Deng X, Noailles PH, Ladenheim B, abd cadet JL. [2004] Methamphetamine induces neuronal apoptosis via cross-talks between endoplasmic reticulum and mitochondria-dependent death cascades. *FASEB J* 18: 238–251.
- [93] Darvesh AS, Yamamoto BK, Gudelsky GA. [2005] Evidence for the involvement of nitric oxide in 3, 4-methylene dioxymethamphetamine-induced serotonin depletion in the rat brain. *J Pharmacol Exp Ther* 312:694–701.
- [94] Virmani A, Gaetani F, Imam S, Binienda Z, Ali S. [2003] Possible mechanism for the neuroprotective effects of L-carnitine on methamphetamine-evoked neurotoxicity. *Ann N Y Acad Sci* 993:197–207.
- [95] Hanson GR, Rau KS, Flekenstein AE. [2004] The amphetamine experience: A NIDA Partnership. *Neuropharmacol* 47: S92–100.
- [96] Sulzer D, Sonders MS, Poulsen NW, Galli A. [2005] Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* 5:406–433.
- [97] Bowyer JF, Ali S. [2006] High doses of Methamphetamine that cause disruption of the blood brain barrier in limbic regions produce extensive neuronal degeneration in mouse hippocampus. *Synapse* 60:521–532.
- [98] Thomas DM, Waker PD, Benjamins JA, Geddes TJ, Kuhn DM. [2004] Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *J Pharmacol Exp Therap* 311:1–7.
- [99] Hauser KF, El-Hage N, Buch S, Berger JR, Tyor WR, et al. [2005] Molecular targets of opiate drug abuse in neuroAIDS. *Neurotox Res* 8: 63–80.
- [100] Cui Y, Chen Y, Zhi JL, Guo RX, Feng JO, Chen PX. [2006] Activation of p38 mitogen-activated protein kinase in spinal microglia mediates morphine antinociceptive tolerance. *Brain Res* 1069:235–243.
- [101] Baraona E, Zeballos GA, Shoichet L, Mak KM, Lieber CS. [2002] Ethanol consumption increases nitric oxide production in

rats and its peroxynitrite-mediated toxicity is attenuated by polyethyl phosphatidylcholine. *Alcohol Clin Exp Res* 26:883–889.

- [102] Narita M, Miyoshi K, Narita M, Suzuki T. [2007] Involvement of microglia in the ethanol-induced neuropathic pain-like state in the rat. *Neurosci Lett* 414: 21–25.
- [103] Crews F, Nixon K, Kim D, Joseph J, Sulkitt-Hale B, Qin L, Zou J. [2006] BHT blocks NF-kappa B activation and ethanol-induced brain damage. *Alcohol Clin Exp Res* 30: 1938–1949.
- [104] Lee H, Jeong J, Son E, Mosa A, Cho GH, et al. [2004] Ethanol selectively modulates inflammatory activation signaling of brain microglia. *J Neuroimmunol* 156: 88–95.
- [105] Suk K. [2007] Microglial signal transduction as a target of alcohol action in the brain. *Curr Neurovasc Res* 4:131–142.
- [106] Suuronen T, Huuskonen J, Pihlaja R, Kyrölenko S, Salminen A. [2003] Regulation of microglial inflammatory response by histone deacetylase inhibitors. *J Neurochem* 87:407–416.
- [107] Suzuki T, Hide I, Matsubara A, Hama C, Harada K, et al. [2006] Microglial alpha 7 nicotine acetylcholine receptors drive a phospholipase C/IP3 pathway and modulate the cell activation towards the neuroprotective role. *J Neurosci Res* 83: 1461–1470.
- [108] Bollimuntha S, Singh BB, Shavali S, Sharma S, and Ebadi M. [2005] TRPC1-mediated inhibition of 1-methyl-4-phenylpyridinium ion neurotoxicity in human SH-SY5Y neuroblastoma cells. *J Biol Chem* 280:2132–2140.
- [109] Klóngpanichapak S, Phansuwan-Pujito P, Ebadi M, Govitrapong P. [2007] Melatonin protects SK-N-SH neuroblastoma cells from amphetamine-induced neurotoxicity. *J Pineal Res* 43: 65–73.
- [110] Shavali S, Ren J, Ebadi M. [2003] Insulin-like growth factor-1 protects human dopaminergic SH-SY5Y cells from salsolinol-induced toxicity. *Neurosci Lett* 340: 79–82.
- [111] Shavali S, Carlson CS, Swinscoe JC, Ebadi M. [2004] 1-benzyl-1, 2, 3, 4-tetrahydroisoquinoline, a parkinsonism-inducing toxin increases alpha-synuclein expression and causes nuclear damage in human dopaminergic cells. *J Neurosci Res* 76: 563–571.
- [112] Shavali S, Combs C, Ebadi M. [2006] Reactive macrophages increase oxidative stress and alpha-synuclein nitration during death of dopaminergic neuronal cells in co-culture. Relevance to Parkinson's disease. *Neurochem Res* 31: 85–94.
- [113] Sharma S, Ebadi M. [2008] Therapeutic Potential of Metallothioneins in Parkinson's disease. In *New Research on Parkinson's Disease*. Eds T.M Hahn and Julian Werner, Chapter-1 pp 1–41. Nova Science Publishers USA.

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