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METALLOTHIONEIN EXPRESSION AND ROLES IN THE CENTRAL NERVOUS SYSTEM

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Metallothioneins (MTs) are low-molecular-weight (6–7 kD) nonenzymatic proteins (60-68 amino acid residues, 25-30% being cysteine) ubiquitously expressed in the animal kingdom. In the central nervous system (CNS), three MT isoforms are known, namely MT-I, MT-II, and MT-III. MT-I and MT-II (MT-I+II) are regulated and expressed coordinately and are currently the best characterized MT isoforms. This review will focus on the expression and roles of MT-I+II in the CNS. MT-I+II are implicated in diverse physiological and pathophysiological functions, such as metal ion metabolism, regulation of the CNS inflammatory response, protection against reactive oxygen species and oxidative stress, reduction of apoptotic cell death, and stimulation of neuroregeneration and brain tissue repair in vivo. *Accordingly, brain tissue damage and neurodegeneration during pathological conditions and the accompanying mortality and clinical symptoms are altogether significantly increased in MT-I+II deficient mice, while the opposite is observed after medical MT-II treatment and in MT-I overexpressing mice. Consequently, MT-I+II are likely essential factors in CNS disorders, which suggests a potential therapeutic use of these proteins.*
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INTRODUCTION

In historical reports, a protein with a high affinity for heavy metals and an unusual cysteine abundance was discovered in horse kidney and subsequently, this protein was biochemically characterized and named metallothionein (MT)(1-3). Over the years, it has been shown that MT constitutes a superfamily of proteins, and a growing interest on MT has been evident (4-13).

MTs are low-molecular-weight (6–7 kD) nonenzymatic proteins (60-68 amino acid residues, 25-30% being cysteine) expressed ubiquitous in the animal kingdom and characterized by a high content of zinc (Zn^{2+}), copper (Cu^{+}), and sulfur (present as cysteine)(4,7,10,14).

In the CNS, there are three major MT isoforms, MT-I, MT-II, MT-III (8-10,14-16), of which MT-III (also called growth inhibitory factor) only has been known since 1991 (17).

MT-I and MT-II (MT-I+II) are regulated and expressed coordinately (18,19) and are the best characterized MT isoforms at present (6-9,12,20). Moreover, MT-I+II are the most extensively distributed MT isoforms, being expressed in virtually all tissues (6,7,15,21). The regulation, expression, and putative roles of MT-III differ substantially from those of MT-I+II and are less well understood (7-9,12,15,17,21-27). This review will focus on the expression and functional significance of MT-I+II in the central nervous system (CNS).

MT-I+II EXPRESSION IN THE CNS

Normal CNS

MT-I+II are expressed in the normal CNS, both during development and in adults (7,28-30). There is a general consensus

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that astrocytes are the main cell type expressing MT-I+II, but in addition meningeal cells, ependymal cells, choroid plexus and endothelial cells show MT-I+II positivity. In contrast, microglia, oligodendrocytes, and neurons appear roughly devoid of MT-I+II expression (7-9,28-35). Regarding MT-I+II expression in neurons, the data are conflicting as MT-I+II positive neurons have been described intermittently (36-39). However, it is generally agreed that the levels of MT-I+II in astrocytes are several-fold higher than in neurons (39).

CNS pathology

Pathological conditions in the CNS elicit a characteristic inflammatory response (40,41). Astrocytes are activated and show reactive astrogliosis (41-43), and macrophages are recruited from peripheral monocytes and resident microglia (44-48), as well as T lymphocytes and to a lesser degree B cells, and neutrophilic granulocytes are recruited in response to tissue damage in the CNS (41,44). During the CNS inflammatory response, both resident CNS cells and recruited leukocytes express various cytokines, major histocompatibility complex (MHC) and adhesion/costimulatory molecules, which ultimately lead to generation of reactive oxygen species (ROS) (49-56). ROS are highly toxic in the CNS – when their production is increased to a degree which overcomes the neutralizing effects of endogenous antioxidants, ROS are key mediators of oxidative stress, which is a hallmark of neurodegenerative diseases and cell death (49-51,57-60). Accordingly, the balance between pro- and anti-inflammatory factors determines the intensity and course of the inflammatory response including the levels of oxidative stress and neurodegeneration (48,61-64).

The expression of MT-I+II is significantly increased in CNS inflammation (7-9). In animal models, MT-I+II are increased following traumatic injury (65-70), administration of gliotoxic 6-aminonicotinamide (6-AN)(71-74), excitotoxic kainic acid-induced seizures (31,34,75,76), N-methyl-D-aspartate-induced excitotoxicity (77), and immobilization stress (78). Furthermore, MT-I+II are increased in a transgenic murine model of familial amyotrophic lateral sclerosis (ALS)(84,85), and in the myelin-deficient jimpy mouse (79) as well as in experimental autoimmune encephalomyelitis (EAE), the experimental model of the human demyelinating disease multiple sclerosis (80-83). MT-I+II expression is also upregulated in human neurological diseases - multiple sclerosis (own unpublished observations), Alzheimer´s disease, Pick's disease (29,86-89), and ALS (89).

The cells increasing MT-I+II expression during pathological conditions are mainly reactive astrocytes, and to a lesser degree activated microglia/macrophages (22,31,34,66,67,70-72,79,81,90-92). In addition, meningeal cells, ependymal cells, and endothelial cells become positive for MT-I+II during pathological conditions (7,32,88). The induction of MT-I+II in neuronal cells has not been consistently described (7-9,20).

Regulation of MT-I+II expression in CNS

MT-I+II expression is regulated in a coordinate manner (18). Various factors such as metals (Zn, Cu, Cd, Hg) (4,30,70,90), glucocorticoids (corticosterone, dexamethasone), catecholamines (norepinephrine, isoproterenol) (93-96), stress (78,96), and ionizing radiation (97-99) affect MT-I+II regulation and production (6,7,14,20). Another important factor influencing MT-I+II regulation in the CNS are inflammatory response-related stimuli such as proinflammatory cytokines (8,9,100). The cytokines interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), IL-3, and interferon-α increase MT-I+II expression *in vivo* in the CNS in a cytokine-specific manner as demonstrated by using transgenic mice overproducing these cytokines (91,92,101,102). Thus, by studying mice with genetic IL-6 deficiency [IL-6 knock-out (IL-6KO) mice], IL-6 has been demonstrated as a major inducer of MT-I+II in the CNS during pathological conditions (34,67,69,71,103). Finally, cellular oxidative injury due to formation of ROS is also an inducer of MT-I+II (6,7,104-110).

THE FUNCTIONAL SIGNIFICANCE OF MT-I+II IN THE CNS

The capability to specifically manipulate the MT-I+II genes in cells and mice, and pharmacological MT treatment studies have provided some answers regarding the roles for MT-I+II in physiological and pathophysiological processes as well as the functional significance of MT-I+II during brain disorders. At present, MT-I+II are known to modulate several fundamental processes in the CNS: 1) metal ion metabolism, 2) the neuroinflammatory response, 3) oxidative stress, and 4) neurodegeneration *versus* neuroregeneration.

Metal ion metabolism

It is generally acknowledged that MT-I+II function in metal ion homeostasis in the brain, and particularly in Zn and Cu homeostasis as well as in the protection against heavy metals (1,15,21,30,70,111-114). By acting both as a donor and acceptor of essential metals Zn and Cu, MT-I+II may control Zn- and Cu-dependent proteins, enzymes and transcriptions factors, and regulate cellular Zn and Cu metabolism in response to dietary and physiological changes (6,15,115-118). Hence, MT-I+II could serve as a metal ion reservoir from which apometalloproteins, including enzymes and zinc finger proteins (transcription factors, signaling and adapter molecules), can acquire metal ions. In support of this, rapid exchange kinetics of Zn bound to MT-II, which is far greater than the exchange of Zn in other Zn proteins exists (119,120), and MT-I+II can successfully donate Zn to a number of Zn dependent apometalloproteins *in vitro* (121). Reactivation *in vitro* of Zn requiring apoenzymes such as apocarbonic anhydrase and apocarboxypeptidase by MT-I+II retrieves their enzymatic

activity (120,122). Likewise, MT-II can donate Zn to transcription factors and thereby to modulate DNA binding (123). The exchange reaction possibly occurs by a direct Zn donation from MT *via* protein-protein interaction (120).

Given that MT-I+II regulate Zn and Cu, MT-I+II may have important roles in fetal development and in tissue responses to pathological conditions (70,115,124). Indeed, expression and regional distribution of MT-I+II during CNS development matched those of vesicular Zn (30). Also, Zn levels in plasma are related to the MT-I+II concentration (126,127), and tissue Zn accumulation shows an accurate relationship with MT-I+II expression (125-127), indicating that MT-I+II are involved in the regulation or processing of newly acquired Zn. In cases of dietary Zn or Cu deficiency, the MT-I+II levels in CNS are decreased significantly, resulting in considerable alterations in the neuroinflammatory response to brain injury (70). Accordingly, mice with genetical MT-I+II deficiency [MT-I+II knock out (MT-I+IIKO) mice)] are more sensitive to dietary Zn restriction as well as to Zn toxicity than normal mice (117).

MT-I+II are likely important in the protection against heavy metal ions as MT-I+II are involved in detoxification and storage of these metals (112,114,128). Thus, MT-I+II deficient astrocytes are more sensitive to methylmercury than astrocytes with intact MT-I+II expression, and MT-I gene transfection in MT-I+II deficient astrocytes resulted in increased resistance to methylmercury (114,129). Using transgenic mice with overexpression of MT-I (MTTg* mice), it was demonstrated that MT-I protects against Cd lethality and toxicity (130), and MT-I+IIKO mice are far more susceptible to this metal (10,131,132) relatively to normal mice. Since both excess concentrations of Zn and heavy metals are highly toxic in the CNS (15,129,133-135), the roles of MT-I+II in metal ion regulation are likely neuroprotective (4,21,114).

Immunomodulation

MT-I+II exert immunomodulatory roles *in vitro* and *in vivo*. Induction of MT-II mRNA and MT protein *in vitro* inhibited monocyte activation including cell adhesion, IL-1β levels, and the respiratory burst (136). The MT levels of monocyte-derived cell lines influence cell adherence, invasion and the oxidative burst (137,138), and MT-I+II significantly decrease the ability of macrophages to induce T lymphocyte proliferation *in vitro* (139). In addition, MT-I+II inhibit the antigen-specific humoral response of lymphocytes (140) and decrease the number of IgG secreting lymphocytes (141). MT-I+II reduce cytotoxic T cell activity by more than 70%, the proliferative response of T cells to cytokines, as well as the target cell killing and lymphocytic expression of CD8 (142). MT-I+II have been shown to *in vivo* modulate CNS immune responses in diverse pathological conditions. Hence, the recruitment of macrophages, T lymphocytes and bone marrow progenitor cells in CNS pathological processes was significantly increased in MT-I+IIKO relatively to wild type mice (66,68,83,143), while both endogenous (transgenic) MT-I overexpression and exogenous MT-II treatment during CNS inflammation resulted in the completely opposite pattern with a significant reduction in macrophages and lymphocytes (8,9,65,74,81). This MT-I+II-dependent immunomodulatory effect is likely neuroprotective, since ongoing inflammation may lead to neurodegeneration and neurological disorders (49-51,54,56,91,92,147-150).

The detailed mechanisms of actions of MT-I+II in reducing both macrophage and T cell recruitment are not fully clarified, but MT-I+II may affect these cells by interference with membrane-associated molecules (53,54,56). Indeed, MT-I+II can bind to the surface of macrophages (139), T and B lymphocytes (144) *in vitro*, and probably affect surface molecules important for leukocyte activation and/or recruitment. In agreement with this, MT-I+II reduced the expression of MHC (142) and adhesion molecules such as ICAM and Mac1 (143). On the other hand, the MT-I+II-induced modulation of CNS inflammatory response could likely be caused by altered cytokine levels. During CNS inflammation, MT-I+II reduced the expression of the proinflammatory cytokines IL-1β, IL-6 and TNF-α as well as stimulated both reactive astrogliosis and the levels of antiinflammatory growth factors such as basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), neurotrophin-3 (NT-3) and -4 (NT-4) and vascular endothelial growth factor (VEGF), as it was demonstrated in genetically modified (MT-I+IIKO and MTTg*) mice and in animals treated with MT-II (8,9,31,65,66,68,73,74,81-83). The proinflammatory cytokines IL-1β, IL-6 and TNF-α orchestrate immunoresponses and have several activating functions during CNS inflammation (52,53,56). They are potentially deleterious or beneficial, depending on their concentration as well as the site and duration of action (54,61,63,64,145,146). Hence, excess or prolonged production of proinflammatory cytokines, such as in genetically manipulated mice can lead to chronic inflammation and functional CNS disorders (91,92,147-150). The MT-I+II-induced inhibition of IL-1β, IL-6 and TNF-α could likely regulate the response of inflammatory cells and serve important neuroprotective roles. In addition, the MT-I+II-induced stimulation of the growth factors bFGF, TGF-β1, NT-3 and VEGF in the pathological CNS (65,68,74) may exert supplementary anti-inflammatory and neuroprotective functions (52,56,151-160).

The above-mentioned effects of MT-I+II remind of the immunomodulatory actions of glucocorticoids, which selectively inhibit proinflammatory cytokines and stimulate anti-inflammatory cytokines and thereby push the immune reaction away from a T_H1 and toward a T_H2 pattern (161,162). Since corticosterone and dexamethasone can induce MT-I+II expression in the brain (94-96), it might be that glucocorticoid hormones and MT-I+II have yet unknown additive and/or synergistic actions during pathological conditions. Another possibility is that some

of the immunomodulatory actions of glucocorticoids in the brain are mediated in part by MT-I+II, as intranasal administration of glucocorticoids in humans increased significantly MT-II mRNA in peripheral leukocytes (163). Also, patients with ulcerative colitis, who are steroid-refractory and respond poorly to glucocorticoid treatment, express low levels of MT-II, while patients in remission, who respond to glucocorticoid treatment, show high MT-II levels in inflammatory cells (164). In agreement with this, MT-I+II induction in mice during influenza virus infection could be inhibited by administration of a glucocorticoid receptor antagonist (165), which reveals a direct role of glucocorticoids in MT-I+II induction during pathological conditions. Nonsteroidal anti-inflammatory drugs such as diclofenac, indomethacin and piroxicam also induce MT-I mRNA and MT proteins (166), as do antirheumatic drugs such as D-penicillamine (167). Therefore, it is likely that the mechanisms of action of both steroidal and nonsteroidal anti-inflammatory drugs involve MT-I+II, suggesting a pivotal role for MT-I+II in regulating inflammation. Although the precise molecular mechanisms underlying these effects have yet to be clarified, it is likely that MT-I+II could be a very potent factor for the treatment of inflammatory and/or autoimmune conditions. Indeed, MT-II treatment during toxic neurodegeneration and traumatic brain injury could decrease both clinical and histopathological signs (65,74). MT-II treatment in EAE significantly improved clinical symptoms, induced disease remission and drastically decreased mortality (81).

Oxidative stress

A considerable oxidative stress occurs in CNS inflammatory responses due to the cytotoxic consequences of a misbalance between the formation of ROS and the ability of cells to produce antioxidants. Oxidative stress takes place when the production of ROS increases, scavenging of ROS by antioxidants decreases, or both. It is a major inducer of apoptotic cell death and a hallmark of neurodegenerative disorders (49-51,57,59,60,146). Increasing data have emerged showing that MT-I+II are extraordinarily efficient free radical scavengers and antioxidant proteins with important roles during oxidative stress (4,12,14,15,68,83,105,107,108,110,168-181). *In vitro* studies have demonstrated that MT-I+II inhibit ROS-induced DNA degradation and tissue damage (106,177,178,182-188). In fact, MT-I+II could protect against ROS-induced DNA damage with a much higher molar efficiency (almost 800-fold) than glutathione (GSH)(189). MT-I+II could also be important during oxidative stress by interacting with reduced GSH (184), MT-I+II can inhibit GSH depletion (183) and antagonize the deleterious effects of oxidative stress on catalase (183). MT-I+II can functionally substitute for Cu/Zn-superoxide dismutase (SOD) in the cellular defense against oxidative stress (190), and compensate for Cu/Zn-SOD deficiency in mice (191). In agreement with this, MT-I+II protect against neurotoxicity and oxidative stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which is used in experimental models of Parkinson's disease (192), in which oxidative stress plays a key role (57,59). Cultured cells overexpressing MT-I are protected against oxidative stress relatively to controls (177,178,187,188), and cells derived from MT-I+IIKO mice exhibit an increased sensitivity to oxidative stress (186,188,193). Hence, embryonic or adult cells from MT-I+IIKO mice, despite their normal levels of GSH and normal activity of Cu/Zn-SOD, glutathione peroxidase or catalase, show enhanced ROS production and lipid peroxidation after exposure to tert-butyl hydroperoxide and paraquat (186,193). MT-I+II protect against the actions of electrophilic anticancer drugs and ionizing radiation, which are also agents causing oxidative stress and cell death (97-99,173,186,194-196). MT-I+II play a key role in protection against oxidative stress induced in mitochondria following administration of mitochondrial-specific reactive oxygen generators (171), and MT-II overexpression in cells reduces drastically doxorubicin-induced mitochondrial ROS levels (197). During neuropathological conditions in MT-I+IIKO mice, the levels of antioxidants catalase, Mn-SOD and Cu/Zn-SOD were increased along a significantly increased oxidative stress, as judged by increased levels of inducible nitric oxide synthase (iNOS), nitrotyrosine (NITT) and malondialdehyde (MDA)(31,66,68,83). In MT-I+II deficiency, even an increased expression of other antioxidants could not protect from increased oxidative stress levels, underlining the antioxidative and neuroprotective importance of MT-I+II *in vivo*. In IL-6KO mice with neuroinflammation, significant increases in oxidative stress levels have been detected relatively to normal mice (34,69,71), and in these mice all examined antioxidants other than MT-I+II were unaffected by the IL-6 deficiency, whereas the MT-I+II levels were clearly reduced (69,71). The levels of Cu/Zn-SOD, Mn-SOD and catalase were comparable in IL-6KO and normal mice, while MT-I+II were significantly decreased in the former (34,69), suggesting that MT-I+II are essential antioxidant factors in CNS inflammation. In addition, both endogenous (transgenic) MT-I overexpression and exogenous MT-II treatment during neuroinflammation protect significantly the CNS from ROS formation and oxidative stress *in vivo* (65,74,81,82). As numerous reports suggest that MT-I+II are important antioxidants *in vivo* the methods to increase the MT-I+II levels during neurodegenerative disorders may become a new therapeutic approach.

Neurodegeneration and apoptosis

As oxidative stress is a major inducer of neurodegeneration and apoptosis (49,50,57,59,60,146), the antioxidant effects of MT-I+II may prevent apoptosis. On the other hand, MT-I+II *per se* can exert significant antiapoptotic functions, and the roles of MT-I+II in the control and inhibition of apoptosis have received increasing attention (8,9,12,16,31,65,66,70,74,

82,83,110,111,143,178,198-203). MT-I+IIKO mice exhibit a significantly higher numbers of apoptotic cells (mostly neurons) during various CNS pathological conditions such as traumatic brain injury, excitotoxicity, EAE and during IL-6-induced inflammation *in vivo* (31,66,83,143). MT-I overexpressing mice show significantly reduced apoptotic cell death in CNS inflammation (65,74), and *in vivo* MT-II treatment following traumatic brain injury, neuroglial degeneration or EAE significantly reduced CNS neurodegeneration and apoptosis (65,74,82). Interestingly, the apoptotic neurons observed in the injured brains were always MT-I+II-negative (65,74), suggesting that MT-I+II expression prevents apoptosis and/or neutralizes apoptotic signals. In agreement with this, suppression of the MT-I+II production in human T cells resulted in apoptosis, in a dose-dependent manner (201), and reduction of MT-I+II during ALS increased significantly the loss of motor neurons, also dose-dependently (85). These effects of MT-I+II are very interesting from a therapeutic point of view, in that CNS injury is often followed by a spread of tissue damage (secondary degeneration and apoptosis), resulting in neuronal loss substantially greater than predicted by the severity of the primary injury.

The precise mechanisms through which MT-I+II can affect apoptosis are yet unknown, but several possibilities are likely. At first, MT-I+II are extraordinarily efficient antioxidants, which in itself could explain the mechanism of action regarding apoptosis in the CNS (57,59,60). Secondly, MT-I+II are major factors controlling Zn and Cu (1,15,21,70,113,116,117,204), which in exceeding amounts can cause neuronal toxicity and death (133-135,205-208). Thirdly, the MT-I+II-induced inhibition of macrophages and T lymphocytes in the CNS could reduce the number of cells suffering from apoptosis, in that an ongoing inflammatory response may initiate cytokine-mediated cascades leading to increased levels of proteases, complement factors, eicosanoids, adhesion/costimulatory molecules, vasoactive amines and free radicals, which can affect the apoptosis pathway (49-51,54,56,61,209). To this may be added that apoptosis could also be due to the lack of proper astroglial functioning, as astrocytes are a main source for trophic factors in the brain and exert neuroprotective actions (4,42,68,72,210-213). In addition, MT-I+II presumably could affect directly critical steps in the signal transduction cascade leading to apoptosis, including proto-oncogenes and tumor suppressor protein p53 (57,59,214), as proto-oncogenes protect against apoptosis, while p53 promotes apoptosis (59,60,214-216). Indeed, the levels of p53 were increased in MT-I+IIKO mice relatively to normal mice (200,203), and antisense down-regulation of MT-I+II was followed by decreased levels of proto-oncogenes *bcl-2* and *c-myc* and increased levels of p53 (198). This response of proto-oncogenes and p53 was reversed completely in MT-II overexpressing cells (198), and apo-MT overexpression in tumor cells promoted increased cell survival and growth by inducing a p53-null state (217). One of the mechanisms for this action could be the sequestration of Zn by MT-I+II in the cell nucleus, whereby Zn-dependent gene expression and signaling pathways are suppressed as well as zinc finger-containing molecules are inactivated (5,6,104,218,219). In addition, MT-I+IIKO mice displayed increased immunoreactivity for caspase-1, activated caspase-3 as well as cytoplasmic cytochrome-c (31,83), which are also involved in the critical steps leading to apoptosis (57,59). In agreement with this, caspase-1, caspase-3, and cytoplasmic cytochrome-c were significantly decreased in MTTg* mice and after MT-II treatment during neuroinflammation (74,82). MT-II overexpression in cells effectively inhibits doxorubicin-induced apoptosis and mitochondrial cytochrome-c release and caspase-3 activation (197). Moreover, the levels of nuclear factor kappa B (NFκB), a key signal leading to apoptosis (59,60), were increased after a traumatic brain injury in MT-I+IIKO mice and in rats with decreased MT-I+II levels relatively to animals with normal MT-I+II expression (70). It was demonstrated that MT-I+II can interact with NFκB (221), and NFκB activity and concentration were significantly increased *in vitro* and *in vivo* in MT-I+II deficiency (31,220,222), and MT-I+IIKO mice showed increased NFκB levels during excitotoxicity relatively to controls (31). Taken together, these data support that MT-I+II have major antiapototic roles and may inhibit critical steps in the signal transduction cascade leading to apoptotic cell death.

Given the involvement of MT-I+II in cell proliferation, survival and apoptosis, it is not surprising that much attention has been drawn to the possible role of MT-I+II in cancer. Inhibition of MT-I synthesis in different tumor cell lines inhibits cell growth in a dose- and time-dependent manner (223). Inhibition of MT-II production in carcinoma cells reduced cell growth by 50-60% and induced apoptosis, while MT-II overexpression in these cells increased two-fold cellular proliferation relatively to control carcinoma cells (198). Moreover, MT-I+II positivty in bone marrow and peripheral blood samples of patients with acute leukemia was strongly associated with significant resistance to chemotherapy (202), which mediates its therapeutic effect by induction of oxidative stress and apoptosis (10,185,215). In the MT-I+II-negative cases of acute leukemia, the chemotherapy was more effective and the apoptotic action of chemotherapy was completed much faster than in the MT-I+II-positive cases (202). Interestingly, the study of MT-I+II expression during treatment of acute leukemia also demonstrates, that even though the chemotherapy eliminates leukemic cells, MT-I+II expressing cell populations survived and were able to increase during the treatment period, as they were capable of escaping apoptosis (202). In accordance to this, leukemic cells exhibited resistance to various anticancer drugs due to increased amounts of MT-I+II protein and MT-II mRNA (224), and this resistance could

be reversed by specific inhibition of the MT-I+II synthesis (224). The apoptotic response to anticancer drugs is increased in cells with genetic MT-I+II deficiency relatively to wild-type cells (195,200). Thus, MT-I+II were demonstrated to have a major impact on chemotherapy resistance phenomena. In general, increased levels of MT-I+II in human tumors are associated with a high degree of malignancy and a poor response to anticancer drugs (14,173,185,225-228). These MT-I+II-induced effects could be mediated by more than one mechanism. MT-I+II have significant antioxidative and antiapoptotic roles, which can counteract the effects induced by anticancer treatment. In addition, MT-I+II can sequester electrophilic antineoplastic agents and thereby directly neutralize the anticancer drugs (10,185,225). By regulating the activities of Zn-requiring metalloenzymes, MT-I+II might alter the therapeutic effectiveness of anticancer drugs (5,225). MT-I+II can protect against chemotherapy-induced mutations in cells as antisense-mediated reduction of MT-II levels drastically elevates spontaneous mutation rates (229). Thus, cell clones with clearly reduced MT-II levels were spontaneous mutators, with a mutation rate 5-10 times higher than control cells (229). Transgenic overexpression of MT-I could considerably reduce the spontaneous mutation frequencies, as well as the mutation rate in Zn- and Cd-treated cells was inversely related to MT-I mRNA and MT-I+II protein levels (230).

Thus, a reduction in the MT-I+II levels can very likely inhibit cancer cell resistance to various anticancer treatments as well as the levels of MT-I+II in tumor cells might be used as a prognostic marker. Specific induction of MT-I+II in healthy tissue of cancer patients but not in cancer tissue could be a successful adjunct to anticancer strategy, which may alleviate the problems with tumor cell resistance to chemotherapy as well as the toxic side-effects in healthy cells, which are major obstacles to the curative treatment of cancer. Interestingly, treatment with bismuth compounds has the ability to induce MT proteins in normal, healthy tissue, but apparently not in cancer tissue (231-233). This is due to the fact that bismuth accumulates in healthy tissue but is hardly taken up by cancer cells, and MT proteins are thus induced only in healthy tissue, which thereby becomes resistant to chemotherapy and radiation, while the simultaneous antitumor activities of anticancer treatments remain uncompromised (231-233). The manipulation of MT-I+II in both healthy and cancer tissue may result in more successful treatment of cancer. Hence, gene-directed techniques leading to cell-specific and tissue-targeted MT-I+II regulation deserve to be investigated with highest priority in the future.

Neuroregeneration

A major problem following brain injury is that neurons are susceptible to degeneration and injured axons hardly regenerate. In this regard, it is interesting that MT-I+II significantly promote reactive astrogliosis. Accordingly, during neuropathological conditions, MT-I+IIKO mice display a significantly reduced number of reactive astrocytes (31,66,68,73,83), while reactive astrogliosis is clearly increased during transgenic MT-I overexpression or after exogenous MT-II treatment in CNS inflammation *in vivo* (65,74,81,82). This MT-I+II-induced stimulation of astrocytes is likely important, because astrocytes have major functions during the acute phase and later stages of CNS inflammation (41,42,56,146,210-212,234,235). Astrocytes produce various neuroprotective factors and contain the highest level of various antioxidants in the brain (4,34,68,70,72,210-212,236-237). Astrocytes from different brain regions have the potential to influence growth and maintenance of adult neurons (213) and protect neurons against injuries (210,234). In addition, astroglia modulate the extracellular matrix and contribute to the formation of glial scar tissue and CNS tissue repair following brain damage (4,42,66,68,69,237).

The growth factors bFGF, TGF-β1, NT-3 and VEGF are anti-inflammatory and neuroprotective factors involved in angiogenesis and tissue regeneration (52,56,151-160). In CNS pathology, MT-I+II induce the expression of bFGF, TGF-β1, NT-3 and VEGF (65,68,74), and thus MT-I+II may further stimulate tissue recovery and neuroregeneration. Accordingly, brain repair after a traumatic lesion to the cortex was drastically reduced in MT-I+IIKO mice, which showed a lesion cavity in the cortex even at 90 days after the injury. In contrast, normal mice, in which MT-I+II levels are increased, showed pronounced angiogenesis and preservation of the brain tissue by 10 days after injury as well as a complete tissue recovery after 20 days (66,68). Neuroregeneration was significantly increased in MTTg* mice and in mice receiving MT-II treatment following a similar brain lesion (65). MT-I+II stimulated the expression of growth-associated protein (GAP-43)(a marker for synaptogenesis) as well as axonal plasticity and repair during neuronal injury (M. Penkowa, unpublished observations). Mice overexpressing MT-I showed reduced cerebral edema and a significantly reduced volume of injured tissue (42% smaller than that of control mice) after focal cerebral ischemia and reperfusion (39). Three weeks after reperfusion, MTTg* mice exhibited reduced sensorimotor defects and a significantly better motor performance relatively to normal mice (39). In EAE model, rats receiving MT-II treatment had a significantly reduced mortality and clinical symptoms in a dose- and time-dependent manner, and MT-II administration resulted in EAE disease remission (81). Treating mice with MT-II during neuroglial degeneration totally eliminated the mortality relatively to mice receiving control treatment (74). On the contrary, MT-I+IIKO mice were more susceptible to kainic acid administration and showed more convulsions and longer convulsion periods when compared to those of control

Table 1. *MT-I+II actions in the CNS during pathological conditions*

MT-I+II inhibit	**MT-I+II stimulate**
Recruitment of macrophages and lymphocytes	Reactive astrogliosis
Production of proinflammatory cytokines (IL-1, IL-6, TNF-α) Free radicals/oxidative stress	Production of anti-inflammatory cytokines (bFGF, TGFB1, NT-3, NT-4/5, VEGF)
Apoptotic cell death and neurodegeneration	Glial scar formation and angiogenesis
Mortality and clinical symptoms	Neuroregeneration and neuronal plasticity/sprouting

mice (31). MT-I+IIKO mice with EAE showed more severe symptoms and increased disease incidence and mortality relatively to normal EAE-sensitized mice (83). Reduction of MT-I+II clearly promotes the onset of ALS clinical signs and mortality in a dose-dependent manner as shown in a transgenic murine model of familial ALS (85). Taken together, the above studies strongly indicate that MT-I+II have cardinal roles in neuroprotection after brain damage (Table 1).

CONCLUSION

The expression of MT-I+II is essential following CNS pathological conditions, while normal mice are able to develop and function independently of their MT-I+II expression. Hence, MT-I+II may not have critical roles during normal conditions or there might be a back-up system to compensate for MT-I+II deficiency. In contrast, during neuropathological conditions, MT-I+II have a fundamental role. MT-I+II influence significantly the inflammatory cells recruited to the CNS, levels of oxidative stress, neurodegeneration and cell death. MT-I+II stimulate CNS angiogenesis and neuroregeneration following brain injury. These functions provide some rationale for the highly preserved and represented in nature MT-I+II proteins. Since MT-I+II effectively address more than one process contributing to CNS pathology, MT-I+II-based therapies might become a new approach in the treatment of neurological disease.

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