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Commentary

Methylene blue and Alzheimer's disease

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ABSTRACT

The relationship between methylene blue (MB) and Alzheimer's disease (AD) has recently attracted increasing scientific attention since it has been suggested that MB may slow down the progression of this disease. In fact, MB, in addition to its well characterized inhibitory actions on the cGMP pathway, affects numerous cellular and molecular events closely related to the progression of AD. Currently, MB has been shown to attenuate the formations of amyloid plaques and neurofibrillary tangles, and to partially repair impairments in mitochondrial function and cellular metabolism. Furthermore, various neurotransmitter systems (cholinergic, serotonergic and glutamatergic), believed to play important roles in the pathogenesis of AD and other cognitive disorders, are also influenced by MB. Recent studies suggest that the combination of diverse actions of MB on these cellular functions is likely to mediate potential beneficial effects of MB. This has lead to attempts to develop novel MB-based treatment modalities for AD. In this review article, actions of MB on neurotransmitter systems and multiple cellular and molecular targets are summarized with regard to their relevance to AD.

1. Introduction

Historically, methylene blue (MB) is the first synthetic compound ever used as an antiseptic in clinical therapy and the first antiseptic dye to be used therapeutically [see 1,2 for reviews]. In fact, the use of MB and its derivatives was widespread in chemotherapy before the advent of sulfonamides and penicillin [see 1 for review]. MB has also been a lead compound in drug research against various bacterial and viral infections [1], and cancer [1,3]. Investigations into its structure and therapeutic activities have played a major role in the development of the phenothiazines [2–4], a large class of drugs employed as antihistamines and neuroleptics.

The beneficial effects of MB in the treatment of cognitive disorders occurring in psychoses have been known for more than a century [5]. Recently, its potential to slow down cognitive decline in Alzheimer's disease (AD) has attracted attention [6–8]. AD is the most common cause of dementia in the elderly. Clinically, it is characterized by progressive cognitive impairment and severe neuropsychiatric disturbances [8,9]. Histopathological hallmarks are extracellular deposits of β-amyloid protein (Aβ, a 40–42-amino acid proteolytic fragment of the amyloid precursor protein, APP) in neuritic plaques, intracellular neurofibrillary tangles caused by the abnormal aggregation of tau protein and neuronal cell loss, particularly affecting the cholinergic system [9].

The present review summarizes the data suggesting that MB is a promising candidate that may help prevent cognitive decline in AD.

2. Biochemical pharmacology

MB, a cationic dye with the chemical name tetramethylthionine chloride, belongs to a class of compounds known as phenothiazines. It is soluble in water and can also dissolve in organic solvents [10]. Its color is deep blue in its oxidized state (MB) with a maximum absorption at light wavelengths of 609 and 668 nm [11], and colorless when reduced to leucoMB, which does not absorb in the visible region. These two forms of the dye exist as a redox couple in equilibrium; together they form a reversible oxidation–reduction system or electron donor–acceptor couple.

In clinical practice, MB is available as a solution (1%, w/v; 10 g/l or 26.74 mM) and the recommended safe dose appears to be between 1 and 4 mg/kg, depending on the source [12]. Typically, MB is administered i.v. or orally (50–300 mg), but interosseous MB

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infusion has also been described [12–14]. In the treatment of methemoglobinemia, it is usually given as 0.1–0.2 ml/kg of a 1% solution administered intravenously (i.v.) over 5–10 min.

In healthy volunteers, mean plasma concentration of 5 μM MB was achieved after i.v. bolus injection of 1.4 mg/kg MB [13]. After oral administration of 100 mg MB, the dose suggested for the oral treatment of methemoglobinemia, whole blood concentrations of up to 25 ng/ml (7–8 μM) were reached in healthy individuals [15]. It is noteworthy that whole blood measurements of MB may not reflect its bio-phase concentrations, since MB binds to blood cells and is extensively taken up by them [16]. Thus, MB concentrations in whole blood have been found to be 4–5-fold higher than in plasma [15,17]. MB also binds to bovine serum albumin with a stoichiometry of 1:1, with the dissociation constant being 2.90 μM [16]. It is estimated that the plasma concentration of free MB is only about 60 nM under the assumption that the total MB concentration is 10 μM, the total albumin concentration is 500 μM and competing ligands of albumin are absent [16]. Thus, not surprisingly, MB has an exceedingly high volume distribution of 21.0 l/kg in rabbits [18]. Interestingly, many of the pharmacokinetic properties of MB show significant dose- and species-dependent variations [15–19].

After oral administration of 100 mg MB, whole blood MB concentrations in healthy individuals were reported to be one order of magnitude lower than after i.v. administration of the same dosage [15]. However, a recent study comparing the administration of single doses of MB (50 mg i.v. versus 500 mg orally) indicated that the absolute bioavailability of MB after oral administration was 72.3% [20]. Reasons for the discrepancies between these studies, such as mode of application (gel capsules versus aqueous oral formulation), blood versus plasma measurements, different methodologies and cellular uptake, have been discussed by the authors [20].

There is strong indication that MB passes the blood–brain barrier [15]. In rats, 60 min after oral administration or i.v. injection, MB concentrations are about 10 times higher in the brain than in plasma [15]. When MB is injected supravitally into the left cardiac ventricle of mice or golden hamsters, it penetrates well into the brain, but possibly both in the MB and the reduced leucoMB forms [21]. After this mode of administration, MB penetrates neurons differentially and is found both in the cytoplasm of a subset of neurons and in the extracellular matrix [21]. Furthermore, clinical studies show that serious transient neurological signs and symptoms develop in patients, who are given MB infusions after parathyroidectomy, if they are taking serotonin reuptake inhibitors or other antidepressants; this also strongly suggests that MB crosses the blood–brain barrier [22]. Passage of MB into the brain despite ionization may be facilitated by the dispersed charge distribution around the molecule, which may facilitate its passage through membranes [10]. Isobolic potential curves encompassing the MB molecule indicate that charges on the nitrogen and sulfur atoms are not localized and are almost equally distributed on the surface of the molecule (Fig. 1).

Other possible factors influencing membrane penetration of MB are differences in ionization and lipophilicity between MB and leucoMB, its reduced form. The pK_a of MB is about 0 to −1; hence, it is completely ionized in the pH range of 1–8 found in the gastrointestinal system [23]. Furthermore, the partition coefficient (log P) of MB was reported to be negative (−0.96); substances with a log P value below 0 are considered hydrophilic [10,23]. From the chemical point of view, given its hydrophilicity and its positive charge, MB would not be expected to pass lipid bilayer membranes [24]. However, cationic MB is reduced to the neutral leucoMB by redox systems in erythrocytes and peripheral tissues [12,15,16,23]. In isolated perfused rabbit lungs, approximately 16% of the MB entering the pulmonary artery was shown to be reduced before emerging from the pulmonary veins [25]. LeucoMB has a pK of 5.8, resulting in only 33% protonation, as opposed to MB with a pK value close to zero. Uncharged leucoMB is more than 20 times more lipophilic than MB [26]. Since lipophilic compounds easily pass the blood–brain barrier [24] and there are significant differences in the biological activities of MB and leucoMB, it is currently not clear, which form of MB mediates its biological actions.

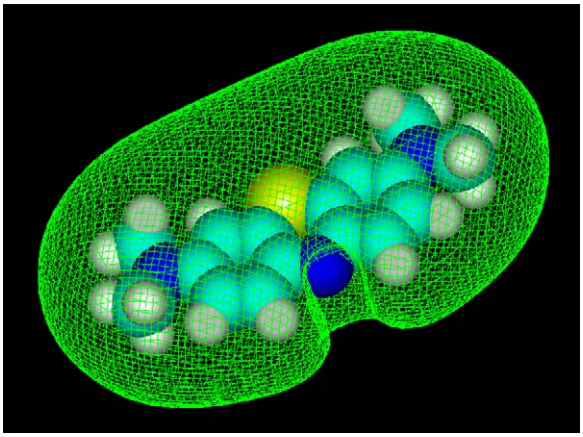

Fig. 1. The isopotential surface surrounding the methylene blue molecule was modeled in 3D space. Carbon, hydrogen, nitrogen, and sulfur atoms are represented by the colors cyan, white, blue and yellow, respectively. The green mesh represents the isopotential surface. The image was produced by Hyperchem version 6, using charges computed after minimizing the structure of MB by the semi-empirical method in the program.

MB has been shown to be taken up by cells, accumulate and dimerize in the cytoplasmic organelles, and to cause cell toxicity and DNA damage in various cell types [10,12,16]. At concentrations higher than 10 μM, MB forms dimers, with the dissociation constant being 170 μM [10,16], and causes cell toxicity. MB accumulation in various tissues is thus a complex process reflecting changes in MB reduction rates by the tissue, dye uptake, and the status of the tissue metabolic functions [10,12,16,25].

When administered i.v., MB demonstrates multi-compartmental pharmacokinetics with a terminal plasma half-life of 5–7 h [15]. Interestingly, i.v. administration of MB differs markedly with regard to organ distribution compared with oral administration. While oral MB results in higher intestinal and liver concentrations, i.v. administration results in higher MB concentrations in the brain [15].

Total urinary recovery of MB ranges from 53 to 97% of its oral dose [23]. The color of the urine following oral administration of MB was shown to be unrelated to the amount absorbed, since a considerable percentage of the dye (33–78%) recovered in the urine was excreted as stabilized leucoMB [15,23]. In clinical applications, MB usually produces blue-green urine, blue sclera and stained clothing. This benign discoloration can be alarming to patients, although it is self-limiting and disappears within a few days of discontinuing the drug.

From the toxicological point of view, MB is relatively nontoxic and has an enviable safety record [27,28]. In one notable pediatric case report [29], a child was given a dose that was 16 times the recommended maximum. The child's skin was intensely blue, but there were no other documented ill effects. Most side effects of MB appear to be dose-dependent and do not occur with doses <2 mg/kg, a dose range that is widely used in the clinical applications of MB. In *in vitro* studies, MB demonstrates biological actions at a wide range of concentrations, from 0.1 nM to 10 μM, and toxic effects have only been reported at concentrations higher than 100 μM. The oral LD_{50} of MB has been estimated to be 1180 mg/kg

in rats and 3500 mg/kg in mice [30]; the intraperitoneal LD_{50} is 150 mg/kg in mice and 180 mg/kg in rats; and the i.v. LD_{50} is 77 mg/kg in mice and 42.3 mg/kg in sheep [19,30,31]. MB easily crosses the blood–milk barrier [32]. In neonates, MB toxicities include hemolytic anemia, respiratory distress, and phototoxicity. The use of MB during pregnancy and intra-amniotic procedures is not recommended, due to its association with a high percentage of fetal intestinal atresia and other teratogenic effects [31]. The results of comprehensive studies by the National Toxicology Program [33] indicated that MB is mutagenic in several bacterial strains and that long term MB administration (1–24 months) in rodents is associated with lymphomas as well as various intestinal malignancies.

Currently, MB is approved by the Food and Drug Administration for oral or i.v. administration in the treatment of methemoglobinemia, the relief of lower genito-urinary tract discomfort, and as a surgical 'tracer dye' for the detection of fluid leaks, mainly from the gastrointestinal and urinary systems. In 2008, eleven clinical trials registered in the United States were investigating the clinical utility of MB in areas ranging from oncology to depression and psychosis. Recently, both preclinical [28,34,35] and clinical studies have confirmed the beneficial effects of MB in the treatment of cognitive disorders caused by psychoses [36–39]. In the following, the influence of MB upon the key neuropathological features of AD, β-amyloid deposits and neurofibrillary tangles, will be reviewed. In addition, its effect upon different neurotransmitter systems affected in AD will be summarized.

3. MB and amyloid plaques

Aβ aggregation which leads to extracellular fibrillar deposits known as amyloid plaques is a complex process involving soluble monomers, oligomers and fibril formation. Although the mechanisms underlying oligomerization and fibril formation are not clearly elucidated, MB has been shown to inhibit $A\beta_{42}$ oligomerization with an IC_{50} value of 12.4 μM in *in vitro* biochemical studies [40]. MB-inhibition of $A\beta_{42}$ oligomerization was observed concomitant with a dose-dependent promotion of fibrilization, suggesting that oligomer and fibril formation may represent different cellular processes. Addition of MB to preformed $A\beta_{42}$ oligomers also resulted in oligomer loss and promotion of fibrilization. This study concluded that MB inhibited $A\beta_{42}$ oligomer formation by promoting fibril formation. Interestingly, in another *in vitro* study, MB has been reported to inhibit $A\beta_{40}$ fibrilization with an IC_{50} value of 2.3 μM [41]. Further influences of MB upon amyloid formation are discussed in the context of the cholinergic system (see below).

The structure–activity relationship regarding the effects of MB has also been investigated. It is noteworthy that both inhibition of $A\beta_{40}$ and the increase of $A\beta_{42}$ fibrillation by MB were also mimicked by a wide range of phenothiazine-class compounds [40–42]. Phenothiazines related to MB have also been shown to inhibit the conversion of soluble prion protein into the protease-resistant form [43,44]. However, in the case of prion protein, although several phenothiazine-class compounds are effective inhibitors of the conversion process, MB was found to be ineffective [44]. Apparently, further studies on the structure–activity relationship are likely to reveal significant potency and efficacy differences among phenothiazine-class of compounds.

4. MB and neurofibrillary tangles

The intracellular neurofibrillary lesions consist of paired helical filaments and straight filaments, which are made of the microtubule-associated protein tau in a hyperphosphorylated state [45]. The temporal and spatial accumulation of hyperphosphorylated tau protein correlates with nerve cell loss and the severity of dementia. In an earlier study [45], the formation of aggregates made of truncated tau protein was inhibited by a number of phenothiazines, including MB (IC_{50} = 3.7 μM) and its desmethylated derivatives, such as azure A, azure B and tolonium chloride (IC_{50} = 60–110 nM). In a recent study [41], the effect of MB was investigated for its ability to inhibit heparin-induced assembly of tau protein into filaments *in vitro*. MB and several other phenothiazines, such as azure A, azure B and quinacrine mustard, inhibited tau filament formation with IC_{50} values in the low micromolar range (2–3 μM), without an effect on the ability of tau protein to interact with microtubules. In this study, comparing the structures of active and inactive phenothiazines, with the exception of quinacrine mustard, compounds lacking a side chain at the phenothiazine ring were found to be strongly inhibitory [41,42], whereas those with a side chain were either weakly inhibitory or had no effect, unlike the prion study where phenothiazines with a side chain were found to be inhibitory [43,44].

Tau protein includes the microtubule-binding domain (MBD) of three or four 31–32-residue repeats. Since the three- or four-repeat microtubule-binding domain (MBD) in tau protein plays an essential role in filament formation, the importance of these repeats in the inhibitory effects of MB on heparin-induced filament formation of MBD was recently investigated in *in vitro* studies [46]. The N-unsubstituted phenothiazine ring of MB was shown to be necessary for the inhibition of heparin-induced tau filament formation. In this study, it was found that the inhibitory responses with respect to heparin-induced filament formation to the second and third repeat peptides of MBD were not altered by MB, suggesting the importance of the first and fourth repeat peptides in the inhibitory activity of MB for MBD filament formation [46].

Given its tau-dissolving properties, the clinical use of MB as a therapeutic of AD has recently been tested by Wischik et al. [6]. Preliminary results of a phase II clinical study in 332 patients with mild to moderate AD suggest that MB significantly improves cognitive functions, compared to placebo controls, and slows the progression of AD over the course of a year. However, up to now, the results, which have been presented at a conference [6], have not yet been published in a peer-reviewed journal. In their study, MB was administered three times per day in three different doses: 30, 60 and 100 mg. Since the gelatin capsule containing the 100 mg dose was reportedly defective, the data of this dosage group were folded into the placebo arm [7]. In addition, problems with blinding have been put forward [7], since MB turns the urine blue. Furthermore, a phase II trial aims at assessing toxicity, but not efficacy. Therefore, further clinical studies are warranted.

5. MB and the cholinergic system

The cholinergic system plays an important role in the regulation of learning and memory. Furthermore, both *in vitro* and *in vivo* studies have consistently demonstrated a link between cholinergic activation and the protein metabolism in amyloid plaques. Lesions of cholinergic nuclei cause a rapid increase in cortical amyloid plaques [for reviews see 47,48]. Similarly, reduction in cholinergic neurotransmission leads to amyloidogenic metabolism and contributes to the neuropathology and cognitive dysfunction in AD [48]. Activation of M1/M3 muscarinic receptors promotes processing of the amyloid precursor protein, APP, into the non-amyloidogenic secreted form of APP (sAPPα) and decreases total Aβ formation both *in vitro* and *in vivo* in AD patients [for a review see 49]. Thus, the cholinergic system has been a common target for drug design in the treatment of AD. Recently, cognitive deficits induced in mice by scopolamine, a potent muscarinic receptor antagonist, have been shown to be reversed by MB [50].

Furthermore, when combined with rivastigmine, a cholinesterase inhibitor, the effect of MB was potentiated, suggesting that some of the side effects of rivastigmine can be avoided by adding low doses of MB [50].

The modulation of cholinergic transmission by MB occurs at multiple levels. At the receptor level, MB competitively displaces the binding of muscarinic ACh receptor antagonists, such as [^{3}H]quinuclidinyl benzylate in cardiac myocytes (K_D = 187 nM [51]) and [^{3}H]-N-methylscopolamine (K_D = 550 nM [52]) in cardiac membrane homogenates. At present, there are no reports on the effects of MB on cholinergic receptors in neuronal structures, neither muscarinic nor nicotinic. However, it is likely that synaptic ACh concentrations of 1–2 mM and the below mentioned MB-inhibition of acetylcholinesterase (AChE) activity significantly diminish the extent of competitive MB-inhibition of muscarinic receptors in cholinergic transmission.

AChE is an enzyme that hydrolyses acetylcholine, thereby terminating the action of this neurotransmitter at cholinergic synapses. By increasing synaptic acetylcholine levels, AChE inhibitors enhance cholinergic neurotransmission indirectly and are presently used as long term symptomatic treatment for patients with AD [48,53]. MB-inhibition of AChE activity has been known for many decades. Early studies demonstrated that AChE activity in cow erythrocytes is competitively inhibited by MB with an IC_{50} of 0.57 μM [54]. Interestingly, the extent of inhibition by MB was significantly decreased when the incubation time of MB was increased. Since the oxidized form of MB was ineffective on AChE activity, it was concluded that the formation of leucoMB during prolonged incubation periods causes decreased efficacy of MB in *in vitro* assay systems. In a more recent study, MB inhibited the esterase activity of human plasma, pseudocholinesterase and bovine AChE in a concentration-dependent manner, with IC_{50} values of 1.1, 5.3, and 0.42 μM, respectively [52].

In the mammalian brain, the second major form of cholinesterase is butyrylcholinesterase (BuChE). The two forms, AChE and BuChE, differ genetically, structurally, and in their kinetics. Butyrylcholine is not a physiological substrate in mammalian brain, which makes the function of BuChE difficult to interpret. In human brain, BuChE is found in neurons and glial cells, as well as in neuritic plaques and tangles of patients with AD. In rodents, selective inhibition of BuChE elevated brain ACh, improved learning processes and lowered β-amyloid protein [55]. Whereas, AChE activity decreases progressively in the brain of patients with AD, BuChE activity shows some increase [47,48]. BuChE inhibitors cause several-fold increases in extracellular acetylcholine levels in the rat brain [47]. It has been suggested that BuChE may compensate decreased AChE activity for hydrolyzing brain acetylcholine in AD patients [47]. Therefore, mixed AChE and BuChE inhibitors have been suggested for the treatment of advanced AD [47,56]. In a recent study [57], the inhibition kinetics of electric eel AChE (K_i = 17 nM) and of human BuChE (K_i = 0.4 μM) by MB were compared. The results of this study indicate that MB inhibits BuChE activity and that the interaction between MB and BuChE is mediated through multiple (at least two) binding sites, whereas AChE inhibition is mediated by a single site. Further studies on the mechanisms of MB binding to BuChE point to a complex, nonlinear inhibition, suggesting a cooperative interaction between these binding sites [58]. Comparison of the inhibitory effects on horse and human serum BuChEs strongly suggests that the two enzymes have distinct microstructural features. Significant differences in the extent of MB-inhibition between bovine erythrocyte AChE and human serum cholinesterase activities were also reported in early studies [54]. Similarly another study reported that MB induced complete inhibition of esterase activity of human plasma and human pseudocholinesterase, whereas bovine acetylcholinesterase was maximally inhibited by 73% [52], indicating that the effects of MB upon AChE activity differ significantly between species. Finally, besides MB, several other phenothiazine derivatives also block the activity of BuChE with K_D values ranging from 4 nM to 10 μM [59–62].

In addition to the above-mentioned hydrolytic activities, noncholinergic functions of AChE have been reported to play an important role in the progression of AD. In the brain, most of the AChE is found as a tetrameric form bound to neuronal membranes. Cytochemical studies have demonstrated that AChE associates with senile plaques and induces amyloid fibril formation by interacting with the peripheral anionic site of the enzyme, thus forming stable and highly toxic AChE–amyloid–Aβ complexes [48,53,63]. The pro-aggregating AChE effect appears to be associated with the intrinsic amyloidogenic properties of the corresponding Aβ peptide. The neurotoxicity induced by AChE–Aβ complexes is higher than that induced by the Aβ peptide alone, both *in vitro* and *in vivo*. Furthermore, AChE inhibition attenuates AChE–Aβ complex formation and increases the expression of nicotinic receptors, both of which correlate with cognitive improvements in AD patients. The neuroprotective effect of nicotine, presumably mediated via nicotinic receptors, against the toxicity and the production of Aβ has previously been shown [for a review see 48]. Thus recently, dual binding site AChE inhibitors, which might inhibit Aβ peptide aggregation through binding to both catalytic and peripheral sites of the enzyme, are being investigated in the treatment of AD [53,63]. However, the effects of MB on AChE–Aβ complex formation and on the activity of the peripheral binding site of the AChE molecule have not been investigated.

It appears that multiple mechanisms, including influences upon the formation of neuritic plaques and of neurofibrillary tangles as well as inhibition of AChE, contribute to the beneficial effects of MB in AD and probably in other neurodegenerative diseases. Traditional pharmacological intervention in neurodegenerative diseases, such as AD and Parkinson's disease, has typically sought to increase or mimic the synaptic activity of specific neurotransmitters, such as acetylcholine, which are reduced due to neuronal degeneration.

6. MB and the serotonergic system

Whereas the decline in cognitive functions in AD can be largely related to cholinergic dysfunction, the behavioral and psychological symptoms associated with AD are most probably caused by an impaired balance between several other neurotransmitters, with serotonin (5-HT) playing a pivotal role [64]. AD patients show numerous abnormalities of the serotonergic system, including a marked depletion in 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the frontal and temporal cortices as well as a significant decrease in cortical neurons expressing 5-HT receptors [64]. In addition, a reduction in serotonergic projection fibers has been reported in the cerebral cortex. It is due to a significant loss of serotonergic neurons in the dorsal and median raphe nuclei, which are a preferential site for neurofibrillary tangle formation.

MB has been shown to increase extracellular levels of 5-HT in the rat brain after local or systemic administration [65]. The main mechanism involved is reversibly inhibition of the function of the 5-HT metabolizing enzyme monamine oxidase (MAO) type A, with IC_{50} values ranging from 27 to 180 nM [11,66]. 5-HT is considered to be a relatively specific substrate for MAO-A, but not for MAO-B. Consequently, when administered together with serotonin reuptake inhibitors (SRIs), such as citalopram, clomipramine, duloxetine, imipramine, sibutramine, and venlafaxine, i.v. infusion of MB, especially at doses exceeding 5 mg/kg [11,22,67–69], may precipitate 5-HT toxicity, formerly known as serotonin syndrome

[70]. For this reason, the use of MB is contraindicated in patients treated with SRIs [71].

7. MB and the glutamatergic system

Another important neurotransmitter system influenced by MB is the glutamatergic system. In rat hippocampal slices, glutamate-mediated synaptic transmission is abolished by relatively high concentrations (5–50 μM) of MB [72]. On the other hand, MB is known to enhance memory retention [73,74] and other brain functions in which ionotropic glutamate receptors are involved [75,76]. Considering the important roles of NMDA receptors in long term potentiation and other events related to synaptic plasticity, it is possible that MB differentially modulates the functions of AMPA/kainate and NMDA-type ionotropic glutamate receptors. The beneficial effect upon cognitive functions observed in AD patients after MB administration [6] may therefore be in part attributable to influences upon the glutamatergic system.

8. MB and mitochondrial function

Drugs which are successful at targeting impaired mitochondrial respiration have also been shown to improve neuronal energy production and memory consolidation [for a review see 77]. In fact, MB is one of such compounds as well. It penetrates through cellular and mitochondrial membranes, accumulates within mitochondria [78], and improves mitochondrial respiration at low concentrations (0.5–2 μM) by shuttling electrons to oxygen in the electron transport chain, and corrects for perturbed mitochondrial metabolism induced by mutagens [79]. When MB acts as an alternative electron acceptor in mitochondria, it also inhibits the production of superoxide by competing with molecular oxygen [80,81]. Cytochrome oxidase is the terminal enzyme in the electron transport chain and is tightly coupled to neuronal metabolism and ATP production. Cytochrome oxidase activity has been shown to decline in AD [77]. MB administration leads to enzymatic induction of cytochrome oxidase, enhancing oxidative metabolic capacity in the brain during the post-training period of memory processing [74]. It appears that MB increases the enzymatic activity of cytochrome oxidase in a use-dependent manner. While performing a particular learning task, brain regions with the highest metabolic demand during memory consolidation show the largest increases in cytochrome oxidase activity [75]. An increase in cytochrome oxidase activity results in increased oxidative metabolic capacity of neurons because it allows more oxygen consumption and ATP formation in the brain [82]. Furthermore, in a recent study, it was demonstrated that MB, at nM concentrations, delays cellular senescence, increases oxygen consumption, heme synthesis, and resistance to oxidative stress and cadmium [83]. MB also increases the cellular content of cytochrome *c* oxidase (complex IV) relative to the other mitochondrial respiratory complexes, and induces antioxidant defense enzymes. Experiments on lysates from mitochondria suggest that MB recycles between oxidized and reduced forms through interactions with specific mitochondrial electron carriers, which may contribute to its antisenescence action [83].

9. Conclusion

MB has many properties desired from a drug expected to act in the brain. Of these, its high solubility in aqueous media, its low toxicity, its ability to cross the blood–brain barrier and cellular membranes and its approval for use in humans make it especially attractive as a potential therapeutic agent. Influences of MB upon the cholinergic, serotonergic and glutamatergic neurotransmitter systems, upon mitochondrial function and upon the formation of amyloid plaques and of neurofibrillary tangles make it a promising candidate for the treatment of AD. Results of recent clinical trials in a relatively small number of patients seem to be promising. However, these findings need to be confirmed by more extensive studies.

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