Review Article

Therapeutic Approaches to Delay the Onset of Alzheimer's Disease

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The key cytopathologies in the brains of Alzheimer's disease (AD) patients include mitochondrial dysfunction and energy hypometabolism, which are likely caused by the accumulation of small aggregates of amyloid-β (Aβ) peptides. Thus, targeting these two abnormalities of the AD brain may hold promising therapeutic value for delaying the onset of AD. In his paper, we discuss two potential approaches to delay the onset of AD. The first is the use of low dose of diaminophenothiazins (redox active agents) to prevent mitochondrial dysfunction and to attenuate energy hypometabolism. Diaminophenothiazines enhance mitochondrial metabolic activity and heme synthesis, both key factors in intermediary metabolism of the AD brain.The second is to use the naturally occurring osmolytes to prevent the formation of toxic forms of Aβ and prevent oxidative stress. Scientific evidence suggests that both approaches may change course of the basic mechanism of neurodegeneration in AD. Osmolytes are brain metabolites which accumulate in tissues at relatively high concentrations following stress conditions. Osmolytes enhance thermodynamic stability of proteins by stabilizing natively-folded protein conformation, thus preventing aggregation without perturbing other cellular processes. Osmolytes may inhibit the formation of Aβ oligomers *in vivo*, thus preventing the formation of soluble oligomers. The potential significance of combining diaminophenothiazins and osmolytes to treat AD is discussed.

1. Introduction

1.1. An Overview of Alzheimer's Disease. Alzheimer's disease (AD) is an irreversible brain disorder that slowly destroys memory and eventually a person's ability to perform the daily life tasks and activities. Memory problems are one of the first signs of AD, and as it progresses, decline in other cognitive abilities such as poor judgment and mood changes starts to surface. Eventually people with severe AD cannot communicate and become completely dependent on others for their care. Most people with AD have lateonset of disease, which usually develops after age 60. However, a silent preclinical phase which precedes the development of AD clinical symptoms may span 2–8 years.

AD is a disease with a complex etiology and no single therapeutic approach is likely to prevent or lead to a cure. All current treatments focus on several different aspects, including management of behavioral symptoms or temporarily slowing the progress of the disease. However, none of these treatments changes or alter the inevitable course of the disease. Thus, even after almost three decades of research, AD is still dementia with a progressive failure to form new memories, and thereby interfering with the basic mechanism of the disease.

In recent years, it has been well-accepted that one of the pathological mechanisms of AD relates to the accumulation amyloid-β (Aβ) peptide in certain brain regions [1]. Aβ is a product of Aβ precursor protein (AβPP) via natural proteolytic processing. AβPP is processed by three different proteases known as α-, β-, and γ-secretase. Each of these three proteases cleaves AβPP at different sites resulting in various Aβ species ranging from 39–43 amino acid residues [2]. It has been shown that long species of Aβ (A$\beta_{42,43}$) are strongly amyloidogenic and form aggregates readily compared to the short forms (A$\beta_{39,40}$). Aβ are the building blocks of insoluble extracellular Aβ deposits or senile plaques formation [3], a neuropathological hallmark of AD [3]. AD is also marked by neurofibrillary tangles, an intracellular filaments of highly

phosphorylated tau protein. Impairment of cellular function in AD is demonstrated by a set of cytopathologies (reviewed in [4]) such as decline in cytochrome c oxidase (complex IV), mitochondrial dysfunction, abnormal iron homeostasis, oxidative stress, dimerization of AβPP and synaptic dysfunction [5–9], and energy hypometabolism. Several lines of evidence point towrads strong connection between small aggregates of Aβ and mitochondrial dysfunction.

1.2. Role of Amyloid-β and Oligomers in Alzheimer's Disease. According to the oldest so-called "cholinergic hypothesis", AD is caused by reduced levels of the neurotransmitter acetylcholine, which is important for memory. However, medications intended to treat acetylcholine deficiency have not been very effective in modifying the course of the disease. In the early 90s, the "amyloid hypothesis" was postulated according to which Aβ deposits arising from AβPP cause AD. As a result, specific proteases that process AβPP were the focus of drug development as a possible mean to lower Aβ production. However, several complications stem from the significance of these proteases for other biological functions creating serious obstacles in this front.

It is now well-accepted fact that AD is a neurodegenerative disorder associated with protein aggregation and misfolding of AβPP, which may be triggered by genetic polymorphism, age-dependent alteration to Aβ metabolism, or environmental factors that may promote accumulation and aggregation of Aβ peptide [10–13]. Thus, therapies directed at reversing Aβ aggregation appeared promising. However, several limitations reduced the excitement in this approach as was the case for immunotherapy targeted using specific anti-Aβ antibodies. Additionally, the lack of chemical safety of the available drugs to prevent Aβ aggregation was also a major concern.

There are reports showing a correlation between an early cognitive impairment in AD and increased oligomerization of Aβ, which precede the appearance of senile plaques [14–16]. Oligomers of Aβ correlate with early cognitive impairment in AD [17, 18]. In recent years, studies using cells, mouse models, and human brain tissues strongly suggest that soluble Aβ oligomers could be a toxic forms of Aβ [19, 20]; however, experimental data for their direct in vivo toxicity is lacking [21]. Thus, preventive approaches may include also preventing the formation of Aβ aggregates. However, it is not entirely clear which cellular compartment is the primary target of Aβ toxicity. Interestingly, in addition to other extra- and intracellular compartments, Aβ is also found in the mitochondria [22, 23], suggesting that neurotoxic effects of Aβ may be widespread [24], unlike earlier views that the extracellular senile plaques are the only main neurotoxic factor in AD. Energy hypometabolism and synaptic dysfunctions are proposed to be the primary target of Aβ neurotoxicity [25, 26]. Thus, preventing mitochondrial dysfunction by identifying the primary metabolic pathway, specifically targeted by Aβ is a plausible approach to delay AD [27]. We propose that preventative approaches are more promising for lowering the prevalence of AD. These approaches could target the formation of Aβ oligomers and enhance mitochondrial activity to counter energy deficiency in AD.

2. Role of Mitochondrial Dysfunction in Alzheimer's Disease

Several lines of evidence suggest that impairment of mitochondrial function plays important role in the development of neurodegenerative diseases including AD. Mitochondrial dysfunction and impaired cellular energy is an early decline in metabolism seen in AD patients. In addition to the biochemical physiological changes, the brain mitochondria of AD patients exhibit substantial structural changes that included abnormal cristae, accumulation of osmophilic material, and smaller size compared to normal controls [23, 28]. It has been reported that mitochondrial fragmentation damages regions of nerve cell synapses. Excessive fragmentation of mitochondria causes synaptic injury leading to eventual nerve cell death. Since synapses are critical for learning and memory, their impairment leads to the dementia in AD patients. We have recently proposed that strong binding of Aβ with heme is a key factor associated with Aβ-mediated neurotoxicity [29, 30], which could be the primary metabolic pathway targeted by excess Aβ production [31–33], and thus interfering with mitochondrial structures and functions by increasing the production of nitric oxide (NO) leading to mitochondrial damage and impaired energy metabolism [34, 35]. One of the key cytopathologies of AD include decline in cytochrome c oxidase (complex IV) and αKGDH, which seems to contribute to mitochondrial dysfunction [36–38]. This, abnormal biology of the mitochondria may contribute to energy deficiency in AD [35, 39]. There are reports showing an abnormal interaction of Aβ with key brain metabolites such as zinc, copper, cholesterol, mitochondrial protein import machinery, HrtA2 protease, ABAD, and heme [22, 31, 40–43]; however, their relation to cytopathologies of AD is not clear.

The decline in complex IV, which occurs in heme deficient cells, leads to similar structural consequences on mitochondria (unpublished observations). Several lines of experimental evidence provided support that heme metabolism may be a specific metabolic pathway that is targeted by Aβ peptides [43–46]. We showed recently a specific heme-binding motif in human Aβ peptides [27]. Based on our recent work and other laboratories, we propose that depletion of regulatory heme and the formation of Aβ-heme peroxidase are key factors of mitochondrial dysfunction in the brains of AD patients. Due to phylogenic variation in the amino acid sequences of Aβ, differential heme-binding of Aβ could also explain why humans, but not rodents, develop AD-like neuropathology (Reviewed in [4]). Heme is responsible for the metabolic integrity of complex IV, which is a key factor in mitochondrial gene regulation systems; therefore, pathways that depend on heme may be properly regulated by lowering Aβ oligomeric forms, and enhanced heme synthesis may improve neuronal energy metabolism.

3. Therapeutic Approaches for Alzheimer's Disease and Challenges

AD has a devastating impact on both personal and community levels. AD is the most common age-related dementia manifested by widespread progressive cognitive deterioration and impaired behavioral skills. With the aging US populations, and widespread prevalence of AD in these populations warrants immediate need for the management of this deleterious disease. In spite of efforts from scientific community for several decades, the available drug therapies for AD are only remedial without proper understanding of the underlying mechanisms involved therein. Unless new treatments are developed to decrease the likelihood of developing AD, the number of individuals with this disease in the United States is expected to be more than 10 million in next three decades.

There is currently no specific cure for AD patients, but scientific research is unraveling the mysteries of AD, including the causes and the mechanisms of the disease progression, which might one day effectively solve the Alzheimer's puzzle. In recent years, some understandings have already provided critical information about how to prevent, delay, or slow the nerve cell damage that leads to AD, which may help maximize quality of life of these patients. However, drug treatments currently available are used to only manage the cognitive symptoms of AD by slowing the progression of symptoms for a while.

Despite the intensive research on AD, a therapeutic or preventive strategy for AD remains elusive or limited at the most [47], which has been a key obstacle for the development of effective therapies for AD. The efficacy of currently available drugs has further been hampered by the fact that the effectiveness of these drugs progressively declines with the progression of disease. Thus, there is an urgent need for a new generation of drugs to prevent or delay the onset of AD.

Except for imaging techniques, a precise and accurate diagnostic biomarker for AD is also lacking [48]. However, images results are excellent in following disease progress, but limited in revealing preclinical before protein deposits occur AD. The ultimate biomarker would preferably be a blood- or CSF-borne metabolite that indicates the risk for AD in advance of the clinical signs or protein deposits. Aβ-heme peroxidase could serve a unique biomarker if found in blood or CSF of AD patients. Aβ-heme has the advantage of being dependant on Aβ and it is tightly linked to a key brain metabolite (heme as mitochondrial metabolite), thus could indicate impairment in brain metabolism that depends on Aβ accumulation.

4. Mitochondria As Targets for Delaying the Onset of Alzheimer's Disease

Mitochondrial dysfunction in AD could serve as a therapeutic target an interest in developing mitochondrial drugs is emerging. Enhancing mitochondrial function and maintaining structural integrity of mitochondria could delay the onset of AD. Below, we discuss pharmacological approaches to
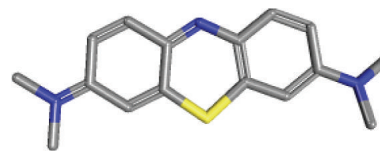

Figure 1: The chemical structure of methylene blue; a diaminophenothiazin.

enhance mitochondrial function and prevent the formation of Aβ toxic oligomers.

Mitochondria are a major energy source, and it has been known that energy deficiency can result in synaptic dysfunction and neurodegeneration of the hippocampus and cortical regions of the brain [49]. The brain is particularly sensitive to mitochondrial dysfunction, the resulting oxidative stress, and impaired energy metabolism [50–52]. Thus, improved energy metabolism through enhanced mitochondrial activity in the brain might be an effective approach to delay the onset of AD.

Mitochondrial dysfunction in AD is associated with a decline in mitochondrial complex IV and energy deficiency. Due to involvement of mitochondria in cellular senescence and aging, it may contribute to neural dysfunction with age. Therefore, targeting mitochondria is an emerging field of research in finding therapeutic strategies to combat aging and neurodegenerative disorders. In fact, recent developments support this idea (reviewed in [53]), and potency of pharmacological agents to prevent or delay age-related neurodegeneration is under investigation [54]. Our recent results with methylene blue (3,7 Bis-dimethylamino-phenazathionium; MB) in countering some mitochondrial dysfunctions including mitochondrial complex IV formation, enhanced cellular oxygen consumption and heme synthesis, and reversed premature senescence are aimed at enhancing mitochondrial function, which could contribute to the antisenescence activity of MB [55–57]. Due to the ability of MB to cross the Blood Brain Barrier [58], our studies may provide a potential future therapeutic tool for AD and other related diseases using MB.

MB is known as a redox indicator with a low redox potential, which allows it to cycle readily in mitochondria (Figure 1), and is easily soluble in both water and organic solvents, thus, MB and its derivative MBH_2 can enter the mitochondria and other intracellular compartments such as lysosomes [59]. MB is the first chemical to induce mitochondrial respiratory complex, and we propose a new medical use for MB by increasing brain's reserve of both complex IV and the capacity to synthesize heme [56]. Increasing the activity of complex IV is intriguing as a decrease in complex IV activity causes cytotoxicity leading to increased oxidants production and decreased energy charge of the mitochondria [60–62]. MB in turn may elevate the levels of complex IV and improve mitochondrial function. Complex IV consumes more than 95% of the O_2 that reaches cells, and thus, excess complex IV may play a key role in lowering the production of oxidants by decreasing the steady-state concentration of intracellular O_2 in the mitochondria. Complex IV activity correlates well with the metabolic activity of cells and thus

could improve cognitive performance. On the other hand, enhancing heme synthesis should help neural cells in delaying the onset of the consequences of sequestration of heme by huAβ. Together, these findings suggest that MB may delay the onset of Alzheimer's dementia. Further, MB at higher concentrations (μM range) are neurotoxic, and our findings show that MB is effective in improving mitochondrial function at nM range of concentrations, which is consistent with the intrabrain concentration that can be achieved upon chronic treatment with MB [56]. MB has a long-standing, extensive history of medical uses [63] with an extended medical and safety record in humans, and its FDA approval for clinical trials in connotation to aging and age-related disorders may not be difficult to obtain on safety grounds.

5. Mechanisms of Action of Methylene Blue and Its Clinical Applications

In spite of widespread clinical uses of MB for decades, the mechanism(s) of its diverse biological actions are not clear. MB is readily absorbed by various organs including brain [64], and has a long history of clinical uses including chronic treatments of congenital methemoglobinemia, methemoglobinemia, psychiatric disorders, and more recently in the prevention of the side effects of ifosfamide-induced encephelopathy chemotherapy [65], and hypotension in septic shock [66–68]. MB has also been shown to protect against cyclosporine injury to kidney [69], streptozotocin injury to pancreas [70], ischemic-reperfusion injury [71], radiation [72], and enhances β-oxidation of long chain fatty acids [73]. Clinical doses of MB ranges between 1-2 mg/kg/day for up to 6 times over 24 hrs [74]. Higher doses (>7.5 mg/Kg) of MB cause the formation of Heinz bodies in erythrocytes [75]. In addition, MB administration has been reported to improve the cognitive function in rats, and increase the activity of cytochrome c oxidase (complex IV) [76, 77] and decrease of monoamine oxidase activity in the brain, which may result in an increased dopamine concentration [78]. Some reports have proposed that MB may be acting by inhibiting the NO-activating soluble guanylate cyclase [79], nitric oxide synthase [80], and MAO activity [78]. There are also reports suggesting that MB may be acting as an antioxidant precursor [81, 82]. However, in recent years, it has become quite clear that the biological effects of MB are not consistent with these mechanisms [55, 56, 83]. This discrepancy may in part be due to different doses of MB used in these experimental conditions, which have ranged from >10 μM to <1 μM, suggesting that uses of MB at doses in the nM concentrations may follow a different mechanism [55, 56]. For example the effect of MB on complex IV depends on the dose used [55]. Thus, interpretation of the experimental findings should take into consideration MB's dose especially when high (non-therapeutic) dose is used [84]. Based on our data and others, for the first time, we proposed a molecular mechanism explaining how MB might be affecting mitochondrial function [55–57]. We propose that diaminophenothiazins cycling between the reduced and the oxidized forms may explain, in part, their mitochondria-protecting activity (Figure 1). This mechanism is proposed in

Figure 3 and in [55]. It is now an open secret that adequate assembly and activity of complex IV depends upon heme-a [85]; thus, the increased rate of heme synthesis with MB treatment could provide cells with heme to support the assembly of complex IV that could result in delaying mitochondrial dysfunction, cellular senescence, aging, and AD.

6. Preventing Aggregation and Oligmerization of Amyloid-β with Osmolytes

Protein misfolding and/or instability leads to aggregate formation. Many neurodegenerative diseases exhibit deposits of aggregated proteins such as Aβ oligomers, senile plaques, phosphorylated tau (p-tau), α-synuclein, and polyglutamine; and all are key neuropathologies in many CNS disorders. Aβ oligomers in AD may progress to form large insoluble fibrils that form the plaques, a key hall mark of AD. Accumulation of these protein deposits exacerbate neurodegeneration due to the fact that the human brain has a limited capacity to prevent the formation or removal of these protein aggregates. It is known that limited protein degradation may contribute to the accumulation of these peptide deposits with age [86]. The aging brain also exhibits limited antioxidant activity and self-repair capacities (e.g., limited neurogenesis).

Our knowledge of the kinetics of the formation of protein aggregates *in vivo* in brain disorders as well as the limited understanding for the mechanism by which these aggregates interfere with neuronal metabolism function has impaired our capabilities for the development of preventative therapeutic strategies. In general, the intermediate species in the cascade of protein misfolding appear to be highly toxic and may interfere with basic metabolic activity of the brain. As for Aβ oligomers it is not clear how toxic they would be in vivo [21]. However, removal or preventing the formation of such intermediate species may prove to be of great clinical value. We demonstrated that the binding of Aβ monomers and oligomers with heme results in sequestration of regulatory heme leading to impaired cellular metabolism. Therefore, preventative approaches to treating AD could be targeted at blocking the formation of Aβ oligomers, enhancing the synthesis of heme and complex IV, in addition to the use of antioxidants.

6.1. Oligmers as Targets for Delaying Alzheimer's Disease. Protein functions depends on maintaining and stabilizing their active conformation(s) under physiological and stress conditions [87]. Thus, under severe physical and chemical stress conditions, biological systems created mechanisms to maintain their functional conformations. These mechanisms are directed at preventing structural perturbations in proteins due to thermodynamic or chemical stressful conditions [88–91]. Biological systems that fail to provide proteins structure stabilizing conditions also fail to adapt to such conditions. As a result, often protein misfolding and aggregation occurs leading to a partial or complete loss of function, in addition to the formation of protein aggregates [92, 93]. Both conditions have serious consequences on cell function and metabolism.
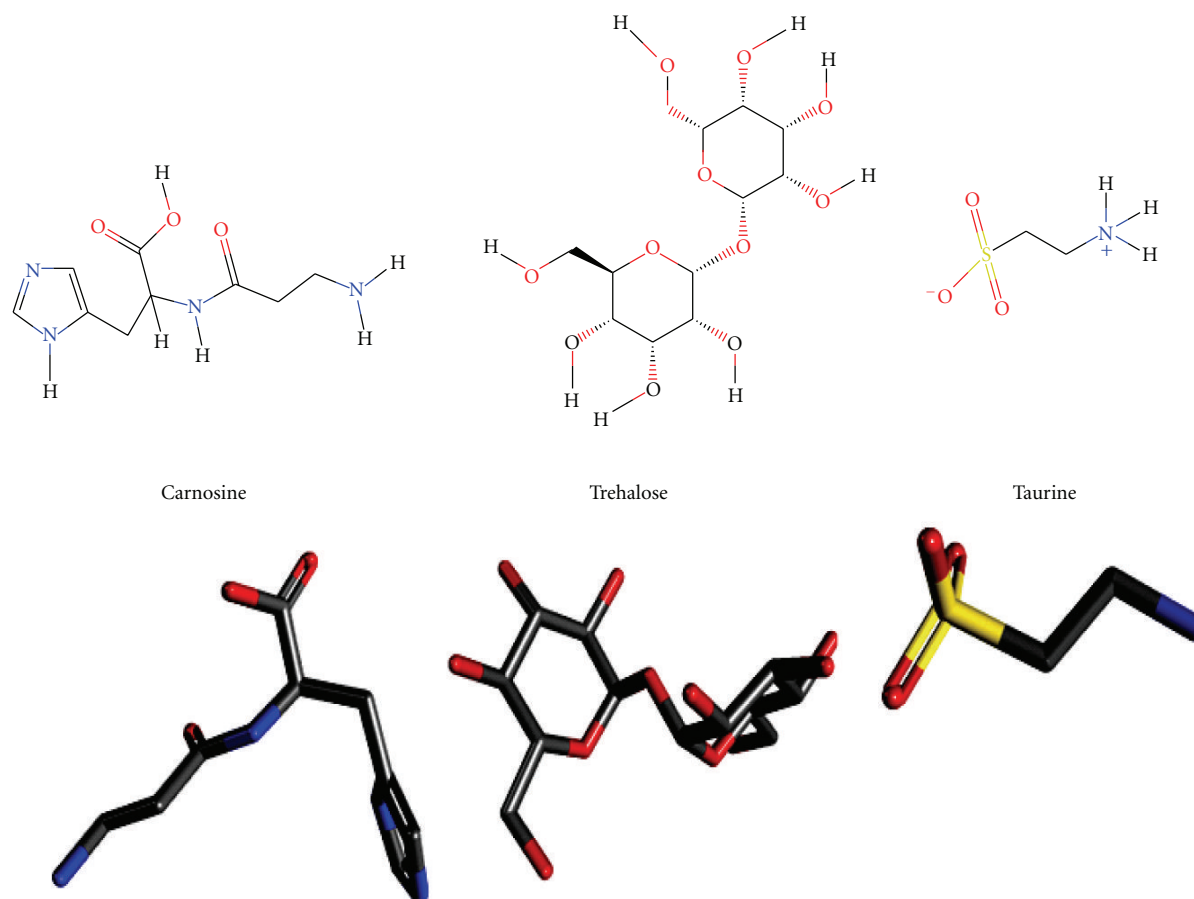
Carnosine   Trehalose   Taurine

FIGURE 2: Chemical structure of selected osmolytes. Upper panel: structure in 2D; lower panel, structure in 3D configurations, respectively.

In order to adapt to stressful conditions, tissues created certain mechanisms such as degradation of misfolded proteins or accumulation of small organic solutes at high concentrations. These solutes can serve as antioxidants or play stabilizing role for intracellular structures of macromolecules. Osmolytes [88, 89, 94] are group of endogenous chemicals produced by cells and accumulate to concentration as high as millimolars (Figure 2). The exact function of these metabolites and significance for intermediary metabolism and organ function is still under investigation. We are interested in finding scientific reasoning that links to identify the role of osmolytes and the high tissue connections. Experimental evidence points that osmolytes maintain stability and folding of proteins without perturbing other cellular processes, an action that requires relatively high intracellular concentrations [90, 91, 93, 95–97]. It is well known that following protein synthesis, a highly disordered unfolded state of the polypeptide chain passes through well-defined partially structured transition states before the fully folded protein forms. Molecular chaperones that deter aggregation of incompletely folded species also play role in correctly folding newly synthesized proteins [98]. If under certain conditions, the cellular environment becomes less than optimal for proper protein folding, then newly synthesized proteins become prone to aggregation [99–101]. Similarly, under these conditions, intermediates in protein processing

pathways may also be subject to accumulation, misfolding, and aggregation (e.g., Aβ). Protein aggregates, if not quickly removed, may transform to fibrils and other possible aggregates that accumulate in tissue and interfere with cell metabolism [102–107]. It is likely that small aggregates, as well as the highly organized fibrils and plaques, can give rise to pathological conditions, a common feature among many neurodegenerative diseases, including AD [97, 108].

The biological significance of naturally occurring osmolytes has intrigued scientists for many years. There are a number of well-known naturally occurring osmolytes, which fall into three chemical classes: methylamines (trimethylamine-N-oxide, Choline-O-sulphate, and sarcosine), polyols (sorbitol, glycerol, sucrose and trehalose), and certain amino acids and their derivatives (glycine, taurine, proline and betaine) (Table 1). The role of osmolytes in protein folding, cell senescence, cell homeostasis, and mitochondrial structure has been described in various studies. However, more investigations are still needed to evaluate the role of osmolytes in health and diseases and their therapeutic potential.

Osmolytes interact with the peptide backbone and amino acid side-chains [109]. The potency of an osmolyte to promote protein folding and solubility is determined by the balance of these interactions and the solvophobic effects of the osmolyte. There are several studies to support the view

TABLE 1: List of some known naturally occurring osmolytes in each class with their major presence in protecting the stability of specific proteins under harsh conditions.

Type	Name(s)	Used by
Polyols	mannitol, glycerol, sorbitol, inositol, pinitol	plants, algae, mammalian kidneys, insects, reptiles, fish
Amino Acid	glycine, alanine, and proline	mammalian cells
Amino Acids Derivatives	taurine, octopine, alanine	marine invertebrates, prokaryotes
Methylamines	trimethylamine-N-oxide, sarcosine, phosphorylcholine, glycine betaine	marine invertebrates, plants, mammalian kidneys

that the powerful solvophobic effects of osmolytes on the peptide backbone dominate, such that the relative Gibbs free energy (ΔG) of the unfolded state is less favorable than that of the folded state (ΔG of the peptide folding is more negative).

The presence of several osmolytes inside cells raises questions about their role in protecting intracellular macromolecules under stressful conditions. The antioxidative activity of the osmolytes has been also proposed. Since the protection provided by an osmolyte does not depend on specific chemical interactions with the macromolecules, in principle, any of the osmolytes should be capable of replacing each other, depending upon either endogenous or exogenous availability of particular osmolyte(s) [110]. Since the role of protein backbone is critical in determining thermodynamic stability and folding of proteins in osmolyte solutions [111–115], designing these small molecules (osmolytes) appears to be an excellent strategy and could be a critical step in preventing various critical proteins from misfolding or aggregation (6). This may have far-reaching consequences in understanding and preventing several deleterious diseases that are caused by protein misfolding/aggregation [116, 117]. Since organic osmolytes are naturally occurring molecules, they may have potential therapeutic applications without concerns of major toxic side effects [118].

Because of their capabilities to fold proteins into native-like functional species, osmolytes have been the focus of several studies related to neurodegenerative diseases in which the pathogenesis is associated with the misfolding of specific proteins [119, 120]. These diseases include AD, Huntington's disease (HD), and muscular dystrophy (MD). Trehalose, an osmoluyte, can significantly inhibit polyglutamine-mediated protein aggregation when orally administrated to the transgenic mouse model of Huntington's disease (a neurodegenerative disease) [114], and can increase the life span of Huntington's disease mouse model [114], indicating that trehalose is readily bioavailable. Osmolytes have similar effect of the folding of androgen receptor containing elongated polyglutamine chain length. Elongated polyglutamine chain in androgen receptor leads to formation of its aggregation and thus play role in causing neurodegeneration in Kennedy's disease [116].

Experimental finding suggests that AD toxicity could be linked to the formation of oligomeric forms of Aβ peptides and AD progression correlates with increasing aggregate formation of Aβ. At physiological concentration, Aβ_{40} peptide incubated in the presence of trehalose inhibits aggregation of this peptide in a dose-dependent manner [121], and this osmolyte-mediated inhibition of Aβ_{40} peptide aggregation correlates with its toxic effects in neuronal cell system [121].

In these pathological conditions, specific misfolded aggregate-prone proteins are resistant to the normal cellular processes of protein folding and turnover and we propose that osmolytes may interfere with the production and/or the removal of these toxic intermediate aggregates. Further, these osmolytes can be attractive molecules for delaying the onset of neurodegenerative diseases characterized by protein misfolding and toxic aggregation. In particular, osmolytes may have therapeutic potential for treating AD, because of their effect on Aβ oligomers. Osmolytes may also help stabilize the senile plaques, preventing the shedding of Aβ oligomers. Osmolytes are also known to function as antioxidants [122, 123], and their level seems to decline in AD patients [124].

The research on naturally occurring osmolytes suggests that they have a protective role in promoting brain health, including resistance to neurodegeneration. Thus, there is potential for both prevention and treatment of neurodegenerative diseases using osmolytes [125, 126]. The prospect of using natural osmolytes as a therapeutic tool for AD appears to be quite exciting and can have far reaching consequences in developing therapeutic tools for its prevention and/or management. However, while these proposals appear to be quite promising, more studies are needed to validate their effectiveness as a potential therapeutic target.

7. Summary

The mitochondrial role in health and disease has recently received immense scientific interest, particularly, because of the emerging field of mitochondria as a potential therapeutic target. Mitochondria play intricate role in energy, redox, and intermediary metabolism positioning them in cross road for health or disease. Genetic, environmental, and life style factors can lead to impairments in mitochondrial function. Impairment to mitochondrial function is found in numerous age-related degenerative diseases and disorders. Mitochondrial dysfunction leads to an increase in oxidative stress, energy hypometabolism, and impairs calcium homeostasis, ultimately leading to inadequate cellular function. Preventing mitochondrial dysfunction is presumed to have functional benefits, regardless of whether mitochondrial dysfunction is primary or secondary [57]. Thus, therapeutic strategies to improve mitochondrial function and delay the onset of age-related degenerative disorders are currently under investigation. We have shown that diaminophenothiazins (e.g., methylene blue, thionine, Figure 1) can delay cellular senescence by enhancing mitochondrial function, which are impaired in AD brains.
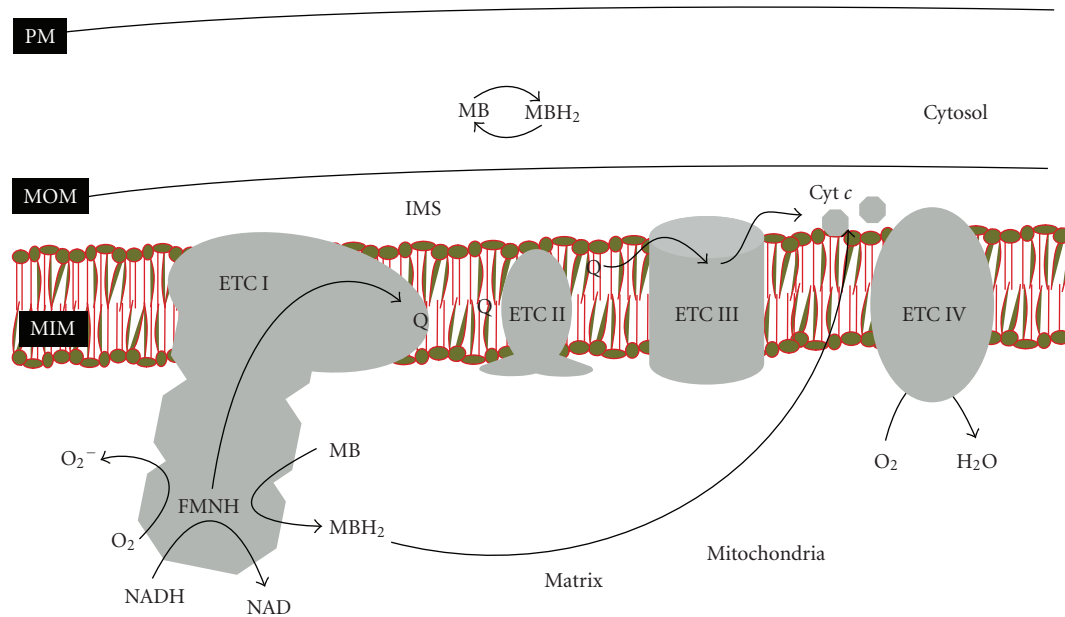
FIGURE 3: The proposed model for the interaction of methylene blue with specific mitochondrial and cellular components. ETC followed by roman number refers to the specific components of electron transport chain (ETC) of the mitochondria. MIM, MOM, and IMS refers to the mitochondrial inner membrane, mitochondrial outer membrane, and intermembrane space, respectively. MB and MBH$_2$ refer to oxidized and reduced forms of methylene blue, respectively. The four complexes are: complex I (ETC I), complex II (ETC II), complex III (ETC III), and complex IV (ETC IV) in addition to ATP synthase (i.e., complex V). The electron transfer through each one of the ETC starts at ETC I, which catalyzes two electrons oxidation of NADH and continues until water is formed on ETC IV. Coenzyme Q serves as low-molecular weight electron carrier from ETCs I and II to III. Cytochrome c (cyt c) serves as electron carrier from ETC III to ETC IV. Production of superoxide radical from complex I is proposed to be prevented by MB, which serves as electron carrier that competes with molecular oxygen on the electrons "leaking" from complex I. During this process MB is converted to MBH$_2$. Then MBH$_2$, a reduce MB, carries the electrons to cytochrome c, which is then oxidized by ETC IV [55].

Mounting evidence suggests a role of small aggregates of amyloid-β (Aβ) in the etiology of AD. Aβ aggregates impair mitochondrial function, synaptic function, Ca^{++} homeostasis and ultimately leading to cellular hypometabolism and neurdegeneration. Aβ peptide can also be localized within the mitochondria of AD patients. Experimental evidence show that osmolytes can stabilize and enhance cellular proteins to adopt physiologically compatible conformation. Osmolytes are efficient antioxidants that may also increase neural resistance to oxidative stress caused by Aβ. Thus, osmolytes may interfere with the aggregation of Aβ, enhance their proteolytic clearance, and counter oxidative stress [57].

We propose two different approaches to prevent or delay the onset of AD. The first is directed at enhancing mitochondrial activity using MB to enhance mitochondrial function (Figure 3). Energy deficiency in AD may be contributed by impaired insulin (glucose) metabolism and mitochondrial function. Thus, concentrating on single impairment at the time would not be enough to resolve the energy hypometabolism in AD. Glucose metabolism depends on adequately functioning mitochondria and vice versa. Since both glucose and mitochondrial metabolism are interconnected, it might be more beneficial for AD patients to develop a therapeutic approach that resolves (or delay) both impairments. Successful merger of treatment with MB and intranasal delivery of insulin to the brain may prove valuable for AD patients. MB exerts its effect at very low (nM) concentration, which in conjunction with its safety record in humans further minimizes any risk of side effects of chronic exposure to MB. The second approach is directed at preventing the aggregation of Aβ by using osmolytes, natural metabolites synthesized in the brain. Preventing the aggregation of Aβ may enhance their proteolytic removal and decrease the risk of their interference with heme and mitochondrial metabolism. MB can also induce heme synthesis, thus, when combined with osmolytes, may assist in preventing heme deficiency. We propose MB and osmolytes could help delay the onset of AD by preventing Aβ oligomers formation, enhancing mitochondrial function, and attenuating heme deficiency.

We propose that preventative approaches for AD could be targeted at blocking the formation of Aβ oligomers, enhancing the synthesis of heme and complex IV, in addition to the use of antioxidants.

Conflict of Interests

Dr. Atamna has applied for patent on MB to treat mitochondrial dysfunction.

Acknowledgments

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