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Review

Methylene blue treatment on Alzheimer and inflammatory bowel disease

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ABSTRACT

High-efficiency oxidative phosphorylation plays a key role in the progression of many diseases. There is growing evidence of methylene blue's protective and reversing actions against neurodegenerative and inflammatory bowel diseases through different mechanisms. In Alzheimer's disease (AD), oligomeric amyloid beta accumulates in the mitochondria and contributes to mitochondrial dysfunction, which occurs before significant plaque deposition. Methylene blue provides an alternative mitochondrial electron transfer pathway, switching from high-efficiency oxidative phosphorylation to the low-efficiency aerobic glycolysis pathway by receiving electrons from NADH in the presence of complex I and transferring them to cytochrome C. The second mechanism is the inhibition of active caspases, especially Caspase-6, a cysteinyl protease causing inflammation and cell death, which has been associated with age-dependent cognitive decline and the pathology of sporadic and familial AD. The third mechanism is the reversal of tau aggregation by oxidizing cysteine residues in tau and forming a more stable monomer, thus blocking tau-tau bindings as well as clearing tau pathology through increased autophagy. In regards to inflammatory bowel disease, reducing oxidative stress and attenuating inflammatory pathways inhibits epithelial destruction in acetic acid-induced colitis. Methylene blue has an anti-colitis effect, mainly relying on its mitochondrial efficacy-restoring, antioxidative, anti-inflammatory, and anti-apoptotic properties. In summary, methylene blue is a promising agent for both AD and inflammatory bowel disease due to its beneficial effects as well as its low cost and high accessibility.

Keywords: Alzheimer's disease, inflammatory bowel disease, methylene blue.

Methylene blue (MB), a heterocyclic phenothiazine-based aromatic chemical compound, was originally synthesized as a textile dye but gained a significant role in the treatment of various bacterial and viral infections, methemoglobinemia, carbon monoxide poisoning, vasoplegic syndrome, and ifosfamide neurotoxicity, as well as in the surgical staining of the parathyroid glands or fistula. The repurposing of previously established drugs is being substantially considered in modern pharmacology, in terms of time- and money-saving features in addition to their previously achieved extensive safety testing records. Consequently, MB has become a promising drug in the treatment and decelerating the progression of certain diseases, such as Alzheimer's disease (AD) and inflammatory bowel disease (IBD).[1]

PubMed, Google Scholar, and UpToDate were searched for the keywords "methylene blue", "Alzheimer's disease", "inflammatory bowel diseases", "methylene blue and Alzheimer's disease", and "methylene blue and inflammatory bowel disease". Abstracts were read by the authors of the present article and the most relevant studies and reviews were analyzed. After the overlapping studies and reviews were excluded, the remaining 62 articles were grouped according to their topics and the similarity of their contents.

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METHYLENE BLUE AND ALZHEIMER'S DISEASE

Alzheimer's disease is a progressive neurodegenerative disorder causing severe disability or death and reduces the quality of life for both patients and families. Alzheimer's disease remains the most common cause of dementia worldwide, and its prevalence continues to rise due to the aging world population, affecting 1 in 9 people over the age of 65 in the United States. This rate approximately matches the estimated prevalence in Turkey.[1-3] As it stands, there is no effective treatment for AD that can halt or significantly slow the patient's inevitable decline. In this regard, this makes AD unique among the world's major killers, as it is one of the few top causes of death with little to no preventative or therapeutic measures.[1]

Alzheimer's disease is histologically characterized by the classical accumulation of senile plaques and neurofibrillary tangles (NFT), consisting of hyperphosphorylated tau protein[4] and amyloid beta (Aβ) oligomers, which are products of the proteolytic cleavage of membrane-bound amyloid precursor protein (APP) by γ-secretase and β-secretase. Amyloid precursor protein processing of Aβ can proceed down one of two pathways, the first of which is the non-amyloidogenic pathway, which starts with APP cleavage by α-secretase that yields a soluble N-terminal fragment, sAPP-α, and a membrane-bound C-terminal fragment (CTF-α). CTF-α is further processed by γ-secretase to yield another N-terminal soluble fragment, p3. The soluble fragments generated by this sequence do not form aggregates and are not neurotoxic. However, the second pathway, the amyloidogenic pathway, commences with APP cleavage by β-secretase, generating sAPP-β. γ-secretase cleavage of the remaining CTF-β fragment generates Aβ, which progressively aggregates from oligomers to plaques that accumulate extracellularly, that interfere with cellular function and activate inflammatory pathways.[5] Oligomeric Aβ also accumulates intracellularly, localizing in the mitochondria, and contributes to mitochondrial dysfunction and energy deficiency in AD pathology.[6]

Until now, the main approach to AD therapeutic research targeted Aβ. As recently as 2007, MB has begun to be studied in this capacity.[7,8] According to the studies investigating its treatment capacity, MB was found to be promising due to certain actions, one of them being improving cognitive function, mainly by promoting complex IV activity and mitochondrial activity.[9] Mitochondrial dysfunction is found to be present before significant plaque deposition and cognitive decline,[10-12] which makes MB a promising agent in the early treatment of AD through its complex IV activity via its electron cycling mechanism as well as its upregulating effect on heme synthesis.[9,13]

The mitochondrial activity of MB has been further investigated in another study, resulting in the discovery of its ability to provide an alternative electron transfer pathway. In this pathway, MB receives electrons from NADH in the presence of complex I and delivers them to cytochrome C. A switch from high-efficiency oxidative phosphorylation to the low-efficiency aerobic glycolysis pathway (Warburg effect) occurs, increasing oxygen consumption, decreasing glycolysis, and increasing *in vitro* glucose uptake, as well as enhancing glucose uptake and regional cerebral blood flow in rats upon acute treatment. In summary, there is growing evidence that enhancement of mitochondrial oxidative phosphorylation via alternative mitochondrial electron transfer may offer protective action against neurodegenerative diseases.[14]

Several studies have demonstrated that MB improves cognitive deficits induced by hippocampal damage by upregulating complex IV and ATP production, decreasing oxidative stress markers through methods such as electron cycling, and inhibiting downstream mechanisms and interactions such as the inhibition of Aβ-ABAD binding, therefore preventing the production of associated reactive oxygen species (ROS), matrix metalloproteinase (MMP) failure, and subsequent cell death,[15-18] as well as the cytosolic release of pro-death factors in several AD models.[15,19,20]

Another study investigated the tau cysteine oxidation mechanism of MB to clarify whether MB may modulate the activity of caspases. Caspases are a group of cysteinyl proteases involved in inflammation and cell death. One of the effector caspases, Caspase-6 (Casp6),

was found to be strongly associated with age-dependent cognitive decline and the underlying pathology of sporadic and familial AD.[21-28] Results of the study showed that MB and its derivatives efficiently inhibited active caspases *in vitro*, in cells, and *in vivo* at concentrations allowing phenothiazine-mediated Tau disaggregation. These results indicated that MB, or its derivatives,[21,29] may have additional effects in AD by inhibiting caspases,[21,30] preventing caspase activation in other degenerative conditions,[21,31] and predisposing individuals undergoing chronic treatment to cancer via the inhibition of effector caspase-3.

Another key factor in AD pathophysiology is the presence of neurofibrillary tangles (NFT), which are aggregates of hyperphosphorylated tau protein (p-tau). Tau is a microtubule-associated protein that stabilizes neuronal microtubules as a natural and prevalent component of CNS. Tau is phosphorylated by kinases such as glycogen-synthase kinase 3 β (GSK3β), c-Jun kinase (JNK), and cyclin-dependent kinase 5 (cdk5)[32-34] as a normal component of the physiological process. However, this process dissociates tau from microtubules and destabilizes them, disrupting cellular function and axonal transport as well as mitochondrial transport as mentioned above. Nevertheless, the excessive phosphorylation of tau aggregates and forms tangles, as the amount of p-tau exceeds the proteasome's clearance capacity. However, it is still under debate whether taupathy precedes Aβ pathology or is a consequence.[35-37]

Targeting this pathological finding, MB was found to reverse tau aggregation by blocking tau-tau binding as a consequence of inhibiting filament formation on the first and fourth repeat peptides on the tau microtubule binding domain[31,38,39] and prevent fibrillization by oxidizing cysteine residues in tau resulting in the formation of a more stable monomer form that is resistant to aggregation.[23] Another study showed that MB clears the tau pathology through increased autophagy in JNPL3 organotypic slices, by interacting with and promoting the oxidation of tau cysteine residues, thus preventing the formation of fibrils and their toxic precursors.[21,23]

Lastly, MB was found to inhibit the aggregation of proteins that take on β-sheet formation *in vitro*, including tau.[31,39] Methylene blue trials *in vivo* have yielded mixed results. In a mouse tauopathy model, 1 mM MB was administered to the right hippocampus using a mini-osmotic pump. This resulted in a decrease in total tau levels paralleled by a decrease in phosphorylated (S202/T205) tau, as well as an improvement in behavior but without any remarkable effect on pathology.[41] When the mice were administered a therapeutically relevant dose of 10 mg/kg of MB in their drinking water for 12 weeks, there was a variable improvement in behavior and a decrease in soluble tau levels, with no change in tau tangle pathology. Interestingly, when the levels of MB in the cerebellum were measured, they were found to positively correlate with Morris water maze performance and inversely correlate with soluble tau levels. These results were compatible with previous findings suggesting that NFTs are likely not associated with functional deficits but that reducing soluble tau levels is beneficial.[40] These results also suggested that the putative therapeutic effect of MB may not be associated with its ability to inhibit aggregate formation. In zebrafish, MB inhibited the aggregation of a mutant form of huntingtin gene, but had no effect on tau pathology when an FTDP-17 mutant tau was expressed, similar to what was observed in the mouse model.[41]

However, in contrast to the mouse model, MB treatment in zebrafish did not prevent or reverse the functional deficits that resulted from the expression of the FTDP-17 mutant tau or the mutant huntingtin.[41]

According to another study that reported a correlation between MB and taupathy, MB was found to clear tau filament by inducing autophagy[42] by reducing tau load in different taupathy transgenic mouse models over short-term and long-term treatment, and this clearance was associated with improvement of cognitive deficits.[43,44] A more stable variant of the MB in reduced form, Leuco-Methylthioninium Bis (Hydromethanesulphonate) (LMTM), was developed[45] for use in human clinical trials to show both cognitive and cerebral blood flow improvements[46] under the name "REMBER". Unfortunately, the study yielded negative results.[47]

METHYLENE BLUE AND INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease is a term mainly used to describe two conditions: ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis and CD are chronic conditions that involve inflammation of the gut. Ulcerative colitis only affects the colon (large intestine), while Crohn's disease can affect any part of the digestive system, from the mouth to the anus.[48] It is estimated that over 1 million residents in the USA and 2.5 million in Europe have IBD, with substantial expenses spent on health care.[49]

Although IBD has been thought to be idiopathic, it has two main attributable causes, including genetic and environmental factors. The gastrointestinal tract where this disease occurs is central to the immune system, and the innate and the adaptive immune systems are balanced in complex interactions with intestinal microbes under homeostatic conditions. However, in IBD, this homeostasis is disrupted and uncontrolled intestinal inflammation occurs.[50]

Methylene blue is conventionally used for staining in endocytoscopy (EC), one of the most novel endoscopic diagnostic procedures that provides up to 1,150 times optical magnification of the gastrointestinal mucosa. This approach allows real-time visualization of tissue and cellular structure. Endocytoscopy, along with confocal laser endomicroscopy, is considered *in vivo* "optical biopsy"[51] as it enables the visualization of cellular atypia of gastrointestinal mucosae[52] and detection of dysplasia or neoplasia.[53] A study on UC patients reported that chromoendoscopy-guided endomicroscopy decreases the number of biopsies performed and significantly increases the per-biopsy yield of intraepithelial neoplasia. Endomicroscopy is an accurate tool for intraepithelial neoplasia detection.[54]

Several pro-inflammatory cytokines are involved in the progression of IBD. One of them, IL-6, activates signal transducer and activator of transcription 3 (STAT3) and has an important function in the inflammatory response. There is elevated production of IL-6 and its soluble IL-6 receptor in UC and CD patients.[55,56] Interleukin-6 also has a key role in the pathogenesis of UC and the carcinogenesis of colorectal cancers related to UC.[57] Moreover, it has been reported that IL-17 can increase the recruitment of T cells into the lamina propria during the inflammatory response.

A previous study revealed that MB can efficiently inhibit epithelial destruction in acetic acid-induced colitis by decreasing oxidative stress and attenuating inflammatory pathways.[58] Other previous studies observed decreased expression of Bcl-2 and elevated levels of Bax protein in UC. These changes were significantly reversed by MB, demonstrating its anti-apoptotic effects. The overproduction of reactive oxygen species in neuronal cells can result in the collapse of cellular components, especially DNA, leading to cell death.[59] This indicates that the anti-apoptotic properties of MB may be partially due to its antioxidative effect.

In a study by El Sayed and Sayed,[60] the anti-colitis effect of MB was tested in a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis model. According to the study, the role of mitochondrial dysfunction is significant in the pathogenesis of TNBS-induced UC. On the other hand, MB restores mitochondrial efficacy and exerts antioxidative, anti-inflammatory, and anti-apoptotic effects. These results indicate that MB is a promising therapeutic agent for the management of UC without harmful side effects.[60] As there are overlaps in the pathogenesis of UC and CD, the mechanisms suggest that MB could also be administered in CD treatment and further related research is warranted.

Declaration of conflicting interests

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