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Therapeutic and preventive effects of methylene blue on Alzheimer's disease pathology in a transgenic mouse model

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ABSTRACT

Methylene blue (MB) belongs to the phenothiazinium family. It has been used to treat a variety of human conditions and has beneficial effects on the central nervous system in rodents with and without brain alteration. The present study was designed to test whether chronic MB treatment taken after (therapeutic effect) or before (preventive effect) the onset of beta-amyloid pathology influences cognition in a transgenic mouse model (APP/PS1). In addition, the present study aims at revealing whether these behavioral effects might be related to brain alteration in beta-amyloid deposition. To this end, we conducted an *in vivo* study and compared two routes of drug administration, drinking water versus intraperitoneal injection. Results showed that transgenic mice treated with MB orally or following intraperitoneal injection were protected from cognitive impairments in a variety of social, learning, and exploratory tasks. Immunoreactive beta-amyloid deposition was significantly reduced in the hippocampus and adjacent cortex in MB-treated transgenic mice. Interestingly, these beneficial effects were observed independently of beta-amyloid load at the time of MB treatment. This suggests that MB treatment is beneficial at both therapeutic and preventive levels. Using solid-state High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HRMAS-NMR), we showed that MB administration after the onset of amyloid pathology significantly restored the concentration of two metabolites related to mitochondrial metabolism, namely alanine and lactate. We conclude that MB might be useful for the therapy and prevention of Alzheimer's disease.

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1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia among elderly people. Clinically, AD results in progressive memory loss and other cognitive impairments, such as aphasia, apraxia, and personality changes. Despite the global nature of the cognitive dysfunction in AD, memory disorder is clearly the most prevalent and prominent feature of the early stages of the disease. The principal hallmarks of AD include extracellular accumulation of beta-amyloid peptide in the core of the neuritic plaque and intracellular accumulation of Tau proteins as neurofibrillary tangles and neuropil threads. Both of them are mandatory to diagnose of AD (Jellinger, 1998). Others histopathological features, such as mitochondrial deficits, do not belong to the diagnosis criteria, but have been also considered important pathological components.

Methylene blue (MB) belongs to the phenothiazinium family. It has been used for more than a century in a wide range of fields, including biology, chemistry, and medicine. In clinical medicine, MB is used in a wide range of indications such as methemoglobinemia, ifosfamide-induced encephalopathy, and thyroid surgery (see Oz et al., 2011 for review). MB has also been reported to have therapeutic effects in psychosis and mania (Deutsch et al., 1997; Narsapur and Naylor, 1983; Naylor et al., 1988). In rodents, Rojas et al. (2012) reviewed the role of MB on memory in normal brain as well as various animal model of neurodegenerative disease including Huntington's disease (Sontag et al., 2012) and AD (Medina et al., 2010; O'Leary et al., 2010). *In vitro* studies support the premise that MB inhibits the formation of beta-amyloid oligomers by promoting fibril formation (Atamna and Kumar, 2010; Necula et al., 2007; Oz et al., 2009) and Huntingtin protein

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aggregation (Sontag et al., 2012). It exerts an anti-tau aggregation effect (Oz et al., 2009; Wischik et al., 2008; Taniguchi et al., 2005). MB crosses the blood–brain barrier and has high bioavailability in the brain (Peter et al., 2000). It has also been demonstrated to penetrate selectively neuronal cell types after systemic administration, in particular hippocampal cells (Müller, 1998). MB has a wide range of activities that is due to its multiple cellular and molecular targets (Atamna et al., 2008; Martijn and Wiklund, 2010; Miclescu et al., 2010; Vutskits et al., 2008; Zhang et al., 2010).

The aim of the present study is to evaluate the therapeutic and preventive effects of MB on AD, using a transgenic mouse model. Different mouse models featuring a range of aspects of AD are available (for review, see Lithner et al., 2011). The double-transgenic mice used in the present study incorporate a chimeric human/mice amyloid precursor protein (APP) construct bearing the Swedish double mutation and the exon-9-deleted PS1 mutation (APP/PS1). Previous studies from our group and from other investigators have showed that these mice develop age-related aggregation of amyloid plaques in the hippocampus and the cortex as well as progressive cognitive impairment (Filali and Lalonde, 2009; Filali et al., 2011; Liu et al., 2002; Wang et al., 2003). To investigate whether MB treatment could have beneficial effects on cognitive impairment, APP/PS1 mice received MB administration. Low doses of MB were administered after (therapeutic effect) or before (preventive effect) the onset of beta-amyloid pathology.

To further gain insights into the mechanisms related to beta-amyloid deposition and the molecular effects of *in vivo* treatment with MB, solid-state High Resolution Magic Angle Spinning Nuclear Magnetic Resonance (HRMAS-NMR) was used to identify metabolic changes. HRMAS-NMR uses intact tissue samples and does not require soluble extraction (Martínez-Bisbal et al., 2004; Sitter et al., 2002). Therefore, it offers the advantages of retaining tissue morphology and eliminating contamination associated with extraction procedures. Multivariate statistical analyses can then be performed with these data, leading to the "metabolic profiling" used today for classification and prediction. It has been successfully applied in various tissues and diseases, including Alzheimer pathology (Mao et al., 2007; Paban et al., 2010; Sitter et al., 2002; Tzika et al., 2002; Woo et al., 2010). Two brain regions were examined, the hippocampus and cortical adjacent areas, which are highly involved in memory and higher cognitive functions and in neurodegenerative disorders. The effect of *in vivo* MB treatment was also investigated at the level of beta-amyloid present in APP/PS1 mice brain.

2. Material and methods

2.1. Animals

Male transgenic mice harbouring the chimeric human/mouse APP gene with the Swedish mutation and the human presenilin I (A246E variant) (strain B6C3-Tg(APP695)3Dbo Tg(PSEN1)5Dbo/J; Jackson Laboratories) were used. The mice were first on a B6C3 background and backcrossed for at least 10 generations to C57BL/6J. All newborn pups were genotyped and included in the different experimental groups. The housing conditions were controlled (temperature 21 °C; light from 7:00 to 19:00; humidity 50–60%), and food and water were freely available. All protocols were conducted according to the Canadian Council on Animal Care guidelines, as administered by the Laval University Animal Welfare Committee.

2.2. Treatment and experimental design

Methylene blue grade was provided by Provepharm S.A.S (Marseille, France) and administered either orally (via drinking water) or intraperitoneally (i.p.) 2 times per week. Because MB is bitter, sucrose was added in the drinking water. We chose a dose of 2 mg/10 ml in drinking water. The daily MB intake was estimated to be about 0.80 mg/day per mouse, on the assumption that the average daily consumption of water is 4 ml for a 35 g adult mouse. For i.p. injection, we chose a dose of 2 mg/kg diluted in 0.2 ml of distilled water. These doses were selected based on positive findings with 0.025% w/w supplemented diet for 16 weeks (Medina et al., 2010), and 1–4 mg/kg i.p. for 5 days (Deiana et al., 2009).

The experimental design is summarized in Fig. 1. To analyze the therapeutic effects of MB (Fig. 1A), treatment was delivered after the onset of beta-amyloid pathology, i.e., in mice of 6 month-old, which developed amyloid deposits in the hippocampus and cortical adjacent areas. Two routes of administration were analysed: orally or intraperitoneally. Note that mice were submitted two times at the behavioral tests battery, i.e., at 6 (before treatment) and 9 (after treatment) months of age. Indeed, we recently reported that APP/PS1 mice had significant impaired performance starting from 6 months old and performed worsened up to 9 months of age (Filali and Lalonde, 2009). So, the question was to investigate whether 3 months period of MB treatment could stabilized these behavioral decline. Group-housed APP/PS1 bigenic ($N = 47$) and littermate wildtype (Wt) mice ($N = 34$) were separated into 6 groups, allowing us to investigate MB's effects in both Wt and Alzheimer's mice model: APP/PS1 sucrose water or saline ($N = 15$, w/o MB), APP/PS1 MB in drinking water ($N = 15$, w/MB Oral), APP/PS1 MB following i.p. injection ($N = 15$, w/MB I.P.), Wt sucrose water or saline ($N = 11$), Wt MB in drinking water ($N = 11$), and Wt MB following i.p. injection ($N = 11$).

To analyze the preventive effects of MB (Fig. 1B), treatment was administered before the onset of beta-amyloid pathology, i.e., in mice of 2 months old. Based on the therapeutic effects of MB showing comparable results no matter what the administration route was, only one way of delivery was studied, we have chosen to

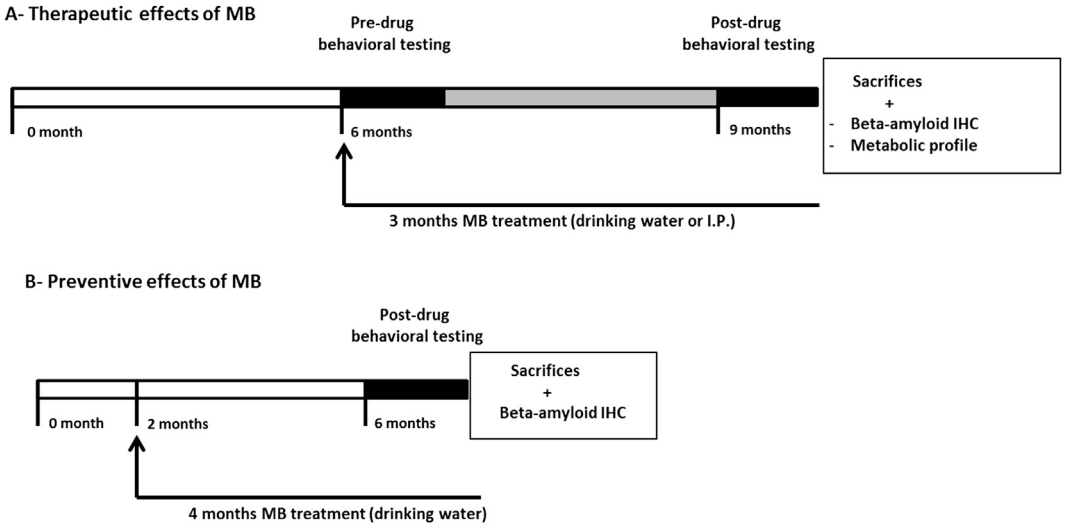

Fig. 1. Timeline of therapeutic (A) and preventive (B) effects of MB in APP/PS1 mice. Behavioral testing consisted in nesting task, left–right discrimination learning, passive avoidance learning, and open field test administered in order. Indications are provided on the analysis done after sacrifices. IHC: immunohistochemistry.