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Review

"Lest we forget you — methylene blue . . ."

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Abstract

Methylene blue (MB), the first synthetic drug, has a 120-year-long history of diverse applications, both in medical treatments and as a staining reagent. In recent years there was a surge of interest in MB as an antimalarial agent and as a potential treatment of neurodegenerative disorders such as Alzheimer's disease (AD), possibly through its inhibition of the aggregation of tau protein. Here we review the history and medical applications of MB, with emphasis on recent developments.

Keywords: Methylene blue; History; Medical application; Alzheimer's disease; tau aggregation inhibitor; malaria therapy; prodrug of azure B

1. Introduction

The 2008 International Congress on Alzheimer's Disease (ICAD) in Chicago disappointed many hopes for treatments of Alzheimer's disease that aimed at reducing the amyloid burden in the brains of patients (discussed previously on the AlzForum, see www.alzforum.org, 30 July 2009). At the same time, new expectations were raised by reports on treatments designed to reduce the neurofibrillary pathology of tau protein. One prominent advocate of this approach was Claude Wischik who presented data arguing that the compound methylene blue (MB) could reduce the aggregation of tau and thereby slow down the disease (Hattori et al., 2008; Wischik et al., 1996, 2008). This prompted an intense public debate on the pros and cons of the "blue wonder" (Gura, 2008). However, it was often forgotten that methylene blue, the first synthetic drug, had already a 120-year history in several areas of medicine.

2. Biochemistry of MB

MB is a tricyclic phenothiazine drug (Wainwright and Amaral, 2005). Under physiological conditions it is a blue cation which undergoes a catalytic redox cycle: MB is reduced by nicotinamide adenine dinucleotide phosphate (NADPH) or thioredoxin to give leucoMB, an uncharged colorless compound. LeucoMB is then spontaneously reoxidized by O_2 (Fig. 1). The typical redox-cycling of MB in vivo can be illustrated in vitro using the famous blue bottle experiment: MB is visibly reduced by glucose to give leucoMB and then, by opening the lid of the bottle it is reoxidized by atmospheric O_2: the color comes back. After closing the lid, there is a lag phase and then MB is reduced again. Analogous phenomena have been observed by pathologists at autopsies (Tan and Rodriguez, 2008; Warth et al., 2009). MB is excreted in the urine as a mixture of MB, leucoMB and demethylated metabolites, e.g., azure B and azure A (Gaudette and Lodge, 2005). MB-containing urine is very clear and has,

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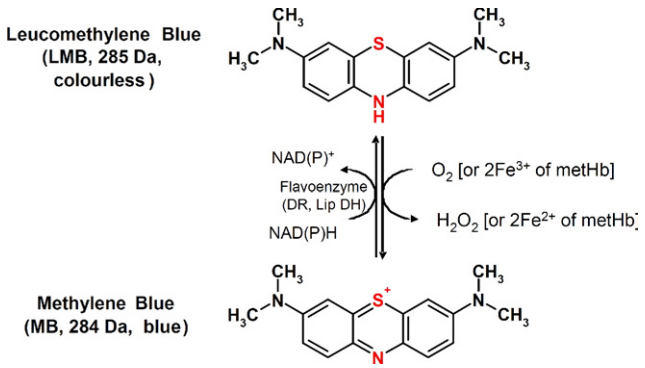

Fig. 1. Methylene blue (MB) as a redox-cycling phenothiazine drug in vivo. MB is spontaneously or enzymatically reduced by NADPH and the resulting uncolored leucoMB is reoxidized by molecular oxygen (O_2) or by iron(III)-containing compounds like methemoglobin. Precious NADPH and O_2 are wasted, and hydrogen peroxide is produced in each round of the cycle. There are also pharmacologic activities of MB which do not depend on its redox properties. Azure B is monodemethylated MB, that is one of the 4 CH_3 groups shown in the formula is replaced by a hydrogen atom. Azure A is the didemethylated form at the same position, converting the $N(CH_3)_2$ to an NH_2-group.

of course, a green or blue color which disappears a few days after the last administration of MB (Guttmann and Ehrlich, 1891).

3. History of MB

MB was the very first fully synthetic drug used in medicine. In 1891 it was applied by Paul Guttmann and Paul Ehrlich for the treatment of malaria, and this application has recently been revived (Coulibaly et al., 2009; Färber et al., 1998; Vennerstrom et al., 1995). The famous Giemsa solution for staining and characterizing malaria parasites and blood cells contains MB, eosin A, and azure B as active principles (Barcia, 2007; Fleischer, 2004). Numerous other microscopic discoveries including the identification of *Mycobacterium tuberculosis* by Robert Koch and the structural organization of nerve tissues (Cajal, 1896; Ehrlich, 1886; Garcia-Lopez et al., 2007) were based on the biochemical properties of MB. Staining with MB was also the beginning of modern drug research (Kristiansen, 1989): Paul Ehrlich argued that if pathogens like bacteria and parasites are preferentially stained by MB, then this staining might indicate a specific harmful effect on the pathogen which could be exploited for fighting disease. This explains why the terms "drug" and "dye" were used synonymously until World War I.

Malaria and methylene blue played a major role also in World War II. In 1943, General Douglas MacArthur, commander of the Allied Forces in the Southwest Pacific theater stated: "This will be a long war, if for every division I have facing the enemy, I must count on a second division in the hospital with malaria, and a third division convalescing from this debilitating disease." Because of the blue urine ("Even at the loo we see, we pee, navy blue"), MB was not well liked among the soldiers (MacArthur, 1964; W. Peters, personal communication).

Going back to the beginning of the twentieth century, MB was used for a wide variety of medical and hygienic indications (Clark et al., 1925). Among others, it was added to the medication of psychiatric patients in order to study their compliance which could be monitored by the observable color of the urine. These studies led to the discovery that MB has antidepressant and further positive psychotropic effects (Bodoni, 1899; Ehrlich and Leppmann, 1890; Harvey et al., 2010). Thus MB became the lead compound for other drugs including chlorpromazine and the tricyclic antidepressants. In 1925 W. Mansfield Clark, famous for the introduction of the pH electrode and the oxygen electrode, was a coauthor of an impressive 80-page review on the application of MB in engineering, industrial chemistry, biology, and medicine. A remarkable aspect of this article is the reference list of illustrious scientists including several Nobel Prize winners — Santiago Ramon y Cajal, Robert Koch, Paul Ehrlich, Alphonse Laveran, Otto Meyerhof, and Heinrich Wieland — who contributed major papers on MB. Thus MB is an example for the high value of observations and articles that were published 100 years ago and are still relevant today.

4. Current medical indications

By 2010, there are more than 11,000 entries for "methylene blue" in the biomedical library PubMed, not counting the studies which had been published in the era not covered by PubMed. Current indications for MB that are approved by the US Food and Drug Administration (FDA) are enzymopenic hereditary methemoglobinemia and acute acquired methemoglobinemia, prevention of urinary tract infections in elderly patients (Table 1), and intraoperative visualization of nerves, nerve tissues, and endocrine glands as well as of pathologic fistulae (O'Leary et al., 1968).

Of great practical importance is also the administration of MB for the prevention and treatment of ifosfamid-induced neurotoxicity in cancer patients (Kupfer et al., 1994). Recommended doses are 3 to 6 times 50 mg/day intravenously (i.v.) or orally (p.o.) as a treatment and 3 to 4 times 50 mg/day p.o. given for prophylaxis, starting 1 day before ifosfamid-infusion and continuing still after oxazaphosphorine-treatment is finished (Pelgrims et al., 2000). Concerning inborn enzymopenic methemoglobinemia (Table 1), the treatment of the Blue People of Troublesome Creek in Kentucky — and other persons worldwide — with the *blue* drug MB was a visible success of knowledge-based medicine (Cawein et al., 1964). The rationale is that MB can be reduced to colorless leucoMB by red blood cell enzymes and that leucoMB reduces the inactive methemoglobin to give hemoglobin (Fig. 1). This conversion turns the bluish tinge of the skin to a rosy complexion — in the early 1960s