Methylene Blue and Nitric Oxide

— Beth Shirley, RPh CCN (January 2023)

Methylene Blue (MB) is the first drug ever used as a medication in modern medicine. It was first discovered in 1876 as a dye and became the first drug treatment for malaria in 1891. Many of the first prescription meds came from MB, from the antipsychotic drug, chlorpromazine, to antibiotics and antiseptics. In fact, MB is the parent compound of chloroquine and hydroxychloroquine. It is considered a grandfathered drug because it was used and available prior to the formation of the FDA. In 1886, Paul Ehrlich coined the words "magic bullet" precisely because of the beneficial physiological actions of MB.[1]

MB is available in every hospital in the world and is used as an antidote to anything that displaces oxygen, such as carbon monoxide and cyanide poisoning that causes methemoglobinemia. Methemoglobinemia is where the iron in hemoglobin is oxidized to Fe3+ and in unable to carry oxygen.

MB is used in low doses, in non-acute situations for chronic, neurological injuries to the brain for neuroprotection against mitochondrial dysfunction.[2]

Critical Importance of Low Methylene Blue Dose

It is misleading to refer to the MB effects without mentioning the dosages being used.[3] MB has a hormetic, bi-phasic, dose response curve having opposite effects when using low vs. high dosages. High IV doses can cause methemoglobinemia and is a prooxidant. Using low doses, MB becomes the treatment of choice for methemoglobinemia and can transfer electrons to oxygen or alternate electron acceptors becoming an electron shuttle.[2]

Used in lower dosages (<3mg/kg), MB is an electron cycler through its reduction-oxidation capacity. It can insert electrons where needed in the electron transport chain (ETC) of the mitochondria. Cytochrome c oxidase (CO), or Complex IV, is the terminal enzyme of the mitochondrial respiratory chain. CO is a heme/Cu metalloenzyme. MB will transfer electrons to oxygen and using protons within the mitochondria, oxygen will be reduced to water. Thus, MB increases CO activity and oxygen consumption. This in turn increases ATP production and energy metabolism. MB rapidly activates this consumption of oxygen, which can lead to a local transient hypoxic state. CO can then change to a nitrite reductase enzyme capable to reducing nitrite to nitric oxide (NO) to improve blood flow and glucose uptake.[3]

The Relationship Between Nitric Oxide and Methylene Blue

Oxidative phosphorylation is when the ETC is coupled with phosphorylation, metabolizing ADP into ATP, our energy molecule. Not all oxygen within the ETC is successfully reduced into

water with some oxygen going into superoxide. In fact, the mitochondria are our main site of intracellular oxygen consumption and one of the main sources of reactive oxygen species (ROS) formation.[4] Nitrite and NO can help recouple this ETC, as does MB, to down-regulate the production of superoxide and ROS.

Blood flow to the tissues can be more important than how much oxygen is carried by hemoglobin. There is a tight coupling between consumption of oxygen and the hemodynamic response which is modulated NO and oxygen. CO activity in the brain has molecular plasticity varying widely in response to energetic demands that change in response to neural activation.[2] In other words, increased brain activity increases the need for oxygen, energy production, and microcirculation.

During hypoxia, acidic pH, or ischemic conditions, nitric oxide synthase (NOS) production of NO is inhibited. In the mitochondria, Complex III or CO have nitrite reductase ability to reduce nitrite to NO. NO can reversibly bind to CO, inhibiting activity and hence mitochondrial respiration. This conserves oxygen and allows oxygen to diffuse beyond the mitochondria and further into the tissue distant from the oxygen source. Once oxygen concentration is restored in the tissue, inhibition is reversed, and respiration is restored. This does not always result in a decreased rate of ATP production.[5] In fact, supplementation with nitrate during exercise can decrease whole body oxygen consumption without changing maximal attainable work rate and increasing exercise endurance.[6]

Also, nitrite reduction to NO by Complex III and CO can act as an antioxidant to prevent lipid peroxidation.[7] This is especially important in our brain tissue because the brain is about 60% fat.[8]

There are a few ways NO plays into this pathway.

First off, hemoglobin requires NO to be attached to the molecule for it to release oxygen to the cells and tissues.[9] Without adequate NO, oxygen delivery is impaired. Even though the brain is only about 2% of our body's mass, it consumes about 20% of our body's requirement for oxygen.[10]

When the body becomes more oxygen deficient or hypoxic, mitochondria are less able to make energy, in other words ATP. The mitochondria become uncoupled as superoxide production increases. This is precisely when CO becomes a nitrite reductase enzyme to slow down oxygen consumption, improve microcirculation, and increase NO production.

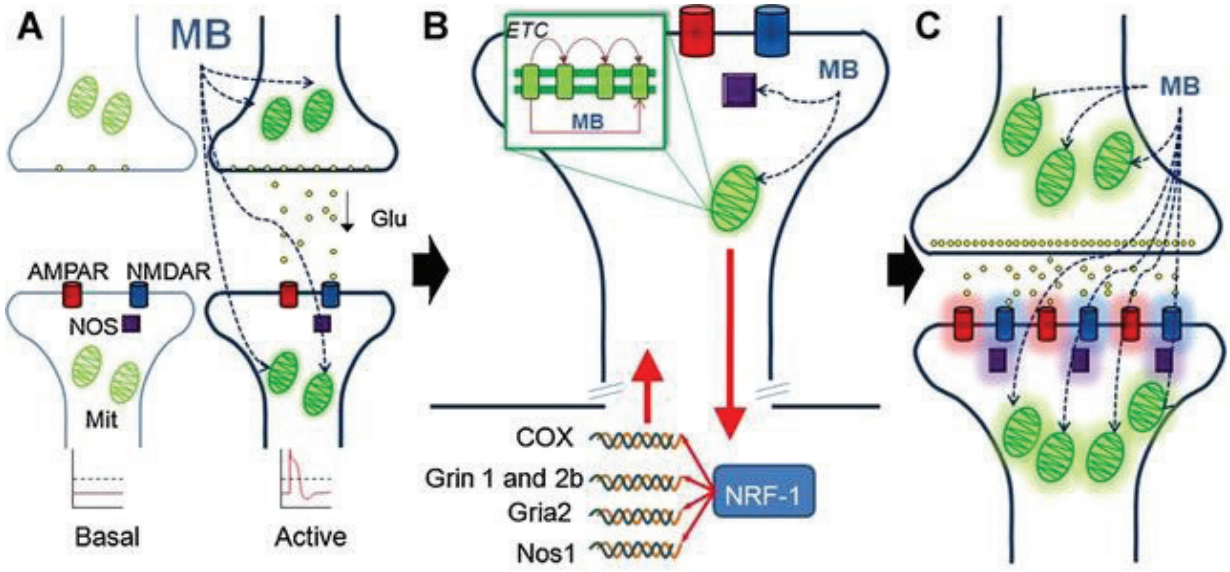
The NOS enzyme's ability to make NO is incredibly environmentally and epigenetically sensitive. So much so that by the time we are 40, NOS production of NO has decreased to 50% and by the time we are 60, it only functions around 15%. Many factors influence the uncoupling of a functional NOS enzyme. These are age, diet, lack of exercise, medications (antibiotics, -azole antifungals, SSRI antidepressants, birth control pills, NSAIDs, PPIs), electromagnetic fields (EMFs), pollution, glyphosate, oxidative stress, genetic single nucleotide polymorphisms (SNPs) (for example: anything that interferes with BH4 production like QDPR, DHFR, MTHFR). Any SNP that increases oxidative stress like SOD, CAT, HFE, etc., and the big one, stress from any source.

This is critical because when NOS is uncoupled or dysfunctional, it becomes a superoxide generator, not an NO producer. Nitrate helps increase the production of BH4, which helps recouple the NOS enzyme.[11]

How Does This All Tie In to Methylene Blue?

MB increases the production of nuclear regulatory factor 1 (NRF1), which upregulates neuronal NOS (nNOS) to increase production of NO. NO dilates microcapillaries and improves oxygen delivery. Neurons cannot store energy so they have lots of CO because they need sustained mitochondrial metabolism and energy production. If the brain is working harder

and processing more information, it uses more energy increasing activity of CO.[2] The caveat here is nNOS must be coupled to produce the needed NO.



(A) MB in mitochondria accumulates in neurons within activated networks with high energy demands and active mitochondria.

(B) MB enhances the activity of electron transport chain and modulates NOS. NOS is activated by glutamatergic receptors (AMPAR and NMDAR) to produce NO, which acts as a second messenger and regulates CO activity. Metabolically active mitochondria communicate with nuclear and mitochondrial genome (red arrows) to up-regulate all CO and NOS1 or nNOS, NMDA receptor and AMPA receptor genes, all under the influence of NRF-1.

(C) Gene expression orchestrated by NRF-1 allows for co-localization of proteins critical for energy metabolism and synaptic transmission aiding in synaptic strengthening and improved memory function.[2]

Low dose MB can support the cerebral metabolic rate of oxygen consumption and metabolic energy production in normoxic and hypoxic conditions in vivo. By the mitochondrial reduction of nitrite to NO during hypoxia, vasodilation is increased, and cerebral blood flow and brain glucose uptake is increased, which is especially beneficial in neurodegenerative conditions.[3] Hypoperfusion of the brain corresponds to cognitive decline and dementia.[12,13]

There is controversy whether MB inhibits NOS as well as inhibiting guanylyl cyclase, blocking the release of cGMP and decreasing NO-mediated endothelial relaxation. Studies show that MB does not interfere with NOS production of NO.[14,15]

There have been numerous studies and books written about the use of MB in vasoplegic syndrome (VS). VS is associated with endothelial dysfunction caused by systemic inflammation.

MB effects are seen only in NO supra up-regulation as seen in increased iNOS activity. One group of researchers wrote, "The pivotal action of MB is not exclusively the guanylyl cyclase blockage, resulting in a cGMP release decrease. This blockage also enhances the 'crosstalk' between cAMP and cGMP pathways, which facilitates the effect of the cAMP dependent vasopressors like epinephrine."[16] There was no effect of MB on the constitutive, Ca2+ dependent eNOS or nNOS.[17]

Nitrate can also down-regulate an up-regulated iNOS.[20] iNOS can produce NO up to 1,000 times greater than eNOS production.[18]

Methylene Blue and a Chronic Inflammatory Pathway

Increased iNOS activity is generally associated with chronic inflammatory processes, infections, or environmental toxins. Bacterial, viral, fungal, and parasitic infections up-regulate iNOS. Some environmental toxins that upregulate iNOS are EMF, aluminum, mercury, uranium, BPA, HFCS, gluten, chlorine, glyphosate, homocysteine, and iron dysregulation. As you can see, an up-regulation of this coupled, functional enzyme can increase production of NO to high levels. MB inhibits the upregulation of iNOS activity without inhibiting the activity of nNOS or eNOS.[17]

When iNOS is upregulated, eNOS and nNOS can be downregulated. Impaired eNOS function results in inadequate tissue perfusion and decreased delivery of oxygen, glucose, and nutrients, and just as importantly, clearing away the cellular debris. This may be harmful to essential vital organs such as the brain, heart and kidneys, leading to multiple organ deficiencies.[18]

Nitric Oxide, Methylene Blue, and Septic Shock

Even though the increased NO production has been held responsible for the progressive and refractory hypotension in septic shock, studies have shown that NO donor treatment may be useful to maintain adequate circulation and perfusion of vital organs. Nitrite significantly attenuates mitochondrial damage, oxidative stress, cytoprotection after ischemia-reperfusion injury and mortality.[19]

MB has been used successfully in septic shock, as infection can decrease blood pressure. Intermediate dosing is required for this treatment at 3mg/kg.[2] This dosage for the treatment of septic shock is well above the 4mg-65mg doses widely used for neurological issues.

Supporting Cerebral Blood Flow

In aging and dementia, we see progressive reduction in cerebral blood flow and energy metabolism that results in cognitive dysfunction. With chronic cerebral hypoperfusion, as seen in neurocognitive disorders, there is decreased CO activity resulting in numerous neurodegenerative effects and impaired learning and memory.[13] There is a decreased supply of glucose and oxygen to brain cells and neurons. MB is used in neurodegenerative issues such as dementia, Alzheimer's, Parkinson's, traumatic brain injury (TBI), strokes, post viral jabs, and anywhere there is an increased oxygen-based energy demand and production.[1]

This reduced cerebral blood flow is the same issue that benefits from improved circulation and microcirculation that NO governs. NO is a mediator of neurovascular coupling, meaning microcapillary diameter enlarges in response to increasing metabolic demands from neuronal activity.[20] Deficits in neurovascular coupling decrease cerebral blood flow and underlie cognitive impairment.

Supporting the Nitrate/Nitrite/NO Pathway with Methylene Blue

Neurons are especially sensitive to oxidative stress because of longevity and limited renewal. With increased ROS, antioxidant defense becomes overwhelmed, and there is increased production of inflammatory cytokines and lipid peroxidation.[21] Oxidative stress interferes with the learning and memory process as well as numerous other neurodegenerative processes. Supporting the nitrate/nitrite/NO pathway as well as MB, downregulates this oxidative stress process and damage.

By supporting the nitrate/nitrite/NO pathway and using MB, one can prevent oxidative damage by blocking free radical formation in the mitochondria. Mitochondrial dysfunction and oxidative stress lead to cellular senescence and aging. Oxidative stress promotes cell senescence by shortening telomere length. NO can prevent cellular senescence and increase telomerase activity, restoring telomere length.[22] Additionally, MB delays senescence by enhancing mitochondrial function.[23]

Both MB and supporting the nitrate/nitrite/NO pathway may provide protection against abundant free radical formation in the ETC and provide protection against numerous pathological conditions and age-related mitochondrial-associated neurodegeneration.

Conclusion

NO touches every single physiological function governing circulation and microcirculation. When microcirculation is impaired, healing cannot and will not happen. Studies show that MB inhibits an upregulated iNOS, and when iNOS is upregulated, eNOS and nNOS become downregulated potentially impairing circulation and microcirculation. Also, with the NOS enzyme becoming uncoupled and dysfunctional by 50% by the time we are 40, and only functioning around 15% by the time we are 60, supporting the nitrate to nitrite to NO pathway with nitric oxide precursors and MB is an effective way to keep energy and oxygen flowing to the cells and tissues, which is essential to healthy longevity and cognition.

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