IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Methemoglobinemia: A Rare Disease"

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Abstract:

Methemoglobinemia (MetHb) is a rare yet significant condition characterized by the unusual accumulation of methemoglobin, an oxidized hemoglobin form that compromises its oxygen transport function. This review comprehensively elucidates MetHb, surrounding its etiological factors, pathophysiological mechanisms, clinical manifestations, diagnostic modalities and therapeutic interventions. The review delineates the diverse causative agents of MetHb, spanning genetic predispositions to exposure to specific pharmaceuticals, chemicals and toxins, with particular emphasis on well-documented substances such as nitrates and aniline derivatives. Additionally the propensity of individuals with methemoglobin reductase deficiencies is discussed. The review further explores the genetic and acquired determinants contributing to MetHb, highlighting the importance of red blood cell metabolism and hemoglobin structure in its genesis. Clinical aspects of MetHb are expounded, ranging from asymptomatic presentations to severe symptoms, including cyanosis, dyspnea, and cardiac arrhythmias leading to circulatory failure in critical scenarios. Remarkably, children with congenital heart diseases and MetHb often manifest ambiguous oxygen saturation readings, accentuating the clinical complexities associated with this condition. Various therapeutic strategies for MetHb are scrutinized, encompassing the use of methylene blue, ascorbic acid and N-acetylcysteine. A relative analysis of these treatment modalities is presented, incorporating dosing recommendations and potential side effects. Moreover, recent advancements in research methodologies, such as Broadband Diffuse Optical Spectroscopy and Photoacoustic Microscopy, are discussed for their contributions to comprehending methemoglobin formation and reduction in animal models. These non-invasive techniques afford real-time monitoring of methemoglobin levels, furnishing valuable insights into the dynamic nature of the disease. In conclusion, this review offers an in-depth exploration of MetHb, elucidating its comprehensive etiology, varied clinical presentations and evolving therapeutic approaches. The insights provided herein serve as a valuable guide for healthcare professionals in effectively managing this elaborate condition.

Keywords:

Methemoglobinemia, Cyanosis, Co-oximetry, Plasmapheresis, Broadband diffuse optical spectroscopy.

INTRDUCTION:

Anemia is a condition characterized by a deficiency of red blood cells or low hemoglobin levels, which results in reduced oxygen transport to body tissues. The symptoms of anemia can vary depending on its type and underlying cause. Acute anemia may manifest with low blood pressure and a rapid heart rate, while chronic anemia can lead to weakness, fatigue, and pale skin. Various forms of anemia exist, including sickle cell, aplastic, hemolytic, iron-deficiency, and anemia due to blood loss, each with distinct causes. Some types, such as hemolytic anemia, can be triggered by enzyme deficiencies or immune system attacks on red blood cells.(1) A functional anemia like methemoglobinemia (MetHb) is the interruption of the ability to transport oxygen capably by the hemoglobin.(2) MetHb a rare condition characterized by elevated levels of methemoglobin in the blood, is intricately linked to the broader context of anemia.(3) Our blood contains a hemoglobin (Hb), which carries oxygen. The normal colour of our skin depends on the amount of hemoglobin and the oxygen it carries. If oxygen levels are low then skin can turn bluish or purplish condition called cyanosis.(2) MetHb is characterized by the conversion of hemoglobin into methemoglobin, rendering it incapable of carrying oxygen due to iron's oxidation from the ferrous (Fe+2) to ferric (Fe+3) form. This shows structural alteration of hemoglobin interferes with oxygen transport shifting the hemoglobin-oxygen dissociation curve to the left and compromising oxygen delivery to tissues that leads to cellular hypoxia. MetHb can develop from both hereditary factors or acquired causes.(4) Methemoglobin which reduces the oxygen in the blood causing cyanosis. Methemoglobinemia occurs when Methemoglobin levels are higher than 1% of total hemoglobin. For adults' normal hemoglobin levels vary but MetHb is usually less than 1%. If it goes higher than the blood can turn chocolate brown. MetHb forms when hemoglobin loses oxygen or reacts with certain substances. The body has ways to convert MetHb back to normal hemoglobin but if MetHb levels go beyond 15 percent symptoms like shortness of breath, headache and weakness can occur. Higher levels can lead to severe problems and even death if not treated.(2)

DEFINITION:

A blood disease that occurs when too little oxygen is provided to the cells of the body is MetHb.

History:

In MetHb is a condition where the blood turns a bluish-gray colour, which can be present from birth in some cases. This colour change doesn't result from heart or lung problems and people with this condition don't necessarily feel sick, even though their blood looks different.

In some cases, MetHb can be life-threatening. The normal level of methemoglobin in the blood is about 1%, but when it's between 3-15% the skin may look slightly discoloured. Patient with 15-20% methemoglobin in their blood may not have many symptoms. However, infants and children can get severe illness if their methemoglobin levels are more than 70%. It's important to ask, if anyone in the family has a history of MetHb or glucose-6-phosphate dehydrogenase deficiency (G6PD). Even if someone has only one gene with a methemoglobin problem, they can still have issues with certain medications that can make their blood turn gray. If someone has stomach problems like nausea, vomiting, or diarrhea, it could mean they have been exposed to a harmful substance. If someone already has anemia (a lack of red blood cells), the symptoms of MetHb can be even worse.(1)

Pathophysiology:

MetHb is a condition where there is an excessive quantity of a substance known as MetHb in the blood, which does not able to carry oxygen, leading to insufficient oxygen delivery to tissues. Normally our body maintains low MetHb levels through enzyme systems. The cytochrome b5-MetHb reductase using nicotinamide adenine dinucleotide converts MetHb back into functional hemoglobin removing 95-99% of MetHb. Another pathway, depending on substances like NADPH, reduces about 5% of MetHb but can be enhanced with compounds like methylene blue. Genetic conditions can disrupt these systems, causing MetHb levels to enhance significantly, up to 40-50%, affecting oxygen transport and potentially becoming life-threatening, especially when triggered by specific chemical agents or drugs.[3]

Pathways:

Methemoglobin Reduction Pathways:

1. The main approach to reduce methemoglobin is through an enzyme called nicotinamide adenine dinucleotide (NADH)-dependent methemoglobin reductase I also known as diaphorase. This enzyme takes electrons from NADH and uses them to convert methemoglobin back into regular hemoglobin. NADH is produced through the main procedure of breaking down glucose in the body.[5]

2. There is another pathway for reduction, which requires the presence of methylene blue and normal levels of G6PD.[6]

Symptoms:

1. The main symptom is chocolate-brown coloured blood.[7]
2. Cyanosis is a bluish colour of the skin which is usually the initial sign of MetHb. However, pulse oximetry can sometimes give misleading outcome by showing lower peripheral oxygen levels.[3]
3. Methemoglobin levels of 10 to 20 percent are usually well-tolerated, but symptoms become more communal as levels increase above this range.
4. At methemoglobin levels of 30 percent or more, symptoms can include shortness of breath, nausea and a fast heartbeat.
5. When methemoglobin levels reach 55 percent, individuals may become lethargic, confused and lose consciousness.
6. Higher levels can lead to heart rhythm problems and circulatory failure.[1]

Generally higher levels of methemoglobin (MetHb) lead to more severe symptoms and an increased risk of health problems or death.[8]

Causes:

Causes of MetHb:

1. Various drugs, chemicals and substances can lead to MetHb like

- Nitrites derivatives (e.g., nitroprusside, amyl nitrite, nitric oxide)
- Sulfonamides
- Dapsone
- Phenacetin
- Phenazopyridine
- Some local anesthetics (e.g., prilocaine)
- Topical anesthetics (e.g. benzocaine)
- Antimalarials

- industrial chemicals and insecticides

Contact with these substances can result into severe MetHb.[9]

2. There are nearly 90 compounds associated with the development of MetHb.

3. However, individuals with methemoglobin reductase deficiency or abnormal hemoglobin may experience severe effects, especially in cases of overdose. [10]

MetHb level	Signs	Symptoms	Causes
<10%	Low pulse oximeter readings, alteration of the skin colour (pale, gray, blue)	Asymptomatic	Acquired
10%-30%	Cyanosis Dark brown blood	Asymptomatic/confusion	Enzymopenic methemoglobinemia, HbM, acquired
30%-50%	Dyspnea, dizziness, syncope	Confusion, chest pain, palpitations, headache, fatigue	Acquired ± hereditary
50%-70%	Tachypnea, metabolic acidosis, dysrhythmias, seizure, delirium, coma	Confusion, chest pain, palpitations, headache, fatigue	Acquired ± hereditary
>70%	Severe hypoxemia, death	-	Acquired ± hereditary[11]

TYPES OF METHEMOGLOBINIMEA:

MetHb can result from either congenital or acquired processes.

CONGENTIAL:

MetHb is a condition where the blood in excess of a substance called methemoglobin, which can't carry oxygen like regular hemoglobin. It can be raised from different sources like certain chemical agents, foods with nitrates or nitrites, medicines and contaminated water sources. There are different types: Type I, Type II, and Hemoglobin M Disease (HbM).

Type I: It is the most common congenital type and causes minor symptoms like bluish skin, similar to HbM.

Type II: It is severe and can be life-threatening, especially in infants. It's rarer and can affect the brain, causing problems like convulsion and developmental delays. Treatment for Type II is usually palliative, which means it focuses on relieving symptoms but doesn't cure the condition. This treatment might include things like ascorbic acid or methylene blue if methemoglobin levels are too high.

HbM: It is another congenital type where there's a gene mutation that makes it difficult to treat MetHb. However, most people with this mutation don't need treatment and can live a normal life, although they may have slightly or normal bluish skin.

ACQUIRED:

Acquired MetHb is more common and usually occurs when you come into contact with substances that can enhance the production of methemoglobin in your blood. This can be little dangerous and even life-threatening. It often happens when people take certain medications or are exposed to chemicals that have this effect.

The acquired type of MetHb some common medicines include nitroglycerin, Dapsone, sulfonamides, phenytoin, phenacetin, and certain local anesthetics. Also, some agricultural chemicals contain solvents that can cause serious MetHb. Babies, especially newborns and infants are at higher risk of developing MetHb when exposed to these medications or chemicals because their body systems are not as good at reducing methemoglobin. So it's critical to be cautious with young children when it comes to above listed substances.[(1)]

Diagnosis:

MetHb can be challenging to diagnose due to its vague symptoms. However, some key features can raise suspicion in certain patients. Blood with high levels of MetHb has a unique chocolate-brown colour, unlike the dark red colour of normal deoxygenated blood. This colour doesn't change when exposed to air, unlike regular blood. An easy bedside test involves placing a drop of bloods on filter paper and observing its colour change after exposure to air. Blood rich in MetHb will remain chocolate brown. Patients with MetHb often have cyanosis (bluish skin) that doesn't match their measured oxygen capacity. This difference is due to how MetHb affects standard oxygen assessments. Most oxygen monitors use pulse oximetry, which measures light absorption at specific wavelengths. MetHb absorbs light at these wavelengths, which can lead to incorrect capacity values. When MetHb levels go above 30%, the measured oxygen capacity plateaus around 85%, even if the actual oxygen content is higher. This is a significant sign for MetHb. In contrast, arterial blood gas measures oxygen capacity differently, considering factors like inspired oxygen levels and lung function. This value is independent of MetHb levels and may differ significantly from the capacity measured by pulse oximetry, creating a "saturation gap." A saturation gap above 5% is abnormal. The chocolate-brown blood colour, lack of improvement in oxygen saturation with additional oxygen and the presence of a saturation gap can all indication at MetHb.[(3)]

Direct measurement of MetHb levels in the blood is the most reliable way to confirm the diagnosis.

Table: Diagnostic tests for MetHb	
1.	Arterial blood gas (ABG)
2.	Co-oximetry (more accurate) or Pulse oximetry
3.	Potassium cyanide test (MetHb and sulfhemoglobin differentiation)
4.	Bedside tests for MetHb (using filter paper for blood colour evaluation or using 100% oxygen to aerate a tube of blood)
5.	Complete blood count (CBC), reticulocyte counts, lactate dehydrogenase (LDH), indirect bilirubin
6.	Chest radiography (pulmonary and cardiac disease exclusion)
7.	CT of the head
8.	Liver function tests
9.	Heinz body preparation
10.	Urine pregnancy tests
11.	Serum level of nitrites or inducing drugs
12.	Enzyme assays[(3)]

Guidelines:

It's crucial to identify MetHb early because the severity of the symptoms will help determine the right treatment. Sometimes, the symptoms can be vague and not very specific. So, at the beginning, the patient should receive initial care, which includes:

1. Giving the person extra oxygen.

2. Figuring out what caused the MetHb.

3. Removing the substance that's causing the problem.

Doctors can run different tests to confirm MetHb, check how well the organs are working, and prevent other health issues.

Table: Guidelines for treatment

	Types of MetHb	Treatment
1.	Acute acquired MetHb	Need to stop administration of inducing agent and emergency therapy
2.	Chronic mild MetHb	Can be fully asymptomatic with cyanosis or not and no specific therapy required.
3.	Chronic MetHb	Medications to reduce cyanosis (Methylene blue, citric acid, oxygen)
4.	Severe MetHb	Need emergency therapy (It is life-threatening)[3]

Current treatment:

1.Methylene blue:

The treatment for MetHb involves removing the substance that caused it and considering the use of an antidote called methylene blue.[12] Methylene blue is a common treatment for MetHb. It's a substance that can convert methemoglobin back to regular hemoglobin. This conversion happens in red blood cells and requires NADPH.[1] Providing high-flow oxygen through a non-rebreathe mask is important because it increases oxygen supply to the tissues and aids in the natural breakdown of methemoglobin. Methylene blue typically works faster and efficiently by interacting with a secondary pathway in the reduction of methemoglobin. In this pathway, NADPH-MetHb reductase reduces methylene blue to leukomethylene blue, using NADPH from the G6PD-dependent hexose monophosphate shunt. Leukomethylene blue then acts as an electron donor to change methemoglobin back into hemoglobin. In cases of acquired MetHb, treatment with methylene blue should begin when methemoglobin levels exceed 20-30%, or even lower if the patient shows symptoms. The assumption to start treatment should be based on clinical signs, and you shouldn't wait for confirmatory lab results. The usual dose of methylene blue is 1-2 mg per kilogram of body weight, administered intravenously over 5 minutes. If severe symptoms continue or methemoglobin levels remain above the treatment threshold, the dose can be repeated in 30-60 minutes.[12] For individuals with inherited MetHb, the typical oral dosage of methylene blue ranges from 50 to 250 mg per day.[1] Healthcare providers should be aware of the possible side effects of methylene blue. Some minor side effects include turning the urine green or blue, which patients should be informed about in advance. More serious side effects are associated with methylene blue's properties as an oxidizing agent and an inhibitor of monoamine oxidase A (MAO-A).[12] Methylene blue in high doses or when not effectively reduced MetHb and It's important to note that methylene blue should not be used for people with G6PD deficiency, it can actually worsen MetHb or lead to hemolysis.G6PD is necessary for producing NADPH,

which is needed for the conversion of methylene blue into its active form.[1] When a patient is already taking serotonergic agents, using methylene blue can increase the risk of serotonin syndrome, so attention is needed. Treating neonates with methylene blue should also be needed carefully because they are highly sensitive to oxidizing agents. Additionally, it's essential to note that methylene blue is classified as a United States Food and Drug Administration (FDA) pregnancy category X drug, indicating a clear risk to human fetal development based on research.[12]

2. Ascorbic acid:

Ascorbic acid also known as vitamin C, was found to have a similar effect in reducing the level of methemoglobin as methylene blue.[13] It can be used to directly reduce methemoglobin (MetHb). However, when used on its own, it works slowly and may require multiple doses over 24 hours or longer to effectively lower MetHb levels. Ascorbic acid is a good choice for treatment when methylene blue (MB) is not available or in cases of MetHb and G6PD deficiency. The dosing can vary widely and is not standardized. In adults, doses have ranged from 0.5 grams every 12 hours for 16 doses, 1 gram every 12 hours for 14 doses, 1.5-2 grams intravenously for 3-4 infusions, 5 grams every 6 hours for 6 doses, or even a single 10-gram dose. In children, doses have ranged from 0.5 grams every 12 hours for 16 doses to 1 gram every 4 hours for 8 doses.[11] This suggests that using ascorbic acid as a treatment for MetHb deserves further investigation and consideration as a potential recommended treatment option.[13]

Supportive therapy:

1. N-acetylcysteine:

The use of N-acetylcysteine in treating MetHb is not entirely clear. In laboratory studies, N-acetylcysteine has shown potential to aid in the reduction of methemoglobin and increase the levels of intracellular glutathione. It has been suggested as a treatment for patients with MetHb, especially those with G6PD deficiency, and in cases of MetHb induced by acetaminophen.

2. When dealing with MetHb, it's important to provide IV fluids and oxygen. In more severe cases, ventilation and medication to support blood pressure might be necessary. To make the body's natural mechanisms for reducing methemoglobin work, it's crucial to have an adequate supply of glucose. For infants who develop MetHb due to diarrhea and acidosis, improvement can often be seen with aggressive hydration and the use of bicarbonate to treat the acidosis. This is effective as long as the level of methemoglobin is less than 20%.[11]

MetHb Overview:

1. MetHb can be chronic or acute, and the normal level of methemoglobin in the blood is 0 to 2 percent.

2. High levels above 70% can be lethal.

3. Symptoms and the rapidity of onset can vary. Some people with lifelong MetHb have no symptoms, while those exposed to certain medications or toxins may suddenly develop severe symptoms.

4. Infants with MetHb may have cyanosis that doesn't improve with extra oxygen supply.

5. Drug-induced MetHb, often associated with certain medications, can also lead to hemolytic anemia (destruction of red blood cells).

6. Hemolytic anemia can have features like Heinz bodies (clumps of hemoglobin in red blood cells), scattered red blood cells and in severe cases, kidney problems and jaundice.(1)

7. Characteristic Cyanotic Congenital Heart Disease from MetHb in Children:

Children with MetHb, on the other hand will have high oxygen levels despite looking blue and having a regular calculated oxygen saturation.[14]

Complications:

- Complications of methemoglobinemia are primarily due to low oxygen levels (hypoxia).

- The type of methemoglobinemia influences the complications which can include seizures, coma, heart attacks and in some extreme cases death.

- People with multiple diseases can face complications and an increased risk of death even with a lower percentage of MetHb.

- Managing methemoglobinemia is more positive for moderate cases but critical cases depend on the extent of damage to vital organs due to lack of oxygen.

- The outcome for severe cases is determined by the result of damage to organs from a lack of oxygen.[2]

In vivo model:

Rabbit model:

Broadband diffuse optical spectroscopy (DOS) to measure the levels of methemoglobin (MetHb) in rabbits. DOS combines various methods to measure how light is absorbed and scattered in tissues. In this case they looked at MetHb, deoxyhemoglobin (Hb-R) and oxyhemoglobin (HbO2) concentrations in real-time while infusing nitrite in rabbits.

They were able to detect very small changes in MetHb levels (as low as 30 nM) over a range of MetHb concentrations (0.80 to 5.72 M) which represented 2.2% to 14.9% of the total hemoglobin. These measurements matched well with data obtained from traditional co-oximetry (a method for measuring hemoglobin types).

The study also tested how MetHb is reduced to functional hemoglobin using methylene blue injections. They could detect changes in levels as low as 10 nM in tissues even when MB levels reached 150 nM.

This research is significant because it shows that broadband DOS can accurately and noninvasively measure real-time changes in MetHb and other related substances, even when their spectral features overlap. These findings have potential applications in evaluating drug delivery and treatment effectiveness in both animal and human models.

Procedure of how broadband DOS technique works:

1. Instrument Setup: The DOS instrument combines steady-state (SS) and frequency-domain (FD) photon migration techniques. It employs six laser diodes that emit light at different wavelengths like 661, 681, 783, 823, 850 and 910 nm and an avalanche photo diode (APD) detector to measure the light that interacts with tissue.

2. Modulation Detection: The APD detector measures the intensity modulated diffuse reflectance signal as it passes through the tissue. This signal is detected at different modulation frequencies ranging from 50 to 550 MHz.

3. Coefficient Calculation: Using the data obtained from the frequency-dependent phase and amplitude measurements the absorption (μa) and reduced scattering (μs') coefficients are directly calculated at each of the six laser diode wavelengths. The reduced scattering coefficient is determined by fitting a power aw to these coefficients.

4. Steady-State Measurement: After the frequency domain measurements a broadband reflectance measurement is conducted from 600 nm to 1000 nm. This steady-state acquisition uses a tungsten-halogen light source and a spectrometer.

5. Calibration: The intensity of the steady-state reflectance measurements is calibrated to the FD values of absorption and scattering, ensuring accurate reflectance intensity.

6. Data Extraction: The absorption spectra are extracted from the absolute steady-state reflectance spectra by subtracting the scattering contribution across the spectral range of interest. This is done by fitting a power-law to FD scattering coefficient measurements.

7. Chromophore Concentrations: Finally, the concentrations of different chromophores (Hb-O2, Hb-R, MetHb, H2O, and MB) are calculated by performing linear least squares fit of the wavelength-dependent extinction coefficient spectra of each chromophore by using known extinction spectra provided by previous research.

In essence, this technique measures how light interacts with tissue extracts information about the concentrations of various hemoglobin types and other substances, and provides insights into the formation and reduction of methemoglobin in real time.[15]

Detection of methemoglobin in vivo:

1. Objective of the Study: Induced methemoglobinemia in 6 male albino Sprague-Dawley rats.

2. Experimental Procedure: Used PAPP (Eastman Kodak Co.) at a dose of 5 mg/kg body weight. Rats were anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally. Intravenous injection and blood sampling via inferior vena cava. PAPP administered as a 0.1% aqueous solution freshly prepared for each rat. Control sample involved 2 ml of whole blood in a heparinized tube.

3. Blood Sampling and Oxygenation:

- Oxygen introduced into the sampling tube.
- Sealed with Para film and rotated for 10 min on a rotating apparatus.
- First blood sample taken before PAPP injection.
- Second sample taken 1 hour after PAPP injection.
- Third sample taken 3 hours after PAPP injection.

4. PAPP Solution Preparation: PAPP dissolved in 0.1 N HCl in 100-ml lots.

5. Measurement Techniques: Total hemoglobin and methemoglobin measured using the spectrophotometric technique of Evelyn and Malloy. Methemoglobin levels also determined by the difference in oxygen capacity between control and unknown blood samples. Oxyhemoglobin measured by the method of Van Slyke and Neill in a Natelson microgasometer.

6. Substitutions in Measurement Methods: Used a 1.0% solution of nonionic polyols as a foam inhibitor. Nonionic surface active agent in 0.3% solution used instead of saponin as a hemolyzing agent.

7. Deaeration of Reagents: All reagents deaerated under vacuum prior to testing.

8. Analysis: Methemoglobin levels measured based on the decrease in oxyhemoglobin, as methemoglobin does not bind oxygen.

Note: The study focused on inducing methemoglobinemia in rats and analyzing methemoglobin levels through various measurements and techniques.(16)

In vivo methemoglobin induction in a mouse model:

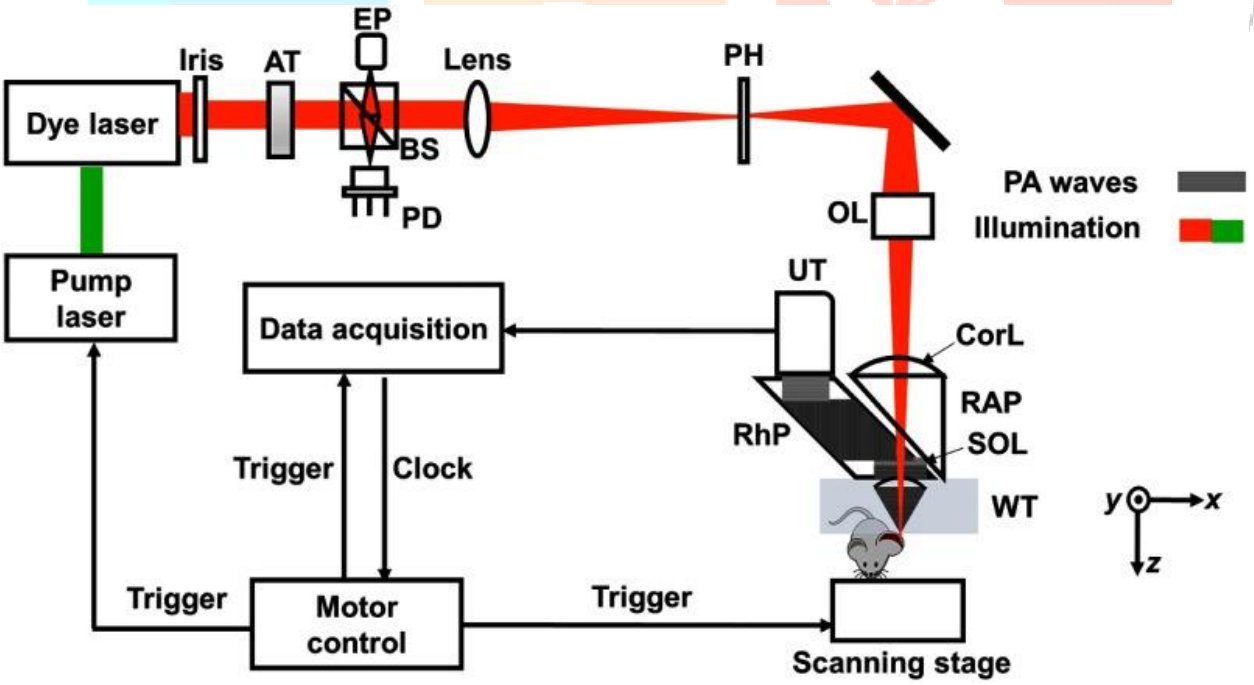
Procedure for inducing methemoglobin formation in a mouse model and performing imaging of the mouse ear blood vessels using the Photoacoustic Microscopy (PAM) system:

1. Preparation: Prepare a well-mixed sodium nitrite solution with a concentration of 10 mg of sodium nitrite per 1 ml of deionized water.

2. Injection: Anesthetize a mouse and access the jugular vein. Inject 60 µl of the prepared sodium nitrite solution into the mouse's jugular vein. The volume and concentration for the injection should be calculated based on the Kohn's model.

3. Imaging: Using a Photoacoustic Microscopy (PAM) system perform imaging of the blood vessels in the mouse's ear. This imaging should be conducted at multiple optical wavelengths.

In summary the procedure involves preparing a sodium nitrite solution then injecting it into the mouse's jugular vein based on a specific model and using a PAM system to image the blood vessels in the mouse's ear at different optical wavelengths. This process is designed to induce methemoglobin formation and visualize the effects on blood vessels.(17)



In Vitro Model:

Procedure of the in vivo reduction of encapsulated MetHb in a rat model:

1. Animal Preparation: The study involved 36 male Wistar rats with an average body weight of 334 grams. All experimental procedures were approved by the ethical committee of animal experiments at Nara Medical University.

2. Anesthesia: Rats were anesthetized using a vaporizer and an anesthetic gas scavenging system. They received inhalation of 1.0% isoflurane mixed with air through spontaneous breathing which was maintained for approximately 6 hours.

3. Arterial Catheterization: A polyethylene catheter containing heparinized saline was annulated into the left femoral artery. The heparinized saline was used to prevent blood clotting.

4. Hemoglobin Vesicle (HbV) Injection: HbV suspension which contained 50%metHb was injected into the rats at a dose rate of 10 ml/kg body weight at a flow rate of 1 ml/min. The HbV suspension had been pre-filtered using a 5 μm filter.

5. Methylene Blue Dye Injection: After a 5-minute interval, a dye solution with a concentration of 2.6 mM was injected at a dose rate of 1.53 ml dye/kg body weight. This dosage was determined to be effective for reducing metHb based on previous studies.

6. Blood Sampling: At various time points approximately 70 micro liters of blood were collected from the arterial catheter into heparinized capillaries. These blood samples were then centrifuged to separate the plasma phase.

7. MetHb Estimation: A 20-microliter sample of the plasma phase was collected and diluted with 4 ml of phosphate-buffered saline (PBS) in a cuvette. The cuvette was sealed with a rubber cap and purged with nitrogen gas for 10 minutes. The level of metHb was estimated using a spectroscopic method.

The study aimed to investigate the reduction of metHb in this rat model.[18]

Case study:

Severe methemoglobinemia caused by prilocaine: A rare case report

A 32-year-old woman went to the emergency room of hospital because she was having trouble breathing, her lips and hands turning blue and she felt dizzy, had a headache, increased or irregular heart rate, shivering, ringing in ears, and tingling in body. She had received injections of a painkiller called prilocaine for fibromyalgia pain about 10 hours before these symptoms appeared. When she arrived at the hospital her lips were still blue, blood pressure was 135/80, heart rate was 101 beats per minute, and she was breathing at a rate of 28 breaths per minute. Her blood tests showed normal results for most things but she had a high level of a substance called methemoglobin in her blood (45%). This can make it difficult for blood to carry oxygen. The doctors started an IV saline solution to hydrate and gave oxygen through a mask, but her lips remained blue. So, they admitted her to the intensive care unit of hospital. To treat the high methemoglobin levels, they gave a medication called methylene blue through an IV and then followed it with more IV saline. Within an hour her symptoms improved and lips and hands returned to their normal colour. She didn't have any problems with low blood pressure or irregular or increased heartbeats. Her blood tests showed that her methemoglobin levels went back to normal, and she was feeling better. After sometime she was released from the hospital in good health condition. She also gave permission for her case to be shared in this report.[19]

Severe Methemoglobinemia and Hemolytic Anemia from Aniline Purchased as 2C-E (4-ethyl-2,5-dimethoxyphenethylamine), a Recreational Drug, on the Internet — Oregon, 2011:

A 33-year-old man collapsed in a fast-food restaurant. He started feeling dizzy and nauseous about 15 minutes after drinking a soft drink with a friend. When he arrived at the hospital he looked bluish, had trouble thinking clearly, and his blood oxygen levels were very low even with oxygen support. His blood had a strange chocolate-brown colour. Tests showed that he had a very high level of methemoglobin (66.7%) in his blood. He also had normal levels of hemoglobin and platelets. The doctors referred a poison center and were advised to give him a methylene blue through an IV. He received multiple doses over a couple of days but his methemoglobin levels kept going up to very high levels. After a few days, his hemoglobin levels dropped significantly and he felt exhausted. Despite getting oxygen his blood oxygen levels remained low (70%-80%). He needed a blood transfusion and was transferred to a specialized intensive care unit of hospital. There he received more blood transfusions and a procedure called plasmapheresis. This process involved removing his blood, separating the plasma, and then putting the RBC back into his body. This helped to improve his condition. Lab tests also found a p-aminophenol in his urine, which is a byproduct of aniline. After about 12 days in the hospital the man was discharged in better health condition. This case showed the serious effects of exposure to harmful substances.[20]

Methemoglobinemia-Induced Acute Kidney Injury:

A 76-year-old man who had a history of lung cancer and had been a heavy smoker was given a medication called benzocaine during a medical procedure. After the treatment, he developed a bluish colour on his lips and fingers. He also had blood in his urine and kidney problems. His conditions got worsen and his kidneys started to fail, so he needed dialysis to clean his blood. They tried giving him a methylprednisolone thinking he had a type of kidney disease but it didn't help. They did a kidney biopsy and found that his kidney damage was due to his kidneys being clogged with blood clots. This occurred because of the benzocaine was given during the procedure. The kidney damage was so severe that he needed dialysis for a long time. After four months he still needed dialysis and his kidneys hadn't improved significantly. Another kidney biopsy confirmed the severe damage caused by the benzocaine, which led to blood clot formation in his kidneys. This damage made his kidneys fail and he had to continue with dialysis.[21]

Methemoglobinemia and hemolytic anemia after COVID-19 infection without identifiable eliciting drug:

A 35-year-old man presented to the emergency room with complaints of severe stomach pain, nausea, fevers, and diarrhea. He disagreed any respiratory symptoms at the time. Upon further investigation, laboratory tests revealed liver enzyme abnormalities, a low white blood cell count and elevated c-reactive protein levels. Imaging studies including a chest x-ray and CT scan showed evidence of lung pathology. A subsequent test for COVID-19 returned positive. The patient was initially treated with antibiotics; however, he subsequently developed respiratory distress and hypoxemia. Further evaluation discovered methemoglobinemia, a low red blood cell count and kidney dysfunction. He was promptly transferred to the intensive care unit and initiated on ascorbic acid therapy. Additional laboratory results demonstrated elevated ferritin levels and low folic acid. After several days of intensive care management, the patient's condition improved and he was eventually moved back to the general medical ward. Subsequent follow-up appointments indicated a positive trend in his laboratory results. After five months, a confirmatory test revealed that the patient had a genetic condition known as G6PDdeficiency. This case highlights the complexity of diagnosing and managing patients with multiple coexisting medical conditions. The presence of G6PD deficiency may have contributed to the patient's atypical clinical presentation and complicated his course of illness. It underscores the importance of considering rare genetic disorders in the differential diagnosis, particularly in cases with unusual or unexpected clinical features.[22]

Reference:

1. Kiese M. Methemoglobinemia: a comprehensive treatise.
2. David SR, Sawal NS, Hamzah MN, Rajabalaya R. The blood blues: a review on methemoglobinemia. Journal of Pharmacology and Pharmacotherapeutics. 2018 Mar;9(1):1-5.
3. Skold A, Cosco DL, Klein R. Methemoglobinemia: pathogenesis, diagnosis, and management. South Med J. 2011 Nov 1;104(11):757-61.
4. Kaymakcı A, Güven Ş, Arpacık M, Şahin C, Esen HK, Yıldız T. The evaluation of the patients with methemoglobinemia due to prilocaine. HaydarpasaNumune Med J. 2019;59(4):351-54.
5. Sonck E, Bourmanne E, Bruteyn J, Dolip W. Methemoglobinemia due to use of poppers: a case report. Journal of Medical Case Reports. 2022 Dec;16(1):1-4.
6. Bahabri A, Moradi J, Choong K, Pai N, Bhatt M. Food protein induced enterocolitis syndrome presenting with life-threatening methemoglobinemia: a case report and review of the literature. International Journal of Clinical Pediatrics. 2020 Jun 18;9(2):35-40.
7. Ashurst J, Wasson M. Methemoglobinemia: a systematic review of the pathophysiology, detection, and treatment. Delaware medical journal. 2011 Jul 1;83(7):203-8.
8. Barash M, Reich KA, Rademaker D. Lidocaine-induced methemoglobinemia: a clinical reminder. Journal of Osteopathic Medicine. 2015 Feb 1;115(2):94-8.
9. Abhilash KP. Methemoglobinemia: When to suspect and how to treat. Current Medical Issues. 2019 Oct 1;17(4):125-8.
10. Alanazi MQ. Drugs may be induced methemoglobinemia. J HematolThrombo Dis. 2017;5(3):1-5.
11. Iolascon A, Bianchi P, Andolfo I, Russo R, Barcellini W, Fermo E, Toldi G, Ghirardello S, Rees D, Van Wijk R, Kattamis A. Recommendations for diagnosis and treatment of methemoglobinemia. American journal of hematology. 2021 Dec;96(12):1666-78.
12. Ludlow JT, Wilkerson RG, Nappe TM. Methemoglobinemia.
13. Keats KR, Robinson R, Patel M, Wallace A, Albrecht S. Ascorbic Acid for Methemoglobinemia Treatment: A Case Report and Literature Review. Journal of Pharmacy Practice. 2023 Jul 8:08971900231188834.
14. Rehman HU. Evidence-based case review: methemoglobinemia. Western Journal of Medicine. 2001 Sep;175(3):193.
15. Tromberg BJ, Lee J, El-Abaddi N, Duke A, Cerussi AE, Brenner M. Noninvasive in vivo monitoring of methemoglobin. J Appl Physiol. 2006;100:615-22.
16. Stossel TP, Jennings RB. Failure of methylene blue to produce methemoglobinemia in vivo. American journal of clinical pathology. 1966 May 1;45(5):600-4.
17. Tang M, Zhou Y, Zhang R, Wang LV. Noninvasive photoacoustic microscopy of methemoglobin in vivo. Journal of Biomedical Optics. 2015 Mar 1;20(3):036007-.
18. Ghirmai S, Bülow L, Sakai H. In vivo evaluation of electron mediators for the reduction of methemoglobin encapsulated in liposomes using electron energies produced by red blood cell glycolysis. Artificial Cells, Nanomedicine, and Biotechnology. 2018 Oct 3;46(7):1364-72.
19. Karabulut A, Sahin M. Severe methemoglobinemia caused by prilocaine: A rare case report. Int J Med Biochem. 2021 Sep 1;4(3):205-7.
20. Kusin S, Tesar J, Hatten B, Horowitz BZ, Hendrickson R, Leman R, Buser G. Severe methemoglobinemia and hemolytic anemia from aniline purchased as 2C-E (4-ethyl-2, 5-dimethoxyphenethylamine), a recreational drug, on the Internet-Oregon, 2011. Morbidity and Mortality Weekly Report. 2012 Feb 10;61(5):85-8.
21. Qureshi AH, Soberon DJ, Asif A, Vachharajani T, Nayer A. Methemoglobinemia-induced acute kidney injury. The open urology & nephrology journal. 2013 Apr 8;6(1).

22. Lopes DV, Neto FL, Marques LC, Lima RB, Brandão AA. Methemoglobinemia and hemolytic anemia after COVID-19 infection without identifiable eliciting drug: a case-report. IDCases. 2021 Jan 1;23:e01013.