Case report

Methaemoglobinaemia: Mystery of the chocolate-brown blood

We present a 23-year-old man with a history of advanced multi-bacillary leprosy treated with dapsone, clofazimine, rifampicin and isoniazid.

The patient presented unconscious to hospital after an overdose of the abovementioned medicines, and required intubation and ventilation. On admission the full blood count and liver and renal function tests were normal, and remained so during the course of the admission. The patient's blood was darkly coloured.

He was extubated after 8 hours of ventilation. Subsequent medical examination revealed a distressed patient (sinus tachycardia 144 beats per minute, respiratory rate 24 breaths per minute, blood pressure 119/62 mmHg) with signs of central cyanosis, and persistently low pulse oximetry readings fluctuating between 80% and 90% despite the patient being on a rebreather oxygen mask. Arterial blood gas (ABG) oxygen saturation was 100%.

In summary, the patient presented with a poly-drug overdose and:

• central cyanosis and low pulse oximetry readings
• concurrent ABG oxygen saturation readings of 100%
• chocolate-coloured arterial blood
• normal liver and renal function and blood count.

A diagnosis of methaemoglobinaemia was proposed. A further arterial blood sample analysed by co-oximetry (which uses a more sophisticated methodology than pulse oximetry and is capable of distinguishing the various forms of haemoglobin, including methaemoglobin) confirmed methaemoglobinaemia of 47%. Methylene blue, the intravenous electron transfer dye used to reduce methaemoglobin, was administered.

The most probable agents responsible for the patient’s methaemoglobinaemia were dapsone and clofazimine.

The first dose of 100 mg methylene blue was given intravenously over 5 minutes. Before the methylene blue administration, the basal pulse oximetry reading was 80%. During administration, the reading fell dramatically to 60% before increasing to 80% over 5 minutes (Table I, Fig. 1).

Six hours later the pulse oximetry reading had risen to 87%, and a second dose of 100 mg intravenous methylene blue was given, which gradually raised pulse oximetry readings to 90% after again initially transiently decreasing them.

The next day repeat arterial blood co-oximetry revealed methaemoglobinaemia of 23%. A third dose of methylene blue was administered and, in addition, the patient was commenced on 50 g of oral activated charcoal 8-hourly to disrupt the enterohepatic circulation of dapsone.

Repeat arterial blood co-oximetry 72 hours after admission revealed a methaemoglobin level of 17%. The patient was then administered his fourth and final dose of methylene blue.

On day 6 he was non-distressed, with pulse oximetry readings of 97% on room air and 100% on regular face-mask oxygen. The patient did not develop further complications and was subsequently referred for controlled re-introduction of his anti-leprosy therapy and psychiatric evaluation.

Discussion

Methaemoglobin refers to haemoglobin of which the haem iron has been oxidised from the normal Fe\(^{2+}\) ferrous haem state to the Fe\(^{3+}\) ferric haem state. In the methaemoglobin state, the Fe\(^{3+}\) ferric haems cannot bind oxygen, impairing the capacity of blood to transport oxygen to the tissues. In addition, the remaining Fe\(^{2+}\) ferrous haems within the haemoglobin tetramer containing Fe\(^{3+}\) ferric haems paradoxically exhibit increased oxygen affinity, causing the oxygen dissociation curve to be shifted ‘to the left’, which further impairs oxygen delivery to the tissues (Fig. 2).

Table I. The patient’s percentage O\(_2\) saturation as determined by pulse oximetry and by extrapolation from arterial blood PO\(_2\) during the course of his admission (note the disparity between percentage saturation obtained by the two methods)

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<thead>
<tr>
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<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
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<tbody>
<tr>
<td>PO(_2) kPa</td>
<td>30.8 (O(_2) mask)</td>
<td>28.4 (O(_2) mask)</td>
<td>33.6 (O(_2) mask)</td>
<td>11.6</td>
</tr>
<tr>
<td>% O(_2) saturation by pulse oximetry</td>
<td>80</td>
<td>80</td>
<td>93</td>
<td>&gt;93</td>
</tr>
<tr>
<td>% O(_2) saturation by extrapolation from PO(_2)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>97</td>
</tr>
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</table>

Fig. 1. Relationship between the patient’s methaemoglobinaemia determined by co-oximetry, his methylene blue doses, and his percentage saturation determined by pulse oximetry and by extrapolation from PO\(_2\) readings on the arterial blood gas analyser during the course of the hospital admission.
Methaemoglobin is produced physiologically, albeit at a low rate, when the electron originally donated by Fe^{2+} to oxygen during oxygen carriage is not returned to the haem moiety upon deoxygenation. This abnormal deoxygenation (Table II) causes an abnormal split of the ferric superoxide complex (Fe^{3+} + O_2) and instead of reforming fresh Fe^{2+} ferrous haems, results in the formation of Fe^{3+} ferric haems (methaemoglobin) and O_2 superoxide radicals. Approximately 0.5 - 3.0% of total haemoglobin in the physiological state is converted to methaemoglobin via this mechanism daily.

The accumulation of methaemoglobin formed physiologically is however prevented by enzymatic reduction back to ferro-haemoglobin. The most clinically important of these reduction pathways are:

- the NADH-dependent cytochrome-b_5 reductase pathway (active under physiological conditions)
- the NADPH-dependent methaemoglobin reductase pathway (negligibly active under physiological conditions).

The cytochrome-b_5 reductase pathway (Fig. 3) is the most important physiological methaemoglobin reduction pathway and is an NADH-dependent enzymatic process. It maintains a very low steady-state level of methaemoglobin by matching methaemoglobin formation by its immediate reduction.

In contrast, the NADPH-methaemoglobin reductase pathway (Fig. 4) does not function physiologically as a methaemoglobin-reduction pathway as it needs an exogenous electron carrier to be present in the red cell for activation. This can be exploited by the use of an exogenous electron carrier substance, such as the redox dye methylene blue. By acting as an electron shuttle, methylene blue thus allows activation of an alternative methaemoglobin-reducing pathway. In pathological states of methaemoglobinaemia, this extra methaemoglobin-reducing pathway acts synergistically with the inherently active cytochrome-b_5 reductase pathway to allow faster reduction of methaemoglobin. A summary of the two pathways is illustrated in Fig. 5.

### Table II. Physiological formation of methaemoglobin occurs in 0.5 - 3.0% of total haemoglobin daily

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#### Fig. 2. Oxygen dissociation curve in methaemoglobinaemia: shifted to the left.

#### Fig. 3. The physiologically active methaemoglobin-reduction pathway: the cytochrome-b_5 reductase pathway (GPD – glyceraldehyde-3-phosphate dehydrogenase; Cyt. b_5R – cytochrome-b_5 reductase).

#### Fig. 4. The NADPH-methaemoglobin reductase (NMR) pathway: inactive in the absence of an exogenous electron carrier such as methylene blue (MB).

#### Table III. Chemical reactions after methylene blue administration (*pentose phosphate pathway).

1. Initially: \( \text{Fe}^{3+} + \text{MB}_{\text{oxidized}} \rightarrow \text{Fe}^{2+} + \text{MB}_{\text{reduced}} \)
2. With activation of pathway*: \( \text{NADPH} + \text{MB}_{\text{oxidized}} \rightarrow \text{NADP} + \text{MB}_{\text{reduced}} \)
3. Final MB reaction: \( \text{Fe}^{3+} + \text{MB}_{\text{reduced}} \rightarrow \text{Fe}^{2+} + \text{MB}_{\text{oxidized}} \)
The NADPH-methaemoglobin reductase pathway, however, takes time to become activated by methylene blue. This accounts for the acute phenomenon of a transient increase in methaemoglobin followed by a gradual decrease to below basal levels after the administration of a methylene blue bolus (Fig. 6).

On entering the bloodstream, methylene blue (administered in the oxidised form) seeks an electron donor. As the NADPH pathway described above takes time to be activated, the initial source of electrons for methylene blue becomes the remaining normal Fe²⁺ ferrous haemoglobin pool which is then paradoxically oxidised to methaemoglobin (Table III), accounting for the transient worsening of methaemoglobinaemia by methylene blue as in the abovementioned case. However, with time the red blood corpuscle’s pentose-phosphate-shunt pathway generates NADPH from NADP⁺. NADPH can then serve as an electron donor for methylene blue, reversing the direction of electron flow so that there is ongoing reduction of methaemoglobin back to the Fe²⁺ ferrous state and decreasing its concentration to below the initial level (Fig. 6, Table III). In essence, methylene blue is acting as an electron shuttle between NADPH and methaemoglobin.

Methaemoglobinaemias are either hereditary or acquired, and arise from increased production or decreased removal of methaemoglobin.

Types of methaemoglobinaemia
A. Hereditary methaemoglobinaemias
- Cytochrome-b₅ reductase deficiency
  - Type 1
  - Type 2

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Fig. 5. Summary of the two clinically relevant methaemoglobin-reduction pathways indicating the flow of electrons (G-6-PD – glucose-6-phosphate dehydrogenase; 1,3 BPG – 1,3-bisphosphoglycerate).

Fig. 6. Graph representing the change in methaemoglobin with time after bolus administration of methylene blue.
Hereditary methaemoglobinaemias include the use of by ingestion of exogenous substances and cause methaemoglobinaemia if there are ‘suspicious’ features (Table V)

### Table IV. Common causes of acquired methaemoglobinaemia

<table>
<thead>
<tr>
<th>Substance</th>
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<tbody>
<tr>
<td>Acetanilid</td>
<td>Naphthoquinone</td>
</tr>
<tr>
<td>p-amino salicylic acid</td>
<td>Naphthalene</td>
</tr>
<tr>
<td>Aniline dyes</td>
<td>Nitrites</td>
</tr>
<tr>
<td>Benzene derivatives</td>
<td>Nitroglycerin</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Chlorrates</td>
<td>Nitrobenzene</td>
</tr>
<tr>
<td>Dapsone</td>
<td>Parquet</td>
</tr>
<tr>
<td>Local anaesthetic agents</td>
<td>Phenacetin</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>Phenazopyridine</td>
</tr>
<tr>
<td>Methylene blue (paradoxically can transiently worsen methaemoglobinaemia)</td>
<td>Sulfonamides</td>
</tr>
</tbody>
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An overdose of dapsone causes acute acquired methaemoglobinaemia and chronic use leads to low-grade methaemoglobinaemia. Cimetidine has been described as effective treatment in preventing and lowering chronic methaemoglobinaemia associated with long-term dapsone use, but is not useful in an acute attack.

### Table V. Features of acquired methaemoglobinaemia

- **Clinical cyanosis with normal ABG oxygen saturation**
- **Dark, chocolate-brown blood that does not change colour with the addition of oxygen (differentiating it from deoxyhaemoglobin)**
- The presence of a ‘saturation gap’ between oxygen saturation determined by pulse oximetry compared with ABG analysis. This is because the standard pulse oximeter cannot differentiate between methaemoglobin and deoxyhaemoglobin based on their absorption spectrum, whereas ABG O₂ saturation is extrapolated from the arterial PO₂, which is unaffected by the presence of methaemoglobinaemia.

An overdose of dapsone causes acute acquired methaemoglobinaemia and chronic use leads to low-grade methaemoglobinaemia. Cimetidine has been described as effective treatment in preventing and lowering chronic methaemoglobinaemia associated with long-term dapsone use, but is not useful in an acute attack.

**Symptoms are directly related to the degree of methaemoglobinaemia and therefore the degree of ‘functional anaemia’ and tissue hypoxia, as described above.** Low levels of methaemoglobinaemia (<20%) are known to cause headache, fatigue and dyspnoea. Higher levels (>20%) can be life threatening, with increasing levels inducing respiratory depression, coma, seizures and ultimately death. Levels >50% are not compatible with life, therefore requiring urgent treatment of the patient.

Clinically acquired methaemoglobinaemia should be suspected when the features listed in Table V are present.

### Treatment and management

Intravenous methylene blue is the treatment of choice for symptomatic acquired methaemoglobinaemia. Methylene blue 1 - 2 mg/kg is administered slowly over 5 minutes. Response is usually rapid; repeat dosages may be required for severe methaemoglobinaemia, as in the case of our patient. As mentioned above, methylene blue can transiently worsen methaemoglobinaemia before effecting its reduction, this phenomenon being more commonly observed with large bolus doses (especially >7 mg/kg).

#### The treatment algorithm for acquired methaemoglobinaemia is as follows:

1. **suspect acquired methaemoglobinaemia**
2. **immediately discontinue use of suspected offending agent**
3. **determine the concentration of methaemoglobin (via ABG co-oximetry)**

**A.** if <20% and asymptomatic

- no therapy needed, monitor methaemoglobin levels

**B.** if >20% or symptomatic

- requires methylene blue administration

- levels >40% are severely life threatening and need to be treated in the high-care or intensive care setting. Requires serial methaemoglobin measurement to monitor response to therapy and to assess need for repeat methylene blue administration.

### Further reading


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