Biochemistry of Blood

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Overview

- Blood as an important diagnostic material
- Transport of blood gases
- Metabolism of RBC

- Iron metabolism
- Haematopoesis from the biochemical point of view
- Anemias

Blood is...

- …easily available material useful for a huge of various assays and measurements
- ... plazma and cells.

Gas transport

- Oxygen is a major e^- acceptor – indispensable for ATP production.
- CO_2 (and water as well) is a major byproduct of energy metabolism
- Gas transport is continuous interchange of CO_2 and O_2 between lungs and tissues.

Oxygen release helps to maintain pH in tissues

- Lungs: $HHb + O2 = HbO2 + H^+$
- CO_2 is formed from plasmatic bicarbonate and proton released from Hb
- Tissues: CO_2 forms proton and bicarbonate:
  - Proton is bound to Hb, when O_2 is released
  - Bicarbonate leaves RBC
- Carboanhydrase plays a key role…
- Cl- / HCO3- interchange is Hamburger effect

Hemoglobin

- 4 peptide subunits (2α + 2β),
  4 molecules of hem (Fe ++)
- Each subunit in R or T state
- Hb disociation curve is % sat. Hb dependency on pO_2
- 1g of 100% sat. Hb contains 1.39 ml O_2
- 1g of 75% sat. Hb contains 1.00 ml O_2

Further forms of Hb

- HbA (2α 2β): 90% of Hb in adult
- HbA_2 (2α2σ): 2-3% of Hb in adult
- HbA_{IC}: glycated Hb – important marker of long-term diabetes compensation
- HbF (2α2γ) - fetal Hb, high affinity to O_2
- Hemoglobinopathies: rare monogenic diseases (HbS –anemia).

Hemoglobine derivates unable to transport CO_2

- Methemoglobine: contains Fe 3+ instead of Fe 2+ (e.g. nitrate/nitrite containing food or water)
- Carboxyhemoglobine – CO poisoning, smokers (cherry red colour)
- Sulfhemoglobine – green

Factors with influence on Hb affinity to O_2

- *Right shift* means higher ability of Hb to release O_2 , but lower ability to bind it.
- Is useful in tissues (site of O_2 release):
  - higher temperature
  - lower pH (Bohr effect)
  - higher 2,3 BPG level

2,3-Bisphosphoglycerate

- Is very important for long-term regulation of Hb affinity to O_2
- 2,3 BPG shunt is a pathway derived from glycolysis.
- Competition with oxygen for binding site on ß-subunits
- Hypoxy stimulates 2,3 BPG synthesis, i.e. improve O_2 release.

There are 3 ways of CO_2 transport…

1. Bicarbonate formation within RBC (carboanhydrase) and Cl interchange…
2. CO_2 dissolved in blood plasma
3. Carbaminohemoglobine formation (reaction with amino groups of globine)

Clinical interpretation of Astrup assay

- Arterial (or capillary) blood sample
- Measurements of *pH (7.35 – 7.45), pO2 = 9.9 – 13.6 kPa , pCO2 = 4.5 – 6.0 kPa and calculation of further ABB parameters…*
- Pulse oxymetry is noninvasive monitoring of Hb saturation.

Metabolic specialities of red blood cell

- No organellae – no mitochondria
- Anaerobic glycolysis (lactate formation) is the only one source of ATP!
- 2,3 BPG shunt is unique for RBC
- 20% of glucose is metabolised via pentosa phosphate pathway

Defense against oxygen radicals

- High tension of oxygen…
- GSH as a defense against harmful oxygen radicals
- Inactivation of O• is coupled with GSH oxidation, back reduction need NADPH

  NADPH + GSSG = NADP + GSH
- Pentose phosphate pathway is a source of NADPH
- Glc-6-P deficiency – haemolytic anemia

Coffee break

Iron metabolism

- **Iron is indipensable for life**

  (either in heme or non-heme form essential for oxygen transport, electron transfer, DNA synthesis, etc.)

- **Iron is insoluble**

  ([Fe] cannot exceed 10^{-17})

- **Iron is potentially toxic**

  (unless appropriately chelated, Fe plays a key role in the formation of oxygen radicals)

Iron storage - ferritin

- Protein, 24 subunits, up to 4 500 Fe atoms per ferritin molecule
- Ferritin is important for intracellular iron storage
- **Ferritin synthesis is stimulated by higher iron stores…**

Transferrin (Tf) transports Fe in plasma

- Glycoprotein with 2 high affinity binding sites for Fe^{3+}
- Tf transports Fe between sites of absorption, storage and utilization
- Cells (esp. Erythroid precursors) strip Fe from Tf by expressing Tf-R
- **Tf synthesis is stimulated by lack of Fe in the body.**

When iron stores are sufficient…

- Ferritin expression in the enterocyte is stimulated. More Fe is then waist with stool.
- Transferrin synthesis is supressed, plasmatic Tf level is low, Tf is highly saturated…
- Only a small part of ingested iron is absorbed.

When iron is needed…

- Ferritin expression in the enterocyte is supressed, only a small part of ingested iron is lost with stool.
- Transferrin synthesis is accelerated, plasmatic Tf level is high and Tf is unsaturated…
- However, iron is absorbed with high efficacy.

It is interesting, that…

- …iron regulates ferritin and Tf –R synthesis at the level of translation (and not transcription)
- IRE of mRNA binds IRP in the presence of Fe and:
  - Activates ferritin translation
  - Block Tf-R translation

Heme synhesis

- 80% of body Fe is used for heme synthesis in developing erythroid cells
- The 1. step is ALA formation from Gly + sucCoA (ALA-S1 –regulatory in liver)
- The 8. step is heme synthesis from proto-IX, (ferrochelatase – regulatory in erythroid cells in the presence of ALA-S2)
- ALA-S2 mRNA contains IRE

Iron overload

- There is no physiological mechanism for the excretion of excess iron!
- Causes:
  - Hemochromatosis: congenital enhancement of iron absorbtion
  - Hemosiderosis: acquired, e.g. regular blood transfusion (aplastic anemias)
- Symptoms (over 28g Fe): diabetes, cirrhosis, hypoadrenalism, slow growth in childhood

Lack of iron causes anemia and microcytosis

- Causes: chronic bleeding (GIT, menstr.), malignancy, extreme diet
- Symptomatology :
  - low hemoglobine level
  - red blood cell count normal or high
  - RBC are small (vol. < 80 fl)

„WHY OUR BLOOD IS RED...“

- Iron stores in the body are regulated only at the level of iron absorbtion…
- Transferrin and ferritin play a key role in iron intake and delivery for tissues…
- Iron overload cause hemosiderosis, lack of iron is the main cause of microcytic anaemia.

Questions...?