What’s new about hemolytic jaundice and iron salvage?

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It’s really all about iron

Objectives

At the end of the session, the participant will be able to:

1. describe hemoglobin catabolism and iron salvage under normal circumstances.
2. describe hemoglobin catabolism and iron salvage during excessive macrophage-mediated hemolysis
3. describe hemoglobin catabolism and iron salvage during excessive fragmentation hemolysis.
Agenda

• Normal red cell death
  – Fragmentation and macrophage-mediated
  – Iron salvage pathways
  – Bilirubin processing
• Hemolysis
  – Causes of fragmentation and macrophage-mediated hemolysis
  – Impacts on iron salvage pathways
  – Bilirubin processing

“We are stardust…”
Joni Mitchell, Woodstock

Because it is all about the iron
The production of bilirubin is tied to the fate of iron in the body

Since most body iron is tied up in RBC heme, the production of bilirubin from protoporphyrin is tied to what is happening to red cells and their hemoglobin

When RBCs die normally after 120 days, most are ingested by macrophages in the spleen and lysed

Iron is tenaciously conserved by the body
Every time an iron molecule is salvaged, a molecule of bilirubin is formed

What can be done with this waste?

Bilirubin is the breakdown product of protoporphyrin (PP)

Diagrams adapted from Rodak’s Hematology: Clinical Principles and Applications, 5e
Good question! Still uncertain.


http://crohnsdiaries.org/2012/12/14/nightmare-before-christmas/remicade-bruise/
Unconjugated Bilirubin  AKA Indirect Bilirubin

Fat soluble so cannot react readily in our test system until it is solubilized like with alcohol (which then includes the direct too)

Water soluble, so it reacts “directly” in our aqueous sulfanilic acid test system

Conjugated Bilirubin  AKA Direct Bilirubin

Total Bili = Direct Bili + Indirect bil

Measured  Measured  Calculated

The “In” is the “Un” is the “Non”

The indirect  Is the unconjugated
Is the non-water soluble

Mnemonic for remembering which is which –
Thank you Jayne Zuhlke, MS, MT(ASCP)
Not all RBCs die by being ingested by macrophages

Some just fragment in the Circulation. So how is their iron going to be salvaged?

When RBCs fragment in the blood stream, the contents are dumped into the plasma. Hemoglobin iron gets oxidized = methemoglobin which can be lost by filtration into the urine.

\[ \text{Hb} \xrightarrow{\text{(met)}} (\text{metHb}) + \text{Haptoglobin (Hpt)} = \text{Hb+Hpt} \]

Haptoglobin prevents the loss of free hemoglobin in urine. Capacity is adequate for the amount of plasma Hb produced daily from normal fragmentation of RBCs

Would result in lost of 5-7% of body iron every day
Although composed as a tetramer of two alpha chains and two beta chains, the Hb molecule functions as two alpha-beta heterodimers.

Hpt binds an alpha-beta dimer much the same way the native Hb dimer does.

Etymology: Gk, haptein, to grasp; L, globus, ball.

**Although haptoglobin saves the hemoglobin from urinary excretion, the iron is still not salvaged until it gets into one of the professional iron regulating cells – macrophages and hepatocytes.**

Remember – in the end it is all about iron.
(met)Hb + Haptoglobin (Hpt) = Hb+Hpt

The Hb-Hpt complex binds to CD163 on the surface of macrophages and the complex is internalized.

When Hpt binds to CD163, Hb affinity decreases so Hb can release

Hpt degraded

Inside the lysosome, the Hb is separated from Hpt and degraded as macrophages do

So every day, the liver produces enough Hpt to replace what was catabolized the prior day while salvaging the iron from hemoglobin dumped into the plasma during a normal amount of fragmentation hemolysis

Remember – it’s all about the iron
BUT...there is a back-up plan just in case the amount of Hb in plasma exceeds the capacity of Hpt

Here comes Hemopexin!

Heme can dissociate from hemoglobin in plasma

Without the protection of globin chains, the ferric iron of metheme is easily able to oxidize other compounds causing damage to blood vessels and tissues

Hemopexin to the rescue!
Hemopexin is able to bind heme with a higher affinity than any known protein. The heme is in a hydrophobic pocket like in Hb which protects tissues from the oxidizing damage of the heme.

Hepatocytes possess CD91, the receptor for Heme-Hpx complex.

Hepatocytes are able to dismantle heme just like macrophages; iron is saved and bilirubin is formed and conjugated.

Hemopexin gets returned to the plasma.


And just in case there is more metheme than hemopexin can bind, albumin is a temporary back-up.

Prevents loss of heme into the urine
BUT the iron is still not recycled into macrophages or hepatocytes.

Metheme will readily dissociate from albumin and complex with hemopexin when available, so then the iron is carried into a hepatocyte for salvage...because it is all about the iron.
How to remember:

- Hapto-GLOBIN binds hemo-GLOBIN
- HEMO-pexin bind HEME

SUMMARY of NORMAL BILIRUBIN PRODUCTION AND PROCESSING

Bilirubin is formed in hepatocytes from Hpx-salvaged metheme

Bilirubin is formed in macrophages from Hb from ingested RBCs or from Hpt-salvaged plasma Hb

But it is really about iron salvage!
What happens when hemolysis is so brisk that there is more hemoglobin (actually the iron) that needs to be salvaged than usual?

When macrophages ingest more than the usual number of RBCs daily, more unconjugated bilirubin gets produced.

A healthy liver can process the added load, so more than the usual amount of direct bili is excreted into the intestine and more than the usual amount of urobilinogen appears in the stool.

More than the usual amount of Uro will be reabsorbed and excreted in the urine.
When more than a normal number of RBCs lyse in the circulation each day, there is more than a usual amount of bilirubin formed in macrophages and processed through the liver.

More than the usual amount of urobilinogen is formed and reabsorbed and excreted in the urine.

Elevation of plasma bilirubin = hemolytic jaundice

Darker than normal stool

Fragmentation or Intravascular Hemolysis

With direct absorption into hepatocytes, no contribution to jaundice

Darker than normal stool

Unlike normal conditions, most of the Hpx will be degraded and little will return to the plasma, so plasma levels fall.
But what if the amount of hemoglobin/heme in the plasma exceeds all these salvage systems?

Methemoglobin/metheme will filter into the urine so the iron may be lost...

Ach, still with the iron!

All is still not lost...

Bring on the Proximal Tubule Cell!
Hb in the urinary filtrate can bind to cubilin, a protein receptor on the luminal side of proximal tubular cells.

Binding is non-specific and competitive; when Hb is in high concentration, more will be re-absorbed.

Proximal cells can dismantle Hb, salvage iron and produce/secrete bilirubin.

NOW – if all the salvage systems are at capacity, any hemoglobin in the urine that is not reabsorbed WILL be lost in the urine with its IRON.
What good is it for proximal tubular cells to salvage hemoglobin? They aren’t major iron-trafficking cells like macrophages and hepatocytes. The iron could just sit in the tubular cell and go no where.

As it turns out...proximal cells CAN return salvaged iron to the plasma

Their basal membrane contains the same iron transporter that enterocytes use to transport iron from the diet and into the blood...ferroportin (Fp); the same protein macrophages and hepatocytes use to recycle iron.

Iron that is not returned to the plasma can be converted to ferritin and if not used, will be converted to hemosiderin; detectable in proximal cells in the sediment during fragmentation hemolysis; stain with Prussian blue

Summary

Fragmentation or Intravascular Hemolysis

Beer colored plasma and urine
Jaundice and icteric plasma
Loss of iron, but not as great as it would be without Hpt, Hx, Cb and Fp

Summary

Macrophage-mediated or extravascular hemolysis

Jaundice and icteric plasma
No concerns for iron loss since the cells are dying inside macrophages
The last word...

The bilirubin story is really about iron salvage; saving iron means generating protoporphyrin waste = bilirubin; measurement of bilirubin and its metabolite, urobilinogen is diagnostically useful

Questions?

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