Bacterial Growth and Non-Transferrin Bound Iron: An In-Vitro Investigation of the Potential Impact of Transfusing Old Red Blood Cell Units

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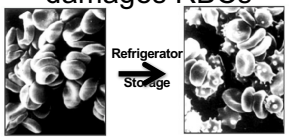
BACKGROUND

Bacteria depend on iron to grow. Part of our human defense against infection involves sequestering iron so that bacteria cannot use this nutrient to grow. When we transfuse patients with old RBC transfusions (stored for >35 days), we introduce red blood cells that have been damaged through storage. This process is referred to as the "Storage Lesion." When these damaged red blood cells are cleared, they introduce a surplus of iron into the body. This excess iron, too abundant to be carried by transferrin, circulates as free iron or non-transferrin bound iron (NTBI) and promotes bacterial growth.

The RBC storage lesion damages RBCs



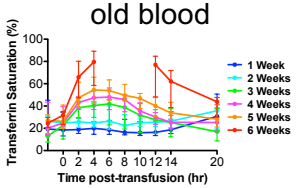
METHODS

- We designed a microtiter plate with 1 x 10^6 CFU luminescent bacteria in serum spiked with increasing transferrin saturations, and NTBI.
- We measured bacterial absorbance and luminescence each hour as a proxy for bacterial planktonic growth.
- After 24 hours, we measured absorbance and luminescence of the biofilm that formed in the plate.
- We did this experiment using healthy volunteer serum spiked with FeCitrate.
- We then did this experiment using serum from a volunteer after a 28-day old transfusion and from a volunteer after a 35-day transfusion.
- We conducted this experiment with Pseudomonas Aeruginosa; Staph Aureus; Staph Epi; and Ecoli.
- We graphed each set of experiments using Prism software.

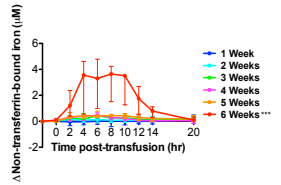
RESULTS

- After transfusing healthy volunteers with blood that was stored for six weeks (42 days old), we monitored serum NTBI levels for 20 hours. For volunteers who received blood stored for less than 5 weeks, NTBI was not detectable. For volunteers transfused with blood stored for more than five weeks, transferrin was saturated and NTBI levels reached 4 µM and remained detectable for at least 6 hours.

Transferrin is saturated after transfusion of 6-week old blood



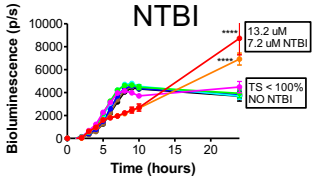
6-week old blood produces non-transferrin-bound iron



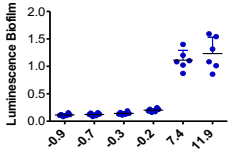
Growth Directly Correlated with NTBI

- When we recreated 6 week old transfused serum in vitro, NTBI had a significant impact on planktonic growth and biofilm growth in all bacterial strains.
- NTBI was directly correlated with increased planktonic proliferation of Pseudomonas Aeruginosa (r =0.93, P <.05) and Staph Aureus (r = 0.89, P <.05).

PA Bioluminescent Planktonic Growth with NTBI



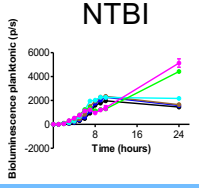
PA Biofilm Luminescence after 24 hours



CONCLUSIONS

Because bacterial growth increases in vitro in serum with increasing concentrations of NTBI, there is reason to reconsider the practice of transfusing blood that has been stored for over 35 days. Circulating NTBI has also been shown to influence thrombosis, cytotoxicity, oxidative damage, and other types of injury. Our findings suggest that though within FDA guidelines, older transfusions may increase infectious risk.

Staph. A Bioluminescent Planktonic Growth with NTBI



Staph. A Biofilm Luminescence after 24 hours