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Leukoreduction of Red Blood Cell Concentrates for Transfusion

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Ex vivo storage imposes an important stress to red blood cells that leads to early evidences of aging. The simultaneous presence of leukocytes in the transfusion bag contributes to the development of the storage lesions through the production of inflammatory mediators including cytokines and oxygen and nitrogen derived oxidants. Meanwhile, leukoreduction has proven to delay this deleterious process. Blood from voluntary donors was collected and fractionated into two samples, leuko-reduced and non-leuko-reduced. Different parameters of aging and stress were analyzed each week until the end of storage at 42 days. The integrity of the red blood cells membrane was investigated by measuring the presence of hemoglobin in the extracellular milieu. The hemoglobin concentration increased more than 30 times in the supernatant after 42 days of storage, while at that time in the leuko-reduced sample the concentration was almost 50% less. The membrane permeability to water, showed an increase higher than 50% after two weeks of storage, independently of the presence of leukocytes in the transfusion bag. The permeability through the lipid fraction, measured in the presence of an aquaporin inhibitor (Hg^{2+}) was the most affected, increasing more than 4 times during storage. Leuko-reduction was also ineffective to protect the barrier function of the membrane. The presence of interleukin-1β (IL-1β) in the extracellular milieu increased in the cell supernatant with the storage time, pointing to an inflammatory environment in the transfusion bag. Strikingly, the cytokine increase was independent of the use of the leukoreduction filter. Although our data showed a decreased hemolysis in the leuko-reduced samples, the lack of effect in other parameters reinforces the need of further analysis into the mechanisms of protection by the use of the leuko-reduction filters.

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Myeloperoxidase and Protein-radicalization are Linked to Insulin Resistance in the Obese Adipose Tissue

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Introduction: Our B6 mouse model of diet-induced obesity showed adipose tissue macrophages (ATM) forming typical crown-like structures in the adipose tissue (AT). These ATM from obese mice express 15-times more myeloperoxidase (MPO) mRNA than those from control mice. MPO protein, but not mRNA, was also found inside adipocytes in the obese AT. Treatment with the nitrone spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) improved insulin sensitivity in obese mice. Objective: Based on these findings, we hypothesized that HOCl produced by MPO inside adipocytes interferes with insulin signaling in the AT. Methods: This may be caused by HOCl-induced radicalization and oxidation of specific proteins involved in insulin-triggered signaling. To test this hypothesis, we differentiated human adipocytes and loaded them with human MPO. Results: Treatment of MPO-loaded adipocytes with H_2O_2 caused intracellular production of HOCl, radicalization of proteins, reduction of insulin-triggered GLUT-4 translocation to the membrane, and reduced glucose uptake. Indeed, DMPO-based immuno-spin trapping and MS-tandem showed the radicalization of specific components of the insulin-triggered signaling (*i.e.,* IRS-1/2, SOCS3 and GLUT-4). These effects were prevented by resveratrol that scavenged intracellularly produced HOCl thus preventing loss of insulin sensitivity. DMPO also prevented HOCl-induced loss of insulin sensitivity in adipocytes by trapping radicalized protein. Conclusion: Scavenging HOCl produced inside adipocytes with resveratrol or preventing protein oxidation with spin traps can protect insulin signaling in adipocytes. Supported by: PICT-2018-3435, PIP-916, PROICO-100218/023418 and PUE-013.

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Peroxynitrite Induces Antibiotic Tolerance in *Staphylococcus aureus*

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