S26: Red blood cell nitric oxide/rheology

S26-1 Nitric oxide synthase activity at various levels and durations of shear stress

Michael Simmonds

Griffith University, Australia

Background: Nitric oxide (NO) is an important free radical that is produced within red blood cells (RBC) by a local NO-synthase (RBC-NOS). The level of shear required for RBC-NOS activation is poorly described, thus we have been exploring the magnitude-duration interactions of RBC-NOS activity, and the resultant levels of intracellular NO produced. Moreover, interactions with other oxygen free radicals has been of interest.

Methods: In a series of studies, we examined RBC-NOS activation via immunohistochemistry, and the level of NO produced via a fluorescent tag, following exposure to discrete shear stress levels (up to 100 Pa) over varying durations (1-45 min). Effects of intracellular superoxide have been explored using phenazine methosulfate incubations.

Results: Physiological levels of shear appear to result in monotonic activation of RBC-NOS. The amount of NO produced within RBC appears to be sensitive to the level and duration of shear exposure. A particularly interesting change in NO concentration within RBC is observed when shear exposure is 5 Pa below an individuals sub-haemolytic threshold (increased NO), when compared with shear exposure above the sub-haemolytic threshold (decreased NO). We have also observed that high levels of intracellular superoxide impairs RBC-NOS activation.

Conclusion: Accumulating evidence indicates that the level and duration of shear exposure regulates RBC-NOS activity, and intracellular NO concentration. Oxygen free radicals appears to negatively impact RBC-NOS activity. The collective findings of our studies highlight special consideration of blood exposure to high shear and oxygen environments, including mechanical circulatory support.

S26-2 Erythrocyte nitric oxide dependent of acetylcholinesterase receptor

Carlota Saldanha, Ana Silva –Herdade

Institute of Biochemistry, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Portugal

The erythrocyte membrane enzyme acetylcholinesterase (AChE) activity is a biomarker of membrane integrity, aging and inflammation. AChE has the particularity to be inhibited by high concentrations of acetylcholine (ACh), meaning its own natural substrate.

Nitric oxide (NO) was observed inside erythrocytes in presence of ACh, by fluorescence microscopy. From all studies done, the data obtained evidence that erythrocyte AChE beyond

its enzyme activity, function as a receptor for signal molecules able to induce rescue or NO efflux from human erythrocytes and its mobilization from or to the reservoir molecules such as S- nitrosohemoglobin or nitrosoglutathione (GSNO) . The signal transduction pathway in dependence of the active, less active or inactive complexes states resulting from the hydrophilic bound of ACh, timolol, indomethacin and velnacrine to AChE receptor and from the entrance on erythrocyte of lipophilic molecules such as adenylyl cyclase , guanylyl cyclase, protein tyrosine kinase, protein phosphatase and phosphatidylinositol 3 kinase inhibitors will be described. The NO efflux , GSNO and its derivative molecules nitrite, nitrate and peroxynitrite

S26-3 Hydroxyurea therapy modulates sickle cell anemia red blood cell physiology by acting as a nitric oxide donor: impact on RBC deformability, oxidative stress and nitric oxide synthase activity.

Elie Nader [a], Marijke Grau [b], Romain Fort [c], Nicolas Guillot [d], Cyril Martin [a], Giovanna Cannas [e], Solène Poutrel [e], Arnaud Hot [e], Alexandra Gauthier[f], Wilhelm Bloch [b], Marc Romana[g], Philippe Connes [a]

[a] *Laboratoire LIBM, Université Claude Bernard Lyon 1, France*
[b] *Molecular and Cellular Sport Medicine, Deutsche Sporthochschule Köln, Germany*
[c] *Service de Médecine Interne, Hôpital Edouard Herriot, Hospices Civils de Lyon, France*
[d] *Laboratoire Carmen Inserm 1060, Université Claude Bernard Lyon 1, France*
[e] *Service de Médecine Interne, Hôpital Edouard Herriot, Hospices Civils de Lyon, France*
[f] *Institut d'hématologie et d'oncologie pédiatrique - Hospices Civils de Lyon, Lyon, France*
[g] *UMR Inserm 1134, Hôpital Ricou, Centre Hospitalier Universitaire, Pointe-à-Pitre, France*

Sickle Cell Anemia (SCA) is a hereditary hemoglobinopathy and the first genetic disorder in the world. It is characterized by the production of an abnormal hemoglobin (HbS), which polymerizes under deoxygenation. SCA patients suffer from hemolytic anemia, repeated vaso-occlusive crises and chronic vascular complications. Hydroxyurea (HU) is the only approved drug and is known to improve the clinical course of patients by raising foetal hemoglobin levels. However, this mechanism cannot explain alone the beneficial effects of HU in SCA.

16 healthy subjects (AA) and 37 SCA patients treated (HU+, n=24) or not (HU-, n=13) with HU were included. Red blood cell (RBC) deformability was measured by ektacytometry. RBC nitric oxide synthase (RBC-NOS) activation was assessed by immunostaining and RBC and plasma nitrite levels were measured by chemiluminescence. Reactive Oxygen Species (ROS) level within RBC were analysed by flow cytometry. Besides, RBC from SCA patients were incubated with Sodium Nitroprusside (SNP) and the same parameters mentioned above were measured.

RBC deformability was decreased in the SCA compared to AA group. HU+ patients had higher RBC deformability, RBC and plasma nitrite levels than HU- patients. RBC-NOS

activation was lower in the HU+ compared to both AA and HU- groups. SCA had higher RBC ROS levels than AA individuals and HU+ patients showed decreased RBC ROS content in comparison with HU-patients. In-vitro, SNP improved RBC deformability and decreased RBC-NOS activation and ROS levels in SCA patients.

HU improves RBC deformability and reduces oxidative stress in SCA. These effects could be attributed to the NO donor effect of HU. The decrease of RBC-NOS activation in SCA under HU suggests a negative retroncontrol effect of HU on the RBC NO production.

S26-4 The multifaceted role of nitrite and the epigenetic nitric oxide donor, RRx-001 on erythrocyte deformability

Selma Cırrık[a] , Ozlem Yalcin[b]

[a]*Ordu University, Faculty of Medicine, Department of Physiology, Turkey*
[b]*Koc University, School of Medicine, Department of Physiology, Turkey*

RRx-001 is an anti-cancer immunotherapeutic that increases the sensitivity of drug resistant tumors via multiple mechanisms including covalent binding to hemoglobin. This binding stimulates the nitrite reductase activity of deoxyhemoglobin, resulting in enhanced nitric oxide (NO) production from nitrite in hypoxia. In present study, the effect of clinically used doses of RRx-001 on erythrocyte deformability was examined.

A dose dependent effect of RRx-001 (0.001 – 1 mM) on erythrocyte deformability was measured by ektacytometer under hypoxia (n=8). The effects of low dose RRx-001 (0.02 mM) on deformability in the presence of ODQ, L-NAME or nitrite were examined both in normoxia and hypoxia. NO release from erythrocytes was measured fluorometrically using a fluorescent probe.

During hypoxia, higher doses of RRx-001 (0.1 and 1 mM) significantly increased SS1/2:EImax ($p<0.01$; $p<0.05$, respectively). Lower dose of RRx-001 (0.02 mM), alone or combination with ODQ or L-NAME, did not affect deformability. However, in the presence of nitrite, RRx-001 (0.02 mM) caused an increase in erythrocyte deformability ($p<0.01$) under hypoxia. NO release was significantly higher during RRx-001 incubation ($p<0.05$) and a further increment was observed after the co-administration of RRx-001 and nitrite ($p<0.05$).

This study show that under hypoxic conditions, clinically used dose of RRx-001 caused a significant increase in erythrocyte deformability in the presence of nitrite. This effect of RRx-001 might be attributed to increased NO production. In conclusion serum nitrite level is an important factor for RRx-001 effectiveness and should be considered during RRx-001 treatment in cancer patients.