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Oxidative stress: Novel insights on red blood cells as redox modulators

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Abstract

Oxidative stress adds to the pathologies of diseases, including hemolytic anemia. In thalassemia it is attributed to iron-overload due to red blood cell (RBC) transfusions and increased ron absorption. Free intracellular iron catalyzes the generation of cytotoxic reactive oxygen species (ROS). RBCs, functioning as oxygen transporters, contain efficient mechanisms against oxidizing substances. In hemolytic anemia, RBC transfusions ameliorate the chronic anemia, but contribute excess iron. Little attention has been given to these opposing effects. To study the antioxidant capacity (AOC) of RBCs, cultured human promyelocytic HL60 cells were labeled with 2'-7'-dichlorofluoresceindiacetate, washed and then incubated with or without RBCs. Cellular fluorescence, indicating ROS generation, was measured by flow-cytometry. The HL60 cells were distinguished from RBCs by their larger size and fluorescence. The results indicated that ROS generation by the HL60 cells was reduced when they were incubated with normal RBCs in a dose-, time- and temperature-dependent fashions. RBCs from β-thalassemia patients showed reduced AOC, unrelated to their smaller size or hemoglobin content. The RBC-AOC was reduced by iron or oxidants, conditions that generate oxidative stress, but was increased by iron-chelators or antioxidants. Thus, normal RBCs serve as redox modulators, but when they are under oxidative stress, their AOC is defected.

Introduction

Oxidative stress plays an important role in the pathophysiology of many diseases, including hemolytic anemias such as thalassemia and the myelodysplastic syndrome (Fibach & Rachmilewitz 2008). In these diseases, oxidative stress has been mainly attributed to excess of iron (iron-overload) (Fibach & Rachmilewitz 2010), which is mainly due to blood transfusions and increased iron absorption. Indeed, iron is well known to catalyze the generation of reactive oxygen species (ROS) through the Haber-Weiss and Fenton reactions. Excess ROS is believed to mediate the toxicity of cells of vital organs (heart, liver) in IO patients.

Blood transfusion therapy to chronic anemic patients ameliorates the anemia but contributes to iron-overload. Multi-transfused thalassemic patients, with less severe anemia but higher iron-overload, have lower levels of oxidative stress (ROS and lipid hydroperoxides) than untransfused patients, with more severe anemia but lower iron-overload (Ferro, Visalli et al. 2012) . However, little further attention has been given to the effect of the anemia proper on oxidative stress.

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The main function of RBC is oxygen transport, for which RBCs and their hemoglobin molecule have evolved efficient and complex mechanisms for protection against oxidizing substances to which they exposed.

Herein, we report on the antioxidant effect on neighboring cells of normal and thalassemia RBC.

Materials and Methods

The human cultured promyelocytic HL60 cell line, which grow as suspended single cells in liquid medium, were labeled with 2'-7'-di-chlorofluorescein diacetate (DCF) for 15 min. as previously described (Amer, Goldfarb *et al.* 2004). They were then washed and incubated with or without RBCs. The intensity of their fluorescence, indicating intracellular ROS generation, was measured by flow cytometry. The HL60 cells were distinguished from RBCs by their larger size (forward light scatter) and fluorescence *(Figure 1)*. Labeling with DCF and incubation with RBCs did not affect the viability of the HL60 cells, as measured by the Trypan Blue exclusion test after the incubation period.

Results and Discussion

HL60 cells were labeled with DCF, washed, and then incubated with normal or thalassemic RBC.

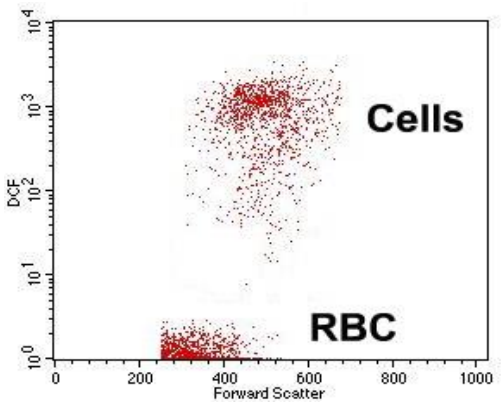

Figure 1. Measurement of ROS in cells in the presence of RBC.

The figure shows a dot plot of cell fluorescence (DCF) vs. size (forward scatter). Two populations are discerned: Larger cells with high fluorescence – the HL60 cells, and smaller cells with low fluorescence – RBCs.

HL60 cells were labeled with DCF, washed, and then incubated with normal or thalassemic RBCs. *Figure 2A*: One concentration ($6x10^6$) of RBC was added and the fluorescence of the cultured cells was measured at the indicated times. *Figure 2B*: Different concentrations of RBCs were added for 30 min. Cell fluorescence without RBCs was taken as 100%.

The results indicate a significant time- and RBC dose-dependent decrease in cell fluorescence with normal RBCs, but less with thalassemic RBCs.

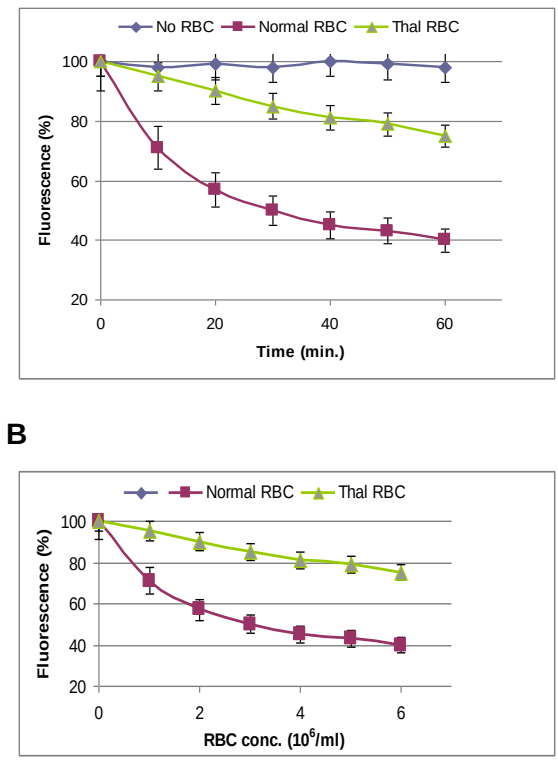

Figure 2. The antioxidative capacity of normal and thalassemic RBC: (A) The effect of time. (B) The effect of RBC concentration.

To study the effects of iron-overload and oxidative stress on the AOC of RBCs, HL60 cells were labeled with DCF, washed, and then incubated with $6x10^6$/ml normal or thalassemic RBCs. Prior to incubation with cells, normal RBCs had been treated for 30 min with an iron source, ferric ammonium citrate (FAC), 1 mM, and thalassemic RBCs – with the iron chelator, Desferal, 5 mM, thus increasing and decreasing iron-overload,

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respectively. Alternatively, normal RBCs had been treated for 30 min with the oxidant H_2O_2, 5 mM, and thalassemic RBCs – with the anti-oxidant N-acetyl cysteine (NAC), 5 mM, thus increasing and decreasing oxidative stress, respectively. The AOC of untreated normal RBCs was taken as control = 100%.

The results indicate that the AOC of RBCs was significantly decreased by iron-overload and by oxidative stress, but could be restored by iron chelation or antioxidants.

In summary: (A) Normal RBCs reduce, in a dose- and time-dependent manner, the ROS in

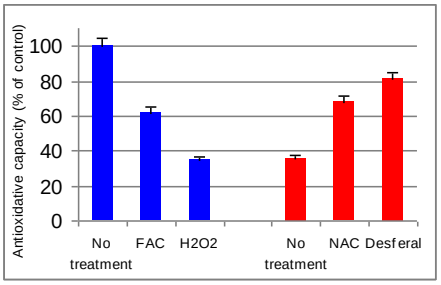

Figure 3. The effects of iron overload and oxidative stress on the antioxidative capacity of RBC.

neighboring HL60 cells. This effect was lower with thalassemic RBCs. Similar effect was in various human adherent and non-adherent cells by RBCs from patients with other forms of hemolytic anemia such as sickle cell disease and myelodysplastic syndrome) (data not shown). The reduce AOC in such RBCs is probably due to their oxidative stress and iron-overload status. A "bystander" effect of cells on the oxidative status of other cells have been described previously in other circumstances, such as oxidative stress induced by ionizing radiation (Mothersill, Stamato et al. 2000) or photo-sensitivity (Chakraborty, Held et al. 2009). (B) The results suggest that transfused normal RBCs in patients with hemolytic anemia patients may function as antioxidants, protecting cells (in the blood and elsewhere) from oxidative stress. (C) The net effect of anemia and iron overload on oxidative stress warrants a careful study in transfused and non-transfused patients.

References

Fibach E. and E. A. Rachmilewitz (2008). The role of oxidative stress in hemolytic anemia. Curr Mol Med 8(7):609-19.

Fibach E. and E. A. Rachmilewitz (2010). The role of antioxidants and iron chelators in the treatment of oxidative stress in thalassemia. Ann N Y Acad Sci 1202:10-6.

Ferro E., G. Visalli et al and A. Di Pietro (2012). Oxidative damage and genotoxicity biomarkers in transfused and untransfused thalassemic subjects. Free Rad Biol Med 53:1829-1837.Amer J., A.

Goldfarb and E. Fibach (2004). Flow cytometric analysis of the oxidative status of normal and thalassemic red blood cells. Cytometry Part A 60A:73-80.

Mothersill C., T.D. Stamato et al and C.B. Seymour (2000). Involvement of energy metabolism in the production of 'bystander effects' by radiation. Brit J Cancer 82:1740-1746.

Chakraborty A., K.D. Held et al and R.W. Redmond (2009). Bystander effects induced by diffusing mediators after photodynamic stress. Rad Res 172:74-81.

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