THE RELATIONSHIP BETWEEN THE BLOOD OXYGEN TRANSPORT AND THE HUMAN RED CELL AGING PROCESS

Michele Samaja[1], Ermanna Rovida[2], Roberto Motterlini[3] and Massimo Tarantola[3]

[1]Dipartimento di Scienze e Tecnologie Biomediche
Università di Milano
[2]Istituto di Tecnologie Biomediche Avanzate del C.N.R.
[3]Istituto Scientifico San Raffaele, Milano, Italy

SUMMARY

We have studied the relationship between the in vivo aging process of the human red cell (RBC) and its main function, the transport of O_2 from the lungs to the tissues. This study included several approaches. First, we observed that the affinity for O_2 in young RBCs was lower than in old RBCs ($p<0.0005$) due to different intracellular concentration of 2,3-diphosphoglycerate, main effector of hemoglobin. Second, we explored whether there are some subgroups of the healthy human population with altered RBC age distribution: females in the age range 25-35 exhibited significantly younger RBCs ($p < 0.0005$) and lower RBC-O_2 affinity ($p < 0.01$) than other groups. Correspondingly, the RBC-O_2 affinity in female blood was significantly lower ($p < 0.002$) than in male blood. Third, we correlated by two independent methods the lowered RBC-O_2 affinity to a more efficient O_2 delivery to the tissues by two independent methods: 1) calculating the size of the cardiac output increase required to sustain the tissue oxygenation after an increase of the RBC affinity for O_2 ; and 2) monitoring the enhanced cardiac function in isolated rat hearts perfused with RBCs at low O_2 affinity. Finally, comparing some hematologic findings relevant for the O_2 transport in two healthy populations with different RBC age distributions, such as age-matched females and males, it appeared that the low RBC-O_2 affinity in females is an adaptive response to their lower [Hb] . All these approaches agreed in indicating that the lowered RBC-O_2 affinity, and by reflect the younger RBC average age, appears associated to more favourable conditions of tissue oxygenation. We conclude that the human RBC aging process is not only a suitable model to study the cellular and tissutal aging mechanisms, but may also have a considerable influence on some physiological and pathological patterns in humans.

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INTRODUCTION

The human red blood cell (RBC) is designed for the specific purpose to transport oxygen from the lungs to the tissues. Nearly all the metabolic, physiological and physical properties of the RBC are aimed to the optimization of this function. For example, the RBC can accomodate large amounts of hemoglobin (Hb), a protein with O_2-carrying properties. In addition, the subcellular structures necessary for the aerobic metabolism, such as the mitochondria and the nuclei, lack in the mature RBC for the dual purpose of accomodating more Hb and allowing greater flexibility in the passage of the RBC through narrow capillaries. Finally, the presence of 2,3-diphosphoglycerate (DPG), intermediate of the RBC glycolytic pathway and major allosteric effector of Hb (Benesch and Benesch, 1967), allows the RBC to regulate its own affinity for O_2 by intrinsic mechanisms. Indeed, erythrocytic DPG regulates the RBC-O_2 affinity in two ways: by its allosteric control on Hb and by lowering the intracellular pH through the Gibbs-Donnan effect (Samaja and Winslow, 1981). Due to the synergism of the two effects and to the high intracellular concentration of DPG, it becomes perhaps the most important regulator of the RBC-O_2 affinity.

The lack of genetic material and of energy-producing compartments gives the human RBC the unique characteristics of a well-defined 120-day life span (Eadie and Brown, 1953), equilibrium set point between the production of new cells by the bone marrow and their removal from the circulation by the spleen. Indeed, human spleen removes selectively from the circulation the oldest RBCs only (Jandl and Cooper, 1983; Mentzer and Clark, 1983), in contrast to other species where the RBC removal is randomized (Clark, 1988). It is still matter of discussion if the primary cause of the RBC aging process is to be searched in the events leading to the metabolic depression, in the stressing factors present in the circulation, or both. Whatever the cause, we believe that it is to be clarified whether the RBC in vivo aging is linked to the main function of the RBC, i.e., the O_2 transport, and whether or how it participates to the organism adaptive responses to environmental and endogenous stimuli. We will here review our approach to this problem.

The O_2 affinity in young and old RBCs

Previous studies have shown that the [DPG]/[Hb] ratio and the P_{50} (PO_2 at which half of Hb is bound to O_2 and useful index of the RBC-O_2 affinity) are higher in the young than in the old RBC fraction (Haidas et al., 1971); Schmidt et al., 1987). Recently, we have confirmed these data (Samaja et al., 1990a) with an approach by which it was inferred that DPG alone does not fully explain the lower RBC-O_2 affinity in the young RBCs, but another unknown intracellular factor must be involved. Nonetheless, DPG always remains the most important allosteric effector of Hb in the RBC. The decreased concentration of DPG in the senescent RBC is related mainly to the age-induced change of the activity of key regulatory enzymes that operate along the glycolytic pathway, but the possibility that the level of DPG may also be set by direct effect of some other factors, for example acute exposure to an hypoxic environment, cannot be ruled out (Mairbaurl et al., 1990).

The RBC age distribution in humans

In the search of a relationship between the RBC aging process and the transport of O_2 to the tissues, it is essential to find a model where the two variables can be easily correlated. For this purpose, one needs a suitable method to characterize the average RBC age distribution in a sample of blood. Several features have been proposed as suitable markers for the RBC senescence, including the metabolic depletion (Seaman et al., 1980), the changes in the RBC deformability and shape (Linderkamp and Meilsen, 1982), and the altered activity of some enzymes (Kadlubovski and Agutter, 1977). The striking correspondence between the age of the RBC and its density is of particular interest because it allows both preparative and analytical studies. The same peculiarity was used to measure the RBC age distribution in some hematological disorders (Nakashima et al., 1973). It was found that iron-deficiency anemia, pyruvate kinase deficiency and polycythemia vera were associated with an increase of the light, young RBC fraction. On the contrary, hereditary spherocytosis, autoimmune hemolytic anemia, aplastic anemia and erythroleukemia were associated with an increase of the dense, old RBC fractions. These features, which are linked to the circulatory stress, or to the actual life span of the RBC, or both, may theoretically provide an in vivo model to study the relationship between the RBC aging process and the tissue oxygenation, but the severity of the underlying disease poses a serious problem because it may induce responses not directly linked to the RBC age distribution.

To explore whether there are subgroups in the healthy human populations suitable to correlate the RBC age distribution to the O_2 transport mechanisms, we have determined the average RBC density in 59 males and females subdivided into the following age classes: 2-12, 25-35, and 62-85. In addition, samples were obtained from 10 pregnant women in the age range 23-34. For the measurements, two isotonic solutions were obtained mixing an hyperosmotic (2.66M NaCl + 0.09 M KCl) solution, water and Percoll (Sigma Chemicals, St. Louis, Mo) in variable ratios to yield densities of 1.099 and 1.102 g/ml. These densities were selected because they appeared the most indicative after running several complete distribution vs density curves. Small tubes were loaded with 0.15 ml of this solution and 0.05 ml of freshly drawn heparinized blood. The tubes were centrifuged at 12 000 rpm for 2 min at 0°C, and the blood layers above and below the Percoll were removed by a syringe and diluted into 5 ml Dabkin's reagent. The amount of RBCs lighter than the Percoll was finally calculated from the absorbance readings.

The adult females in the age range 25-35 exhibited the youngest RBCs with respect to all the other classes of subjects ($p<0.0005$), including the age-matched males and pregnant women (figure 1),leading to the following considerations: 1) the factor(s) shifting the average RBC distribution towards the young RBC is (are) to be searched in the events inherent to menstruating women, possibility the anemia-induced increase of erythropoiesis; 2) either the oxidative factors are less, or the RBC life span is shorter in the blood of adult females than in the other subjects; and 3) the age of the subject per se does not affect the RBC age distribution in healthy subjects.

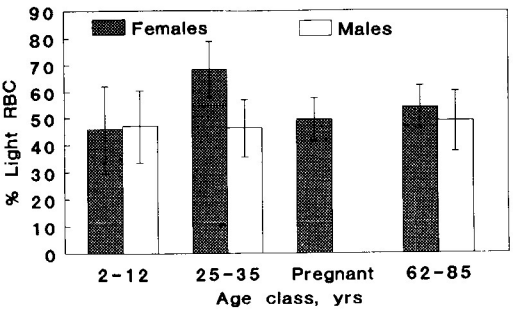

Fig. 1. Percent of RBCs lighter than 1.099 and 1.102 g/ml in various classes of subjects (average between duplicate measurements at the two densities, mean±S.D., n=69).

Some hematologic findings (Fig. 2) obtained in healthy male and female subjects of the same age group (25-35) are highly correlated to this result and to the previously discussed observation that the young RBCs have higher DPG and lower O_2 -affinity than the old RBCs. Whatever the factor for the younger or less stressed RBCs in females' blood, this is an unique opportunity to investigate in healthy well-doing populations whether the altered RBC age distribution has an effect on the RBC-O_2 transport. However, it is mandatory first to understand whether a lowered RBC-O_2 affinity is really advantageous for the oxygenation of the tissues.

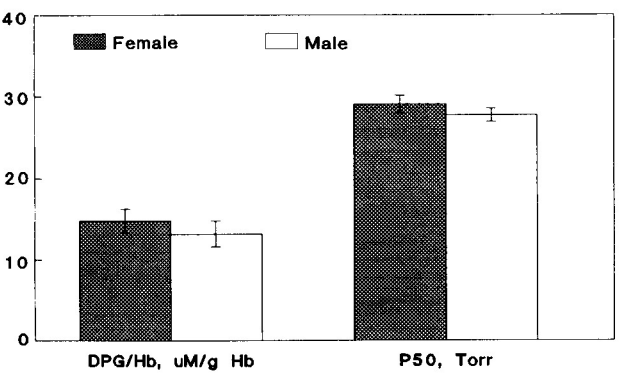

Fig. 2. Some hematologic findings in blood from 9 male and 16 female subjects of the same age (25-35). The [DPG]/[Hb] and P_{50} differences are significant at the $p < 0.005$ level.

The RBC-O_2 affinity and the O_2 transport to the tissues

Theory

Whether a decreased RBC-O_2 affinity, whatever the cause, is advantageous for tissue oxygenation, is a still debated question. Theoretical considerations, sketched in figure 3, indicate that when the P_{50}

increases from 28 to 32 Torr as a consequence of an increase of the [DPG]/[Hb] ratio from 12.9 to 17.7 μM/g Hb, or 0.8 to 1.1 M/M, (Samaja et al., 1981), the amount of O_2 that can be delivered to the tissues at constant arterial and venous PO_2 is augmented, in the case of figure 3, from 43% to 49% of the total O_2 carried by blood. Although it is expected that the organism may adjust the venous PO_2, it is likely that a decreased RBC-O_2 affinity is favourable for the organism because the same amount of O_2 can be delivered to the tissues either at a higher PO_2 or at a lower flow through a specific organ. In the former case, the higher venous PO_2 prevents tissue hypoxemia, and in the latter the lower blood flow reduces the circulatory load. It was calculated (Samaja, 1988) that the blood flow through the major organs may decrease about 10% following an increase of the P_{50} of 2 Torr, without compromising the oxygenation of the tissues at the physiological metabolic level.

The observations that the average RBC age distribution is younger in female than in male blood, and that the young RBC has a lower O_2 affinity than the old RBC, provide an opportunity to test in vivo the theoretical indications that a lowered RBC-O_2 affinity favours the oxygenation of the tissues. As expected, [Hb] is lower in females with respect to males (13.7 and 15.5 g/dl, respectively, on the average). Consequently, the blood O_2 capacity in females is lower and the lower O_2 delivery to the tissues must be compensated with a higher cardiac output. The required increase of the cardiac output when [Hb] decreases from 15.5 to 13.7 g/dl was calculated (Samaja et al., 1986) at constant value of the other factors, such as arterial and venous PO_2's (95 and 40 Torr, respectively), arterial PCO_2 (40 Torr), and O_2 uptake (0.35 l/min): the cardiac output in females should increase from 6.34 to 7.12 l/min to compensate their lower Hb and to support an adequate oxygenation to the tissues. However, since the [DPG] / [Hb] ratio and the P_{50} are higher in females than in males, the enhanced O_2 carrying properties tend to compensate the lower [Hb] in females, and it was calculated that owing to the increase of the [DPG]/[Hb] ratio only, the cardiac output in females may decrease from 7.12 to 6.80 l/min without comprimising the oxygenation of the tissues. This implies a considerable saving of the energy cost of blood pumping.

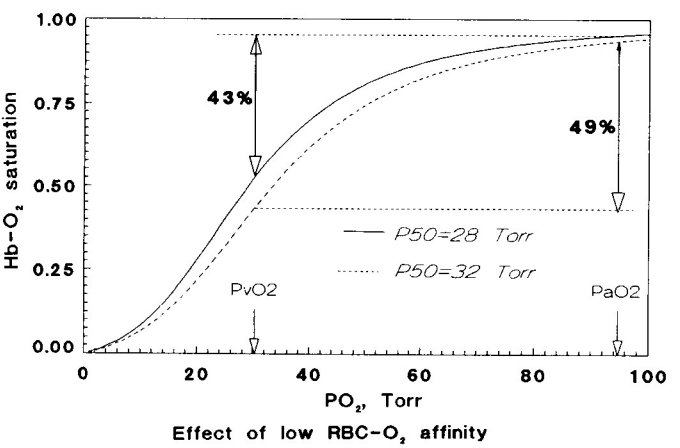

Fig. 3. When the P_{50} shifts from 28 to 32 Torr, the fraction of O_2 delivered to tissues increase from 43% to 49% at constant $P_a O_2$ and $P_{\bar{v}}O_2$

Isolated heart perfusion

The direct experimental confirmation of the above theories is not univocal despite the very large body of experimental studies using in vivo and in vitro (or ex-vivo) models. The in vivo studies aimed to show an advantage of a lowered RBC-O_2 affinity necessarily have the drawback that the studied organisms can compensate the experimentally induced changes of the RBC-O_2 affinity with both circulatory (blood flow vs venous PO_2 adjustment, organ to organ compensation, capillary recruitment) and metabolic (erythropoiesis long-term adaptation, acid/base adjustment) adaptations. On the other hand, the major problem of the in vitro and ex-vivo studies is that the often drastic changes of the induced RBC-O_2 affinity have little physiological relevance. Thus, it is still uncertain whether the relatively smaller fluctuations of the O_2 affinity are physiologically significant in in vivo models. However, the perfused isolated heart is perhaps ideal to test the physiological significance of the RBC-O_2 affinity changes which, under appropriate experimental conditions, may be correlated to a variety of myocardium responses without confounding effects. Nevertheless, the data obtained from these studies still remain quite contradictory. Part of them showed a high degree of insensitivity of the heart O_2 consumption to a decreased P_{50} : Martin et al. (1979) attributed this result obtained in working rat hearts to a 50% increase of the capillary density; and Ross and Hlastala (1981) hypothesized that the drop of the O_2 consumption observed in isolated skeletal muscles was associated with the presence of humoral factors rather than with the RBC O_2 affinity. On the other hand, in experiments in paced isolated rabbit hearts, the myocardial function increased when switching from RBCs at P_{50} =16.9 to RBCs at P_{50} =32.6 Torr (Apstein et al., 1985) showing an evident advantage associated with a decreased RBC-O_2 affinity. In other studies, inositol hexaphosphate (IHP) was used to drastically increase the P_{50} from 18-20 to 42-47 Torr. In perfused isolated rat and rabbit hearts (Stucker et al., 1985; Baron et al., 1987), low O_2 affinity RBCs decreased the coronary blood flow and increased the O_2 consumption again showing an advantage with respect to high-O_2 affinity RBCs, but the allowance for flow compensation, the low arterial PO_2 (184-188 Torr) and a possible direct effect of the IHP-loaded RBCs (Samaja et al., 1990b) do not clarify the question.

In our Langendorff-perfused isolated hearts, the aorta of excised hearts from male Sprague Dawley rats was mounted onto a stainless-steel cannula and the heart perfused retrogradely with an oxygenated Krebs-Henseleit buffer (PO_2=670 Torr, PCO_2=42 Torr, pH 7.4). A ballon connected to a pressure transducer (Harvard Apparatus mod. 52-9966, Natick, MA) was inserted into the left ventricle and filled with saline to monitor the developed pressure. A teflon cannula was inserted in the pulmonary artery to collect the perfusate for the measurement of the venous PO_2 and of the O_2 uptake. An additional pressure transducer above the aortic cannula monitored the coronary pressure. The age-induced O_2 affinity changes were simulated treating human stored blood (3 to 10 days old) from a local blood bank in two different ways. To obtain RBCs with low O_2 affinity, the blood was filtered through a Pall RC-100 Leukocyte Removal Filter (Pall Biomedical Products Corp., East Hills, NY) and incubated 60 min at 37°C in the presence

of 50 ml rejuvenating solution (103 mM sodium phosphate, 100 mM inosine, 100 mM pyruvate, 5 mM adenine, pH 7.0 at 37°C). The RBCs were then washed (IBM 2991 blood cell processor, Hopewell Junction, NY) with 2 l isotonic NaCl. RBCs with high O_2 affinity were obtained using 10 days old blood filtered and washed as above. The resulting P_{50} values were 18 and 30 Torr at pH 7.4, PCO_2=40 Torr, 37°C.

Table 1. Myocardial function change (mean ±SEM) when switching from buffer to RBC perfusion. The change in the developed pressure only is significant at the $p < 0.005$ level.

	RBC-O_2 affinity	
	high	Low
Coronary pressure, Torr	8.1±3.5	14.1±9.5
Developed pressure, Torr	-10.6±5.1	-0.8±4.1
Oxygen uptake, µM/min	0.7±0.4	-0.1±0.2

Table 1 shows the change of the heart performance when the perfusion was switched from the buffer to the RBCs with high or low O_2 affinity at 10% hematocrit. All the other parameters were unchanged, including the arterial gasses, the flow through the heart (15 ml/min) and the heart preload (10 Torr). The RBCs with low O_2 affinity have a positive effect on the perfused hearts, because of the better preserved developed pressure when switching from the buffer perfusion to the low O_2 affinity RBCs. The coronary pressure, index of the vascular state, did not differ appreciably between the two groups as well as the O_2 uptake. It can thus be inferred that an increased RBC-O_2 affinity is not favourable for the oxygenation of the tissues in this experimental model.

CONCLUSIONS

It is possible that under anemic or hypoxic conditions which increase the erythropoiesis, the average RBC population become relatively younger. The size of the alteration of the consequent whole blood affinity for O_2 is sufficient to directly tune up the O_2 transport and adapt it to the new situation. This hypothesis has had three independent lines of confirmation: 1) a theoretical inference based on the calculation of the amount of the O released at constant values of the other factors that regulate the O_2 delivery to the tissues; 2) an experimental approach based on the perfusion of isolated hearts with RBCs of different O_2 affinities; and 3) comparing the relevant hematologic data in two healthy populations that have different RBC age distributions. We conclude that the RBC aging process appears

biologically relevant for the adjustment of the average blood O_2 affinity and transport.

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REFERENCES

1. C. S. Apstein, R. C. Dennis, L. Briggs, W. M. Vogel, J. Frazer and C. R. Valeri, Effect of erythrocyte storage and oxyhemoglobin affinity changes on cardiac function, Am. J. Physiol. 248:H508 (1985).
2. J. F. Baron, E. Vicaut, O. Stucker, M. C. Villereal, C. Ropars, B. Teisserie and M. Duvelleroy, Isolated heart as a model to study the effects of the decrease in oxygen hemoglobin affinity, Adv. Biosciences 67:73 (1987).
3. R. Benesch and R.E. Benesch, The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin, Biochem. Biophys. Res. Comm. 26:162 (1967).
4. M. R. Clark, Senescence of red blood cells: progress and problems, Physiol. Rev. 68:503 (1988).
5. G. S. Eadie and I. W. Brown, Red blood cell survival studies, Blood 8:1110 (1953).
6. D. Haidas, D. Labie and J. C. Kaplan, 2,3-diphosphoglycerate content and oxygen affinity as a function of red cell age in normal individuals, Blood 38:463 (1971).
7. J. H. Jandl and R. A. Cooper, Hereditary spherocytosis, in:"Hematology", W. J. Williams, E. Beutler, A. J. Erslev, M. A. Lichtman ed., McGraw-Hill, New York (1983).
8. M. Kadlubovski and P. S. Agutter, Changes in the activities of some membrane-associated enzymes during in vivo ageing of the normal human erythrocyte, Br. J. Hematol. 37:111 (1977).
9. O. Linderkamp and H. J. Meiselman, Geometric osmotic and membrane chemical properties of density-separated human red cells, Blood 59:1121 (1982).
10. H. Mairbaurl, W. Schobersberger, O. Oelz, P. Bartsch, K. U. Eckardt and C. Bauer, Unchanged in vivo P at high altitude despite decreased erythrocyte age and elevated 2,3-diphosphoglycerate, J. Appl. Physiol. 68:1186 (1990).
11. J. L. Martin, M. Duvelleroy, B. Teisserie and M. Duruble, Effect of an increase in HbO_2 affinity on the calculated capillary recruitment of an isolated rat heart, Pflug. Arch. 382:57 (1979).
12. W. C. Mentzer and M. R. Clark, Disorders of erythrocyte cation permeability and water content associated with hemolytic anemia, in: "Pathological Membranes", A. Nowotny ed, Plenum, New York (1983).
13. K. Nakashima, O. Susuma and S. Miwa, Red cell density in various blood

disorders, J. Lab. Clin. Med. 82:297 (1973).
14. B. K. Ross and M. P. Hlastala, Increased hemoglobin oxygen affinity does not decrease skeletal muscle oxygen consumption, J. Appl. Physiol. 72:211 (1981).
15. M. Samaja, A. Mosca, M. Luzzana, L. Rossi-Bernardi and R. M. Winslow, Equations and nomogram for the relationship of human blood p_{50} to 2,3-diphosphoglycerate, CO_2 , and H+, Clin. Chem. 27:1856 (1981).
16. M. Samaja, P. E. diPrampero, P. Cerretelli, The role of 2,3-DPG in the oxygen transport at altitude, Resp. Physiol. 64:191 (1988).
17. M. Samaja, Prediction of the oxygenation of human organs at varying blood oxygen carrying properties, Resp. Physiol. 72:211 (1988).
18. M. Samaja, E. Rovida, R. Motterlini, M. Tarantola, A. Rubinacci and P. E. diPrampero, Human red cell age, oxygen affinity and oxygen transport, Resp. Physiol. 79:69 (1990).
19. M. Samaja, R. Motterlini, M. Tarantola, M. Beretta, F. Rossi, L. Sabbioneda, M. Porcellati and A. Zanella, Viability of the IHP-loaded red cell in the hypoperfused isolated rat heart, Adv. Biosciences in press (1990).
20. W. Schmidt, D. Boning, and K. M. Brauman, Red cell age effects on metabolism and oxygen affinity in humans, Resp. Physiol. 68:215 (1987).
21. C. Seaman, S. Wyss and S. Piomelli, The decline in energetic metabolism with ageing of the erythocyte and its relationship to cell death, Am. J. Haematol. 8:31 (1980).
22. O. Stucker, E. Vicaut, M. C. Villereal, C. Ropars, B. P. Teisserie and M. A. Duvelleroy, Coronary response to a large decrease of hemoglobin O_2 affinity in isolated rat heart, Am. J. Physiol. 249:H12244 (1985).