Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 399-409 (1998)

A Model-Based Isotopic Analysis of O_2 Transport During Anemia and Transfusion

Hartmut Heller*, Bernd Otto Göbel[+], and Klaus-Dieter Schuster

Department of Physiology and [+]Medical Polyclinic, University of Bonn, 53115 Bonn, Germany

Summary

We studied the relative role of blood flow to limit oxygen (O_2) transport in patients suffering from chronic anemia and with respect to the transfusion of packed red cells. The stable isotopic oxygen molecules $^{16}O_2$ and $^{16}O^{18}O$ were used. $^{16}O_2$ diffuses 3 % faster and in addition passes the respiratory chain 1.3 % more rapidly than $^{16}O^{18}O$, but within convective pathways it is transported as rapidly as $^{16}O^{18}O$. Thus, with increasing limitation by blood flow, the isotopic composition of oxygen changes less. By using a series resistance model we calculated the oxygen partial pressure difference between arterial and venous blood ($PavO_2$) on the basis of the change in $^{16}O^{18}O/^{16}O_2$ ratios. Nine patients suffering from chronic anemia as well as 14 normal volunteers were studied. In five patients transfusions had to be performed periodically. By means of respiratory mass spectrometry, $^{16}O^{18}O/^{16}O_2$ ratios were determined in expiratory gas mixtures. $PavO_2$ dropped more distinctly through transfusion (- 13 mmHg < $PavO_2$ < - 6 mmHg) than due to chronic anemia (- 5 mmHg < $PavO_2$ < - 2 mmHg). We concluded that the amelioration of the hemoglobin concentration of blood did more than compensate for the impediment of O_2 release which is induced by the decrease in the mean 2,3-diphosphoglycerat content of packed red cells and the increase in viscosity due to transfusion.

Key Words: stable oxygen isotopes, transfusion of packed red cells

Introduction

It is well known that the diminution in oxygen (O_2) carrying capacity of anemic blood is compensated by a rise in levels of 2,3-diphosphoglycerat (2,3-DPG) content of red blood cells, decreased viscosity of anemic blood, and redistribution of blood flow to O_2-dependent tissues respectively. In patients suffering from severe anemia it is customary to perform red cell

CCC 0362–4803/98/050399–11$17.50

Received 8 November 1997
Revised 5 January 1998
Accepted 9 January 1998

transfusions in order to ameliorate conditions of O_2 supply. However, apart from enhancing the hemoglobin concentration of blood ([Hb]), viscosity is also increased and the mean 2,3-DPG content of red cells within patients' blood is reduced when transfusing stored red blood cells (9). Thus the changes in 2,3-DPG content as well as viscosity could counteract the benefit derived from increasing [Hb].

We present the development of a series resistance model of oxygen isotope transport from environment to tissues that was undertaken to quantify the contribution of blood flow to the overall resistance to O_2 transport (F_B) shortly before and after transfusion of packed red cells. The change in F_B due to transfusion was compared with the dependence of F_B on variations in the hemoglobin concentration of blood as determined in patients suffering from various degrees of chronic anemia. For this purpose conditions of O_2 transport were assessed by using the naturally occurring stable isotopic oxygen molecules $^{16}O_2$ and $^{16}O^{18}O$, which amount to 99.6 % and 0.4 % of atmospheric oxygen. Due to different molecular weights, the lighter molecule $^{16}O_2$ diffuses 3 % faster (8) and in addition passes through the respiratory chain 1.3 % more rapidly than $^{16}O^{18}O$ (2). Within convective pathways, such as ventilation and blood flow, $^{16}O_2$ is transported as rapidly as $^{16}O^{18}O$. Consequently, the more O_2 transport is limited by blood flow (and/or by ventilation), the less the isotopic composition of oxygen changes during the entire transport to mitochondria.

By applying the series resistance model to the overall change in isotopic composition of oxygen as quantified in our test subjects, we determined the change in F_B caused by transfusion or induced by various degrees of chronic anemia. We believe that this approach provides an additional insight into O_2 transport during pathophysiological conditions.

Methods

Subjects. Participants in this study included 9 patients suffering from various degrees of chronic anemia, and 14 normal volunteers, five females and nine males, whose ages ranged from 18 to 49 years (average age ± SD = 35 ± 10). The patients characteristics are given in Table 1. In five patients transfusion of packed red cells had to be performed periodically. The

normality of healthy volunteers was determined before enrolment by history, physical examination, and laboratory analyses. No normal subject was taking any kind of medication. Each of the subjects gave their informed consent.

Table 1. *Patients characteristics*

Patient	Age yr	Sex	Diagnosis	Transfusion	[Hb] $g \cdot l^{-1}$
1	42	M	immunocytoma	+	66
2	46	M	non-Hodgkin's lymphoma	+	76
3	73	F	osteomyelofibrosis	+	79
4	81	F	plasmocytoma	+	103
5	76	F	thymoma	-	92
6	59	F	osteomyelofibrosis	+	85
7	29	F	hypoferric anemia	-	115
8	20	F	hypoferric anemia	-	110
9	33	F	hypoferric anemia	-	90

[Hb], hemoglobin concentration of blood.

Experimental protocol. In each subject red blood counts were carried out on the day of isotopic analyses. In the patients who underwent transfusion, a routine check-up was performed before and after transfusion, including red blood counts.

Each subject was studied in a sitting position, breathing room air at rest. In order to attain steady state conditions, expiratory gas mixture of the volunteers was obtained 5 min., at the earliest, after the beginning of breathing through the mouth-piece of the gas sampling equipment. Circulatory conditions were roughly estimated by recording the heart rate (pulsoximetry) simultaneously. During the sampling procedure the subjects expired into a gas-tight aluminum bag (Plastigas Beutel, Linde Gase AG, Düsseldorf, Germany). From there the

gas was led through a cooling trap, and was continuously sucked into the inlet system of a magnetic sector respiratory mass spectrometer (M3, Varian MAT, Bremen, Germany) in order to determine the fractional $^{16}O^{18}O/^{16}O_2$ ratio of expired oxygen (X_E). X_E was compared with the corresponding ratio $^{16}O^{18}O/^{16}O_2$ of inspired oxygen (X_I) or room air, and the change in isotopic composition of oxygen was quantified.

Quantification of the change in isotopic composition of oxygen. During steady state conditions, the $^{16}O_2$ and $^{16}O^{18}O$ input due to inspiration is equal to the output via mitochondrial utilization and expiration of oxygen, resulting in the following balance of isotope fluxes

$$X_I \cdot \dot{V}_{IO_2} = X_E \cdot \dot{V}_{EO_2} + X_R \cdot \dot{V}_{O_2} \quad (1)$$

where $\dot{V}_{IO_2}$, $\dot{V}_{EO_2}$ and $\dot{V}_{O_2}$ are the inspiratory flow, the expiratory flow, and the consumption of $^{16}O_2$ respectively, and X_R is the fractional $^{16}O^{18}O/^{16}O_2$ ratio of utilized oxygen. As oxygen isotope transport from environment to mitochondria is considered, the change in the isotopic composition of oxygen, which is defined as overall fractionation factor of respiration α_o, stems from the difference between X_I and X_R, related to 1.0:

$$\alpha_o = 1 + \frac{X_I - X_R}{X_I} \quad (2)$$

As isotopic oxygen composition within mitochondria is inaccessible for analysis, eq. (2) was rearranged by inserting $\dot{V}_{IO_2} = \dot{V}_{EO_2} + \dot{V}_{O_2}$ into eq. (1), leading to eq. (3):

$$\alpha_o = 1 - \frac{\dot{V}_{EO_2}}{\dot{V}_{O_2}} \cdot \left(\frac{X_I - X_E}{X_I} \right) \quad (3)$$

The more oxygen transport is limited by convection, the less X_E differs from X_I, and α_o converges at 1.0.

Developing the series resistance model. According to the steady-state model as commonly used (1, 5, 6), oxygen transport from environment to mitochondria can be considered as consisting of serially connected resistances, each of which represents a component pathway i. Thus each pathway exerts its particular resistances, R_i and R_i*, to the

transport of $^{16}O_2$ and $^{16}O^{18}O$. The change in isotopic oxygen composition through each pathway can then be quantified using the respective single fractionation factor α_i

$$\alpha_i = \frac{R_i^*}{R_i} \tag{4}$$

which amounts to 1.0 for convective oxygen transport, and to 1.03 for diffusion as well as to 1.013 for mitochondrial utilization. In this manner, the overall fractionation factor of respiration α_O is also defined as

$$\alpha_O = \frac{R_O^*}{R_O} \tag{5}$$

where R_O and R_O^* are the overall resistances to $^{16}O_2$ and $^{16}O^{18}O$ transport. R_O and R_O^* are assumed to consist of the serially connected resistances R_i and R_i^*. Therefore, eq. (4) can be used to rearrange eq. (5) into

$$\alpha_O = 1 \cdot F_V + 1.03 \cdot F_{DL} + 1 \cdot F_B + 1.03 \cdot F_{DT} + 1.013 \cdot F_U \tag{6}$$

where F stands for the fraction respectively contributed to the overall resistance R_O (R_i/R_O) by ventilation (F_V), pulmonary diffusion (F_{DL}), blood flow (F_B), tissue diffusion (F_{DT}) and mitochondrial utilization (F_U). It can easily be inferred from eq. (6) that α_O approximates unity more closely with increasing limitation of O_2 transport by convection (F_V, $F_B \rightarrow 1$).

In order to exclude the influence of ventilation, α_O values were normalized (α_O^N), referring to a constant alveolar partial pressure of oxygen (P_{AO_2}) of 100 mmHg (4). Due to Ohm's law, F_V is defined as the oxygen partial pressure difference between inspired (P_{IO_2}) and alveolar gas, related to P_{IO_2}: F_V = (150 mmHg - 100 mmHg)/(150 mmHg) = 1/3. By combining F_{DL} and F_{DT} in F_D, and in order to investigate the dependence of F_B on changes in the hemoglobin concentration of blood (d[Hb]), eq. (6) was differentiated for d[Hb], yielding

$$\frac{d(\alpha_O^N)}{d[Hb]} = 1.03 \cdot \frac{d(F_D)}{d[Hb]} + \frac{d(F_B)}{d[Hb]} + 1.013 \cdot \frac{d(F_U)}{d[Hb]} \tag{7}$$

With respect to the normalization procedure ($d(F_V) = 0$) and

$$0 = d(F_D) + d(F_B) + d(F_U) \tag{8}$$

two extremes are to be taken into account: either the fractional resistance of diffusion or that of mitochondrial utilization are independent of changes in [Hb], meaning that the two cases $d(F_D)/d[Hb] = 0$ (3) or $d(F_U)/d[Hb] = 0$ must be considered. By transforming eq. (7) on the basis of eq. (8), and by using $d(F_B) = d(P_{avO_2})/P_{IO_2}$, we obtain

$$-\frac{P_{IO_2}}{0.013}\left(\frac{d(\alpha_o{}^N)}{d[Hb]}\right) < \frac{d(P_{avO_2})}{d[Hb]} < -\frac{P_{IO_2}}{0.03}\left(\frac{d(\alpha_o{}^N)}{d[Hb]}\right) \qquad (9)$$

where $d(P_{avO_2})$ is the change in oxygen partial pressure difference between arterial and venous blood, induced by variations in the hemoglobin concentration of blood.

We used inequation (9) for assessing the relative role of blood flow to limit O_2 transport during chronic anemia or with respect to transfusion.

Mass spectrometry. Isotopic analysis was applied to room air and expiratory gas mixtures in order to gauge X_I and X_E. The respiratory mass spectrometer used was adapted to measure fractional $^{16}O^{18}O/^{16}O_2$ ratios within expired oxygen. According to Schuster et al. (7), the dried sample gas was repeatedly compared with a reference gas which was equally composed of nitrogen (N_2), $^{16}O_2$ and carbon dioxide (CO_2). In the present study, the relevant gases were detected at the following mass-to-charge ratios (m/e), CO_2 (22), N_2 (28), $^{16}O_2$ (32), $^{16}O^{18}O$ (34), determining $^{16}O_2$ and $^{16}O^{18}O$ simultaneously at the $^{16}O_2$-32 and the $^{16}O^{18}O$-34 ion-collector.

Isotopic analyses on expiratory gas mixtures (average sample volume = 40 l) and room air yielded a coefficient of variation (SD/mean) in X_E/X_I ratios of 6 - 7 %. $\dot{V}_{EO_2}$ and $\dot{V}_{O_2}$ were calculated from the mean expired ventilation and the inspiratory and expiratory fractions of O_2 and CO_2 which were also measured by mass spectrometry. We additionally determined the respiratory exchange ratio (R) by using the O_2 and CO_2 fractions. Alveolar partial pressure of oxygen was calculated by applying the ideal alveolar gas equation to values of R and arterial partial pressure of CO_2 as determined in each subject (blood gas analysis: ear lobe) during the gas sampling procedure.

Data analysis. We calculated the overall fractionation factor of respiration by using eq. (3). As has already been introduced (4), α_o^N was normalized on the basis of $\alpha_o^N = \alpha_o - 6 \cdot 10^{-5} \cdot (P_{AO_2} - 100$ mmHg). We performed regression analysis on the α_o^N-to-[Hb] relation in order to determine the slope $d(\alpha_o^N)/d[Hb]$ in anemic patients and normal volunteers. The result was compared with the mean slope of $d(\alpha_o^N)/d[Hb]$ as respectively obtained from the patients who underwent transfusion, applying Student's paired t-test. A difference between both values was considered significant when $P < 0.05$. In both study groups $d(P_{avO_2})$ was calculated by performing inequation (9) with $P_{IO_2} = 150$ mmHg and d[Hb] = 26 $g \cdot l^{-1}$ which was the mean increase in hemoglobin concentration of blood caused by transfusion.

Results

In Fig. 1 it is shown that isotopic composition of oxygen changes with increasing hemoglobin concentration of blood. Regression analysis on this interrelation revealed $\alpha_o^N =$

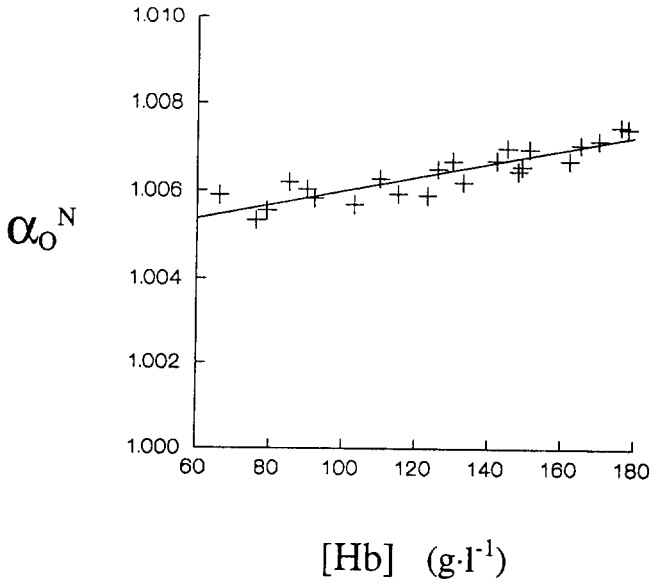

Figure 1. Relation between normalized overall fractionation factor of respiration (α_o^N) and hemoglobin concentration of blood ([Hb]) as determined in 23 anemic and normal volunteers.

$1.0044 + 1.56 \cdot 10^{-5} \cdot [Hb]$ ($r = 0.9$, $P < 0.001$), and the mean ± SD of the slope amounted to $d(\alpha_o^N)/d[Hb] = 1.56 \cdot 10^{-5} \pm 0.17 \cdot 10^{-5}\ l \cdot g^{-1}$. The effect of transfusion is illustrated in Fig. 2. As a result of transfusion, in each of the five patients α_o^N was increased with increasing [Hb], showing $d(\alpha_o^N)/d[Hb] = 4.3 \cdot 10^{-5} \pm 0.72 \cdot 10^{-5}\ l \cdot g^{-1}$, which is significantly higher than the former value ($P < 0.002$).

Using inequation (9), and for $d[Hb] = 26\ g \cdot l^{-1}$, the oxygen partial pressure difference between arterial and venous blood dropped more distinctly through transfusion

$$-13\,mmHg < d(P_{avO_2}) < -6\,mmHg \quad (11)$$

than with respect to various degrees of chronic anemia

$$-4.7\,mmHg < d(P_{avO_2}) < -2\,mmHg \quad (12)$$

Consequently, transfusion of packed red cells led to an amelioration of O_2 transport by blood flow which turned out to be much more effective than expected.

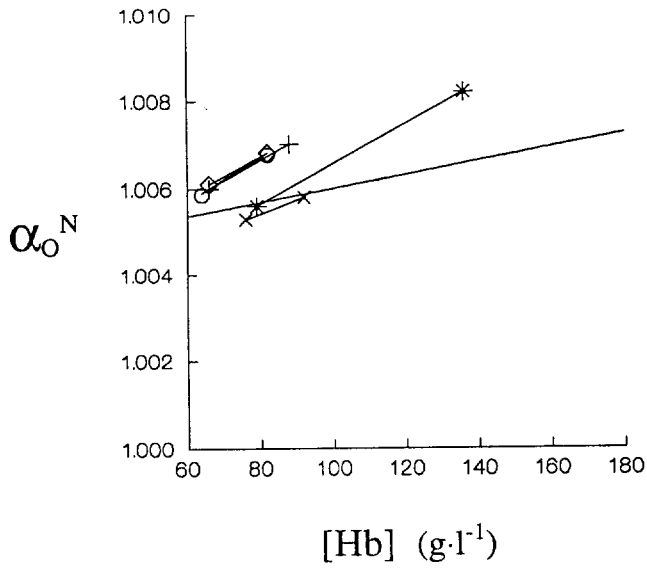

Figure 2. Change in normalized overall fractionation factor of respiration (α_o^N) in five patients suffering from chronic anemia as induced by transfusion of packed red cells, compared to the α_o^N-to-[Hb]-relationship as predicted in Fig. 1 (regression line).

Discussion and Conclusions

The model presented in this paper arose from our efforts to develop tools for differentiating between the influence of the various pathways on O_2 transport under pathophysiological conditions. The present approach thus represents a particular adaptation of isotopic analysis to evaluate the relative contribution of blood flow to the entire resistance to O_2 transport with respect to variations in the hemoglobin concentration of blood. By comparing values of $d(P_{av\,O_2})$, we were able to demonstrate that due to transfusion the decrease in the mean 2,3-DPG content of red cells as well as the increase in viscosity was more than compensated for by the amelioration in the hemoglobin concentration of blood, although heart rate tended to decrease after transfusion (from the baseline value = 106 min^{-1} to 97 min^{-1} 1.5 hours after transfusion).

The separate assessment of blood flow was facilitated by excluding the influence of ventilation on O_2 transport, applying the normalization procedure ($d(F_V) = 0$). This procedure may appear arbitrary, but it is based on experimental data derived from previous work (4). In addition, we neither found a significant dependence of alveolar partial pressure of oxygen on levels of hemoglobin concentration of blood ($r = - 0.4$, $n = 23$, $P > 0.05$) nor did our volunteers show any risk factors with respect to respiratory diseases, permitting us to disregard ventilation.

The series resistance model of fractionating ($\alpha_i > 1.0$) as well as non-fractionating pathways ($\alpha_i = 1.0$) involved a rather rough estimate of values in $d(P_{av\,O_2})$. However, there was no overlap between ranges of $d(P_{av\,O_2})$, even on viewing the data of the patient who showed the biggest gain during transfusion (d[Hb] = 136 $g \cdot l^{-1}$ - 79 $g \cdot l^{-1}$ = 57 $g \cdot l^{-1}$). Although this patient showed an increase in hemoglobin concentration of 72 % and hematocrit rose by 54 %, we determined a decrease in $d(P_{av\,O_2})$ ranging between - 30 mmHg < $d(P_{av\,O_2})$ < - 13 mmHg. Since the reduction in the 2,3-DPG content of stored red blood cells does not revert until 6 to 12 hours after transfusion (9), the 2,3-DPG content should have been low since we performed the experiments no later than 1.5 hours after transfusion. Nevertheless, even in this

patient, the impediment of oxygen release from blood to tissues due to changes in the mean 2,3-DPG content was evidently made up for by the increase in the hemoglobin concentration of blood.

From the pathophysiology of anemia it is predicted that regulatory mechanisms should be able to prevent a detectable limitation of O_2 transport by blood flow until the hemoglobin concentration declines to values of 70 - 80 $g \cdot l^{-1}$. If applicable, and with respect to inequation (9), we should have detected a quite non-linear relationship between α_o^N and hemoglobin concentration, at least showing no significant change in α_o^N (or $d(P_{avO_2})$) within the normal range of [Hb] values. Contrary to this prediction, our findings indicate that even in our normal volunteers the respective value of α_o^N increased, or the oxygen partial pressure difference between arterial and venous blood decreased with increasing [Hb], and the contribution of blood flow to the overall resistance to O_2 transport was reduced still further.

The superiority of the combination of isotopic analysis and the series resistance model lies in its ability to provide a link between the observed change in isotopic composition of oxygen and the relative roles of fractionating and non-fractionating pathways to limit O_2 transport. After having investigated the influence of both convective pathways on α_o we are now able to continue the analysis of the entire O_2 transport system, focusing on the remaining, fractionating pathways.

References

1. Dejours P. - Respir. Physiol. 80: 155-162 (1990)
2. Feldman D.E., Yost H.T. jr. and Benson B.B - Science 129: 146-147 (1959)
3. Hsia C.C.W., Chuong C.J.C. and Johnson R.L. jr. - J. Appl. Physiol. 79 (3): 1039-1047 (1995)
4. Könen M., Heller H. and Schuster K.-D. - Pflügers Archiv 419: R 117 (1991)
5. Otis A.B. An overview of gas exchange. In: *Handbook of Physiology*, edited by L.E. Farhi and S.M. Tenney. Bethesda: American Physiological Society, 1987, Vol. 4, Section 3, p. 1-11.

6. di Prampero P.E. and Ferretti G. - Respir. Physiol. 80: 113-128 (1990)

7. Schuster K.-D., Pflug K.P., Förstel H. and Pichotka J.P. Adaption of respiratory mass spectrometer to continuous recording of abundance ratios of stable oxygen isotopes. In: *Recent Developments in Mass Spectrometry in Biochemistry and Medicine*, edited by A. Frigerio. New York: Plenum Publishing Corp., 1979, Vol. 2, p. 451-462.

8. Schuster K.-D. and Pflug K.P. - Adv. Exp. Med. Biol. 248: 151-156 (1989)

9. Simon T.L. Red cell transfusion. In: *Principles of Transfusion Medicine*, edited by E.C. Rossi and T.L. Simon. Baltimore: Williams & Wilkens, 1991, p. 95-98.

