EXPERIMENTAL GENETICS

Diurnal Rhythmic Fluctuations of the Oxygen Affinity of Hemoglobin in Premature Infants

G. V. Yatsyk, M. D. Mitish, E. V. Syutkina, A. E. Grigor'ev, and A. S. Abramyan

UDC 616.155.16-053.32-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 425-428, October, 1993
Original article submitted April 27, 1993

Key Words: *oxygen affinity of hemoglobin; cosinor analysis; premature infants*

The oxygen affinity of hemoglobin (Hb) is affected by a number of factors: type of Hb, temperature, pH of the blood, the blood content of 2,3-diphosphoglycerate (2,3-DPG), etc. [5]. The presence of fetal Hb (HbF), hypothermia, and alkalosis cause a leftward shift of the oxygen dissociation curve (ODC) of oxyhemoglobin; on the other hand, the presence of HbA, hyperthermia, acidosis, and an increase in the 2,3-DPG concentration are responsible for the shift of the ODC to the right. The position of ODC is characterized by the p_{50} value, that is, the oxygen tension providing for a 50% oxygen binding by Hb. The value of p_{50} is usually determined in a single sample of the patient's blood, and a number of methods of p_{50} determination have been described [2,6,10,14]. Invasiveness is the major drawback of all above methods, making determination of p_{50} in its dynamics difficult or impossible, especially in neonates.

According to published data, p_{50} is lower (19.8-22 mm Hg) in neonate than in adults (27.0±1.1 mm Hg); it increases gradually throughout the first year of life [4,12]. Diurnal rhythms of fluctuations in the pH, pCO_2, pO_2, and SO_2 have been described for the blood of premature infants [9]. This attests to possible diurnal alterations of the oxygen affinity of Hb. The aim of the present study was to investigate the daily time course of p_{50} in premature infants.

Department for Premature and Newborn Infants, Institute of Pediatrics, Russian Academy of Medical Sciences, Moscow. (Presented by M. Ya. Studenikin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Sixteen premature infants with mild and moderate perinatal encephalopathy were followed up; one infant was examined twice. Seven infants were first-borns and nine were from second or later deliveries, including one case of cesarean section. During the follow-up, 12 infants received oxygen therapy. A brief clinical description is presented in Table 1. All measurements were noninvasively performed *in vivo*. The tension of blood gases ($tcpO_2$ and $tcpCO_2$) was transcutaneously measured with the aid of a TCM-222 Radiometer monitor (Denmark) at a transducer temperature of +44°C [11]. The electrode placements were changed every 4-5 h to eliminate the risk of skin burn. The data were continuously recorded on paper tape moving at a speed of 0.5 cm/min, and then the records were manually marked off in 2-min intervals and stored in an IBM PC/AT. The $tcpO_2$ and $tcpCO_2$ were recorded over 24 hours.

0007-4888/93/0010-1290$12.50

Every 15-20 min over 24 h the oxygen saturation of the arterial blood (SpO_2) was recorded with a noninvasive Pulsemate Colin BX-5 monitor (Japan).

The simultaneously measured values of $tcpO_2$ and SpO_2 were approximated to the standard ODC [14] using the method of least squares by varying the "effective" pH and by correcting for the difference between the temperatures of the body and the transducers; on this basis p_{50} was determined. SpO_2 values not exceeding 95% were included in the calculations.

Such a method of p_{50} determination was verified by comparing its results with the p_{50} values of blood samples [10] obtained postoperation from 6 patients, aged 7 to 42, hospitalized in the Department of Wounds and Wound Infection of the A. V. Vishnevskii Institute of Surgery. For recording of the $tcpO_2$ and SpO_2, a single blood sampling was performed, and the p_{50} values were determined at 37°C on a Stat Profile Nova Biomedical device (Austria), and then converted to body temperature; SO_2 was measured with an OSM-2 hemoximeter; p_{50} was calculated using Oxygen Status Algorithm software (Radiometer, Denmark). The mean p_{50} values determined by the two methods did not differ at $p>0.1$ (Table 2), this being evidence of a sufficiently high accuracy of the noninvasive method.

To reveal the circadian (CD) and most pronounced ultradian (UD) rhythms, the p_{50} values determined for consecutive 2-h intervals, as well as the $tcpO_2$, $tcpCO_2$, and SpO_2 values obtained were processed using the method of unit cosinor analysis [8] by determining their amplitudes, mesors, and acrophases. The rhythms were regarded as statistically reliable if the hypothesis of null amplitude was rejected at a significance level of 0.05.

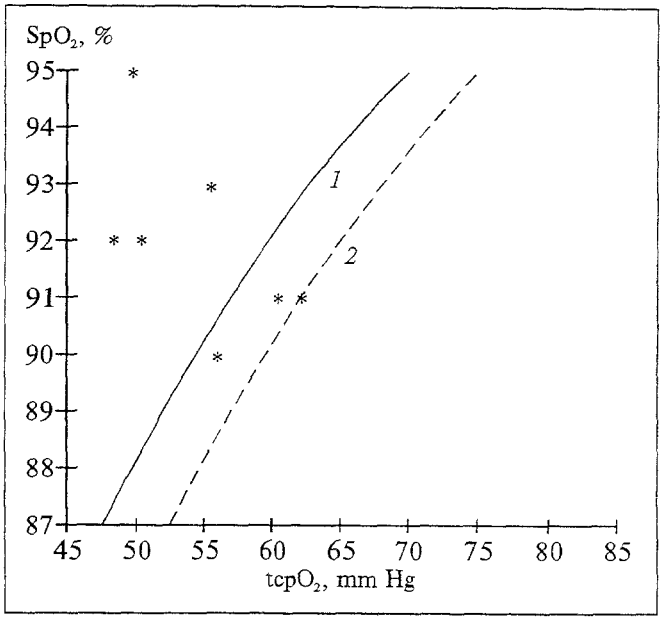

Fig. 1. Oxygen dissociation curve in a premature infant over a 2-h interval. *1*) standard dissociation curve; *2*) approximation curve.

RESULTS

This approximation enabled us to plot statistically reliable ($p<0.05$) ODC for each time interval in all the infants examined (an example of such a curve is shown in Fig. 1). The p_{50} values lay in the range of 16.6 to 36 mm Hg. In most cases reliable UD or CD rhythms were encountered; an infradian rhythm with a 35-h period was strongly expressed in one infant.

TABLE 1. Summary of Clinical Characteristics of Premature Infants

	Age, days	Apgar score	Weeks gestation	Sex	Birthweight, kg	Oxygen therapy
acht	21	6/7	34	F	2.30	–
bnd	7	7/8	34	M	2.20	+
dmh	2	6/7	36	M	2.50	+
gln	5	4/5	36	F	3.00	+
hau	11	7/7	33	F	1.89	+
ign	4	7/7	33	F	2.00	+
kob	4	7/8	35	M	2.75	+
koc	7	8/9	36	F	1.95	–
krh	6	7/7	36	F	2.45	+
lub	4	7/8	37	M	2.15	+
mor	3	6/7	35	M	2.15	+
mor2 12	6/7	35	M	2.15	+	
shr	6	6/7	36	F	2.40	+
sko	5	7/8	36	F	2.55	–
sld	5	7/8	37	F	2.40	–
sta	24	6/7	30	M	1.85	+
vls	5	7/7	36	F	2.10	–

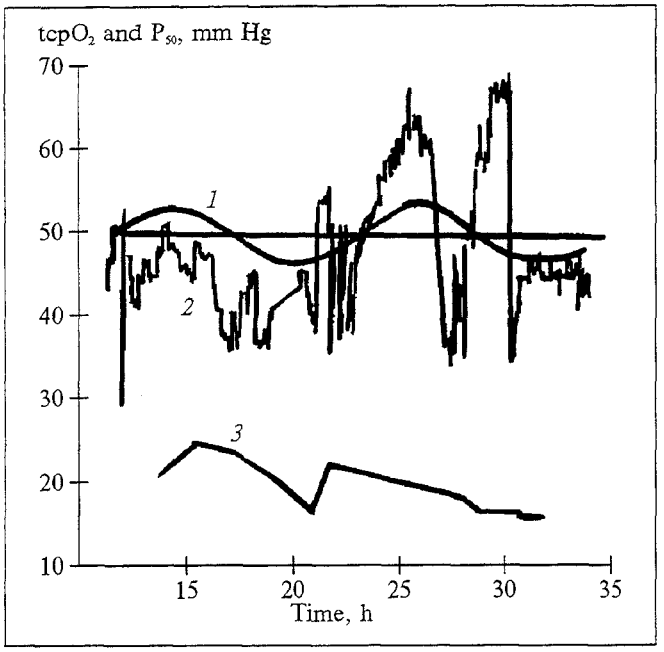

Fig. 2. Twelve-hour rhythms of $tcpCO_2$ and p_{50}. *1*) $tcpCO_2$ rhythm; *2*) $tcpCO_2$ throughout recording; *3*) p_{50} throughout recording.

The parameters of the most marked rhythms of p_{50} are presented, along with the parameters of similar rhythms of $tcpO_2$, $tcpCO_2$, and SpO_2 in Table 3. As is seen, the mesors of the most pronounced rhythms of p_{50} lay in the range of 18.2-33.7 mm Hg; the mean value of the mesor was 24.6±4.2 mm Hg. Fluctuations of p_{50} in the same infant throughout the period of measurements were considerable: the double amplitude of the most pronounced rhythm lay in the range 2.92-17.4 mm Hg, i.e., 12.7-71.2% of the mesor. Reliable rhythms of $tcpO_2$, $tcpCO_2$, and SpO_2 with periods corresponding to the most pronounced rhythm of p_{50} were found in all the infants; the parameters of these rhythms are presented in Table 3.

In the infants who received oxygen therapy, the amplitude of the most marked rhythm of p_{50} (3.8±1.9 mm Hg) was higher than that in the infants not receiving oxygen (2.4±0.6 mm Hg, $p<0.05$). A tendency toward an increase of the mesor was also observed for oxygen therapy: this increase constituted 25.5±4.5 mm Hg (vs. 21.5±1.2 mm Hg in infants not receiving oxygen).

TABLE 2. Values of p_{50} (mm Hg) in Patients during Postoperative Period Obtained Using the Traditional Method (I) and Calculated on the Basis of Measurements of $tcpO_2$ and SpO_2 (II)

Patient	Age, years	I	II
T. S.	30	24.2	21.55
P. A.	27	25.4	21.01
A. D.	7	24.9	25.61
K. I.	28	25.0	24.25
P. V.	33	24.9	20.50
Zh. D.	27	24.6	26.77
Mean		24.8±0.4	23.28±2.4

It should be mentioned that in the infants not receiving oxygen a positive correlation was revealed between all the parameters of the rhythms of p_{50} and $tcpO_2$: the correlation coefficient for the amplitudes was 0.89 ($p<0.05$), for the acrophases 0.98 ($p<0.01$), and for the mesors 0.96 ($p<0.01$). Thus, fluctuations of oxygen tension in the blood were to a large extent determined by the changes of the oxygen affinity of Hb. In infants breathing with oxygen during the examination, only a positive correlation between the mesors of p_{50} and $tcpO_2$ (correlation coefficient 0.96, $p<0.001$) was observed; the time characteristics and the intensity of rhythmic alterations in both parameters did not depend on each other, this indicating that the oxygen supplied from outside contributed greatly to the p_{50} value of the blood.

The values of p_{50} determined in the morning (the traditional time for blood sampling) markedly differed from the mesor of the most marked rhythm in a given infant: the differences between them varied from -2.11 to 3.11 mm Hg (1.6±2.1 mm Hg, on average).

The values of p_{50} calculated for consecutive 2-h intervals correlated positively with the mean $tcpCO_2$ values for the same time intervals in 10 infants: in different infants the increase of p_{50} varied from 0.07 to 3.18 mm Hg for an increase of $tcpCO_2$ by 1 mm Hg. In all the infants the dependence was described by the equation:

$$p_{50} = 0.31 \times tcpCO_2 + 13.09$$
$$(r=0.2695;\ n=75;\ p<0.05).$$

In 7 cases a synchronicity of the rhythms of p_{50} and $tcpCO_2$ was discovered (Fig. 2). In these infants the difference between the acrophases was 0.06±1.1 h. In 6 infants the $tcpCO_2$ maximum was reached 7.6±3.6 h ahead of the p_{50} maximum, while in 3 infants the opposite relationship between the acrophases was observed: the p_{50} maximum was noted 6.2±1.4 h prior to the $tcpCO_2$ maximum. Overall, for all the infants the correlation between the acrophases of the most pronounced rhythms of p_{50} and of the corresponding rhythms of $tcpCO_2$ was slightly below the statistically significant level.

A correlation was established between the amplitude of the most marked p_{50} rhythm and the birthweight (correlation coefficient 0.70; $p<0.01$), this reflecting a dependence between the oxygen affinity of Hb and the degree of maturity of the infant.

Determination of p_{50} in infants during the postdelivery period has interested researchers and cli-

TABLE 3. Double Amplitude (2A) and Mesor (M) of the Most Pronounced Rhythm of p_{50} Fluctuations (with period T), and Amplitude and Mesor of the Corresponding Rhythms of $tcpO_2$, $tcpCO_2$, and SpO_2 in Premature Infants

	Period T, h	p_{50}, mm Hg		$tcpO_2$, mm Hg		$tcpCO_2$, mm Hg		SpO_2, mm Hg	
		2A	M	2A	M	2A	M	2A	M
acht	18	6.54	21.99	8.3	70.9	1.4	39.6	1.1	93.0
bnd	13	4.06	21.64	4.2	66.7	5.7	37.9	0.6	93.1
dmh	22	10.86	29.28	7.5	79.4	4.0	42.6	3.3	89.2
gln	16	17.04	23.91	7.4	68.4	1.6	39.9	1.2	90.8
hau	9	4.90	18.20	5.7	62.0	0.7	32.4	0.5	94.5
ign	9	7.56	24.85	9.2	75.4	4.2	34.4	1.4	92.9
kob	24	11.94	27.44	7.4	77.0	3.1	52.0	0.4	94.6
koc	19	5.08	27.52	8.3	86.8	5.0	37.8	0.4	92.6
krh	10	7.48	27.91	2.1	78.8	3.9	42.0	2.4	91.5
lub	17	3.86	20.31	6.7	63.6	2.2	42.1	0.9	91.1
mor1	35	5.76	33.77	11.9	98.8	2.9	41.3	0.8	91.9
mor2	24	8.22	23.55	5.8	73.2	0.6	42.7	0.6	95.2
shr	18	4.50	30.49	4.5	88.3	0.7	39.9	1.5	92.2
sko	3	2.92	22.88	1.8	75.2	0.7	37.7	1.8	94.4
sld	10	4.86	20.88	7.4	60.5	1.5	48.2	0.8	92.1
sta	7	3.84	24.44	1.7	71.5	1.4	40.0	0.5	91.8
vls	7	4.40	20.25	6.5	62.9	3.3	42.3	0.8	93.9

nicians since the discovery that the oxygen affinity of HbF is higher than that of HbA [1]. In studies of the nature of ODC in the neonate, specificities of the interaction between HbF and 2,3-DPG have been an aspect of primary consideration [3,13]. To date, p_{50} has been regarded as a constant quantity characterizing the oxygen affinity of Hb on the day of investigation; the main emphasis has been on its dynamics (age-dependent or in relation to the changes of clinical status).

The use of the noninvasive method enabled us to visualize diurnal fluctuations of the oxygen affinity of Hb. These proved to be considerable, showing that there are intervals, when the conditions of oxyhemoglobin dissociation are impaired, so that oxygen delivery to the tissues is hindered, and, on the other hand, there are times when the oxygen-binding capacity of Hb is lowered.

The presence of a correlation between p_{50} and $tcpCO_2$, as well as of a synchronicity of the rhythms of these parameters in a number of infants, demonstrate the impact of the tension of carbon dioxide on the shape of ODC. The question as to what causes the rhythmic changes of p_{50} in the remaining infants is still to be investigated.

Values of p_{50} from 18 to 29.5 mm Hg (mean values 25.1±2.7 mm Hg), i.e., close to the mesor values obtained by us, were previously discovered in premature infants with bronchopulmonary dysplasia of the same postconception age as in the present study [7]. On the basis of our investigation performed on a relatively small group of infants, it is hardly possible to speculate whether the p_{50} fluctuations are beyond the range of physiologically normal values. One may just note that in the infant with the highest p_{50} mesor (mor1), oxygen therapy mainly caused an increase in the $tcpO_2$; the oxygen saturation of Hb remained on a relatively low level. On the other hand, in the infant with the lowest p_{50} mesor (hau) a sufficiently high level of SpO_2 but a low oxygen tension were observed against the background of breathing with oxygen.

One may assume that noninvasive p_{50} determination in real time along with transcutaneous recording of the tension of blood gases and of the oxygen saturation of Hb makes it possible to defined more precisely the range of normal parameters of the latter and the clinical parameters beyond the normal range.

Thus, the oxygen affinity of Hb in premature infants is not constant over 24 h; it undergoes rhythmic fluctuations, the amplitude of which attains 12.7-71.2% of the mesor. Rhythmic fluctuations of CO_2 tension in the blood of premature infants markedly affect the rhythms of p_{50}.

REFERENCES

1. K. T. Anselmino and F. Hoffman, *Arch. Gynak.*, **143**, 477 (1930).
2. P. Astrup, *Lancet*, **101**, 1152-1154 (1964).
3. E. G. Brown, G. J. Mendoza, F. A. Chervenak, *et al.*, *Amer. J. Obstet. Gynecol.*, **162**, № 1, 223-229 (1990).
4. M. Delivoria-Papadopoulos, N. P. Roncevic, and F. A. Oski, *Pediat. Res.*, № 5, 235-245 (1971).
5. K. Dziedzic and D. Vidyasagar, *Clin. Perinatol.*, **16**, № 1, 177-197 (1989).
6. M. Edwards and R. Martin, *J. Appl. Physiol.*, **21**, 1898-1902 (1966).

7. S. Furfaro, J. Prosmanne, and H. Bard, *Biol. Neonate*, **57**, 72-76 (1990).
8. F. Halberg, *Ann. Rev. Physiol.*, **31**, 675-725 (1969).
9. D. C. Hillman, Z. Wang, J. Rigatuso, *et al.*, *Biochimica Clinica*, **15**, 151-154 (1991).
10. W. Kasinger, R. Huch, and A. Huch, *Scand. J. Clin. Lab. Invest.*, **41**, 701-707 (1981).
11. P. N. Le Soufe, A. K. Morgan, and L. P. Soutter, *Acta Anaesth. Scand.*, **68**, 91-97 (1978).
12. F. A. Oski and M. Delivoria-Papadopoulos, *Pediatrics*, **48**, 853-856 (1971).
13. F. A. Oski, *Crit. Care Med.*, № 7, 412-418 (1979).
14. W. Severinghaus, *J. Appl. Physiol.*, **21**, 1108-1116 (1966).

Effect of Transcutaneous Irradiation of the Cubital Vascular Bundle with He-Ne Laser on Blood Rheology

N. R. Paleev, V. I. Karandashov, M. A. Voronina, and I. A. Fin'ko

UDC 615.849.19.06:616.151.5].07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 10, pp. 428-430, October, 1993
Original article submitted May 18, 1993

Key Words: *He-Ne laser; blood rheology*

Therapy with low-energy He-Ne laser has been in use for more than 30 years. *In vitro* investigations, experiments on animals, and clinical studies have shown that monochromatic coherent radiation of He-Ne laser (HNL) activates the immune system, the biosynthesis of ATP, RNA, proteins, and prostaglandins, and stimulates cell proliferation [4,15]. These effects have made it possible to use HNL in the treatment of suppurative-inflammatory diseases, trophic ulceration, and gastric or duodenal ulcers [5,6]. In recent years HNL has come into use for the treatment of ischemic heart disease and chronic failure of the arteries of the extremities [8,10]. Here, great importance in explaining the mechanism of the biological effect of HNL on the organism is attributed to vascular reactions and to changes in the blood rheology. Koslov *et al.* [7] showed the stimulating effect of HNL on the microcirculation. According to the data of Aleinikov *et al.* [1], laser treatment blocks the efferent innervation of the vessels, resulting in vasodilation.

Cardiopulmonological Department, M. F. Vladimirskii Moscow Region Research Institute of Clinical Investigations, Moscow

In studies of the effect of HNL on blood rheology Lysov observed a decrease in blood viscosity, increased deformability of erythrocytes, and suppressed platelet aggregation. These results were obtained for intravascular irradiation of the blood with the aid of flexible light pipes [9].

We studied changes in the blood rheology for another method of irradiation, namely transcutaneous. The method is based on the extremely high permeability of living tissue for red light.

MATERIALS AND METHODS

Rapid effects of HNL were investigated, i.e., the blood samples before and immediately after irradiation were compared. A total of 37 procedures of transcutaneous laser irradiation of the blood (TLIB) were performed in 12 patients with acute pneumonia. The source of radiation was a continuous-mode LG-79-2 HNL, power 17 mW. The duration of irradiation of the cubital vascular bundle was 40 min.

Blood viscosity was determined on a Rotovisco-100 rotary viscosimeter (Germany). The osmotic resistance of erythrocytes was studied after Deich. The elastic properties were assessed as the

0007-4888/93/0010-1294$12.50