OPEN ACCESS

GAVIN PUBLISHERS

Review Article

Morpho-Biophysical Study of Erythrocytes of Intact and Vagotomized Rats at Various times after Acute Blood Loss

Tsybulevsky Alexander Yuryevich[1], Dubovaya Tatiana Kleonikovna[1], Maksina Alexandra Genrikhovna[1], Ashrafova Tamilla Raufovna[2], Puzikov Alexander Mikhailovich[2], Lychkova Alla Eduardovna[3*]

[1]Russian National Research Medical University named after N.I. Pirogov, Moscow, Russia

[2]Center AMG-ESTETIC, Moscow, Russia

[3]GBUZ Moscow Clinical Research Center named after A. S. Loginov DZM, Moscow, Russia

*__Corresponding author:__ Lychkova Alla Eduardovna, GBUZ Moscow Clinical Research Center named after A. S. Loginov DZM, Moscow, Russia

Summary

The acute massive hemorrhage (35-37 % of the blood volume) at rats is accompanied by changes of morphological (diameter, the area, polarizations, the form-factor, integrated and specific absorbency) and biophysical (a relief of a surface and microviscosity of a lipide phase of plasmolemma) characteristics of erythrocytes. Thus character and dynamics of response of erythron initially intact and vagotomized (14 days after operation) animals essentially differ: the former demonstrate significant changes in 3 – 10 h and 240 h and the latter in 0,5 h and 96 h.

Introduction

Acute massive blood loss is one of the most common pathological conditions [1]. Some aspects of it require further research, in particular, the state of red blood cells after blood loss in conditions of impaired innervation of the abdominal organs. This is due to the fact that vagotomy is used as a component of surgical intervention in the surgical treatment of perforated bleeding duodenal ulcers [2]. Based on the leading role of hypoxia in the pathogenesis of posthemorrhagic syndrome [3].

The Aim

Is to study the state of erythrocytes in intact and vagotomized rats in dynamics after massive blood loss.

Materials and Methods

The experiments were carried out on 75 mongrel male rats weighing 200-250 g. 4 groups of animals were studied: 1st - intact; 2nd - animals 14 days after bilateral sub-fragmentary stem vagotomy; 3rd and 4th, respectively, intact and denervated rats that suffered blood loss. Bloodletting was carried out from the jugular vein in the amount of 35-37% of the total blood volume. The material for the study was taken through 0,25; 0,5; 3; 10; 24; 96 and 240 (10 days) h after blood loss. The diameter, area, integral and specific optical density (an indicator indirectly reflecting the saturation of erythrocytes with hemoglobin), polarization, erythrocyte shape factor, values of anisocytosis and anisochromia were determined on unpainted blood smears fixed in formaldehyde vapor using computer morphodensimetry (on the complex of automated microscopy MECOS-C2). the percentage of echinocytes and stomatocytes. For a generalized characterization

of morphological changes in erythrocytes, taking into account the totality of the studied parameters, a complex integral indicator was calculated [4]. Using the spin probe method, the microviscosity of the lipid bilayer and the relief of the surface of the erythrocyte membrane were studied (24 hours after blood loss). To do this, the suspension of erythrocytes was incubated with one of three probes: spin-labeled analogues of stearic acid with a nitroxyl fragment at the fifth (probe 1) or fourteenth (probe 2) carbon atom of the acyl chain and spin-labeled benzocarboline (probe 3). It is significant that probes 1 and 2 are embedded in the lipid bilayer in such a way that the carboxyl group is located on its surface, and the fatty acid chain is immersed in the bilayer parallel to the acyl chains of phospholipids. At the same time, the radical fragments of the probes are located at different depths (0.6-0.8 nm and 2.0-2.2 nm, respectively). Probe 3 is localized in the area of peripheral plasmolemma proteins. According to the kinetics of the restoration of its signal by potassium ferricyanide, the relief of the surface of erythrocytes was judged. The indicator of the quenching rate of the probe is the tangent of the angle of inclination of the kinetic line with respect to the horizontal axis (tg α). The greater the tg α, the faster the signal quenching occurs (the more accessible the probe is) and, consequently, the looser the glycocalyx of the plasma membrane of the erythrocyte is. To characterize the behavior of spin probes in the membrane, an ordering parameter calculated from the spectra of electronic magnetic resonance and characterizing the mobility of acyl chains in the localization region of the nitroxyl fragment

of the probe was used. In this case, parameter S1 characterizes the ordering of the membrane at a depth of 0.6 – 0.8 nm from the surface, parameter S2 - at a depth of 2.0 – 2.2 nm. The obtained data were subjected to statistical processing using the Fischer-Student method [5].

Results

Massive blood loss in initially intact rats is accompanied by a significant increase in the average diameter, erythrocyte area, polarization and shape factor (0.5; 3; 10; 24 and 240 h), as well as their integral (3, 10, 240 h) and specific (240 h) optical density (Table 1). There was also a tendency to increase the specific proportion of echinocytes (10 h) and stomatocytes (96 h). Statistically significant deviations of anisocytosis and anisochromia were not found. The calculation of the complex integral index showed that the most pronounced changes in the morphology of erythrocytes are noted after 3-10 h and 240 h. Under these conditions, regular changes in the physico-chemical characteristics of erythrocytes were revealed. Thus, a decrease in the ordering parameter was registered for probes 1 and 2, which indicates a decrease in the microviscosity of the lipid phase along the entire profile of the erythrocyte membrane (Table 2). Analysis of the kinetics of restoring the signal of probe 3 showed that pronounced transformations of the relief of the erythrocyte surface were noted against the background of blood loss.

Deadlines (hour)	0(n=5)	0.25 (n=5)	0.5(n=4)	3(n=5)	10(n=5)	24(n=5)	96(n=5)	240(n=5)
Average diameter (microns)	6.28±0.01	6.35±0.15	6.45±0.17*	6.55±0.08*	6.52±0.12*	6.41±0.06*	6.37±0.32	6.75±0.06*
Square (mm2)	31.10±0.09	31.77±1.55	32.79±1.75*	33.85±0.87*	33.57±1.20*	32.35±0.56*	32.16±3.20	35.96±0.63*
Polarization	0.050±0.01	0.058±0.01*	0.053±0.01	0.064±0.01*	0.064±0.01*	0.051±0.01	0.059±0.01*	0.055±0.01
Form Factor	14.72±0.52	13.94±0.10*	14.05±0.17*	16.70±1.01*	16.39±0.60*	15.49±0.30*	14.92±0.17	15.20±0.10*
Integral optical density	5.84±0.18	6.40±0.91	6.28±0.77	6.52±0.56*	6.57±0.36*	5.99±0.32	6.07±0.33	7.68±0.57*
Specific optical density	0.188±0.01	0.200±0.02	0.191±0.02	0.193±0.02	0.196±0.01	0.186±0.01	0.191±0.02	0.213±0.01*
Anisocytosis	6.6±0.2	6.8±0.2	6.6±0.2	6.0±0.1	7.2±0.4	6.4±0.6	6.6±1.6	8.0±0.6
Anisochromia	18.4±1.3	21.4±0.2	19.4±0.6	17.2±0.4	17.2±0.4	15.4±0.6	17.0±2.4	21.0±2.0
Complex integral indicator	0	0.19	0.13	0.31	0.3	0.21	0.18	0.39

Table 1: Morphological parameters of erythrocytes of intact rats at various times after blood loss, (m±m).

Series	Parameters		
	S_1	S_2	tg α
Intact rats	0.639 ± 0.09	0.516 ± 0.014	0.0105
Intact rats + blood loss	0.587 ± 0.011*	0.467 ± 0.006*	0.0394
Vagotomized rats	0.636 ± 0.013	0.519 ± 0.005	0.0112
Vagotomized rats + blood loss	0.614 ± 0.003*	0.508 ± 0.008	0.0289

Table 2: Biophysical parameters of the plasma membrane of erythrocytes at various times after blood loss.

It was found that vagotomy is accompanied by an increase in the average diameter, area and integral optical density of erythrocytes (Table 3). At the same time, no significant changes in the microviscosity of the lipid bilayer and the relief of the surface of the erythrocyte membrane were detected. The dynamics of morphological parameters of erythrocytes in denervated animals under conditions of blood loss is characterized by a number of peculiarities compared to those in intact rats. Thus, the average diameter and the area of erythrocytes significantly decrease after 0.5 h, while in intact cells they increase. Similarly, the polarization indices (after 3 and 10 hours) and the shape factor (0.5 and 10 hours) of erythrocytes are changed. Unlike intact rats, blood loss in vagotominated animals does not lead to a significant increase in the integral optical density of erythrocytes (with the exception of 240 h) (Table 3). The restructuring of the qualitative composition of the population of erythrocytes of de-nerved rats also has certain features: after 0.5 h, there is a tendency to increase the content of stomatocytes, and after 10 hours - echinocytes. The greatest deviations of the complex integral indicator of erythrocyte morphology were registered after 0.5-3 hours and 96 hours. There were no significant differences in the changes in the physico-chemical characteristics of erythrocytes under conditions of acute blood loss in vagotomized rats compared to the initially intact ones. One can only note a slightly smaller degree of changes in the surface profile of the erythrocyte plasmalemma in rats subjected to vagotomy.

Deadlines (hour)	0 (n=5)	0.25 (n=5)	0.5 (n=4)	3 (n=5)	10 (n=5)	24 (n=5)	96 (n=5)	240 (n=5)
Average diameter (microns)	6.59±0.01**	6.49±0.02	5.86±0.02*	5.68±0.02	6.66±0.01	6.54±0.01	6.76±0.02	6.94±0.01*
Square (mm2)	34.30±1.97**	33.24±2.74	27.25±3.80*	25.70±4.90	35.03±1.91	33.71±1.93	36.11±1.32	38.04±0.72*
Polarization	0.051±0.01	0.062±0.01	0.053±0.01	0.056±0.01	0.052±0.01	0.053±0.01	0.061±0.01	0.047±0.01
Form Factor	14.55±0.26	15.97±0.89*	14.68±0.24	14.59±0.21*	14.27±0.12	16.02±1.13*	16.22±0.23*	14.90±0.72
Integral optical density	6.64±0.59**	6.33±0.63	5.74±0.42	5.65±0.59	6.91±0.59	6.08±0.21	7.82±0.96	8.00±0.46*
Specific optical density	0.193±0.01	0.191±0.01	0.215±0.02	0.226±0.03	0.197±0.01	0.181±0.01	0.216±0.02	0.210±0.01
Anisocytosis	6.8±0.2	7.0±0.4	7.4±0.2	7.8±0.8	6.5±0.4	6.6±0.2	7.5±0.3	8.3±0.4
Anisochromia	20.8±1.0	17.6±1.8	14.6±0.6	17.0±0.8	18.0±0.8	17.4±0.8	16.2±0.6	21.0±1.0

Complex integral indicator	0	0.28	0.55	0.51	0.17	0.25	0.41	0.3

Table 3: Morphological parameters of erythrocytes of vagotomized rats at various times after blood loss, (m=m).

Conclusion

The increase in the average diameter and area of red blood cells during blood loss is most likely due to the activation of erythropoiesis and the accelerated flow of reticulocytes from the red bone marrow into the bloodstream, which are known to be large in size compared to normocytes [6]. At the same time, there is a distortion of the shape of red blood cells, as evidenced by an increase in the values of polarization and shape factor. The probable cause of the change in the geometric characteristics of erythrocytes may be a decrease in the microviscosity of the lipid phase of their plasmolemma, as indicated by a decrease in the order parameter for probes 1 and 2. This phenomenon is presumably based on violations of the chemical structure and physico-chemical characteristics of the erythrocyte membrane. Thus, it is known that under conditions of blood loss, activation of free radical oxidation of lipids of the erythrocyte membrane is observed [7] and the restructuring of the lipid composition towards an increase in the proportion of acid phospholipids and cholesterol [8]. Plasma lipoproteins can also make a certain contribution to the change in the lipid profile of the erythrocyte membrane. It is believed that as a result of their interaction with the erythrocyte membrane, the content of cholesterol and triacylglycerols in its lipid matrix changes [8]. Lipid peroxidation products, biogenic amines and other physiologically active substances, the content of which in the blood significantly increases with blood loss, can be considered as the most likely agents leading to such changes [9]. It is suggested that shifts in the chemical composition of the lipid matrix of the erythrocyte plasmolemma and related changes in the viscoelastic properties of these structural elements can be considered as a non-specific inflammatory reaction of the body to hypoxia of various etiologies. The fact is that an increase in the content of acid phospholipids in the membrane improves its permeability to respiratory gases, and an increase in cholesterol concentration leads to an increase in the total surface area, which ultimately increases the efficiency of the gas transport function of erythrocytes [10,11]. Also an important element of the response of the blood system to blood loss is an increase in the hemoglobin content in red blood cells. In our study, this is indirectly indicated by an increase in the specific optical density of erythrocytes. It should be borne in mind that the development and outcome of this pathological condition significantly depends on the rate of elimination of "old" and defective erythrocytes [12]. Indeed, in our work and in the studies of other authors [13], an increase in the proportion of aging forms (echinocytes, stomatocytes, etc.) with blood loss has been demonstrated.

Assessing from a biological standpoint the revealed maladaptation processes in the blood system under conditions of blood loss, first of all it is necessary to point out the special role of hypoxia of the central nervous system and the disorders of integrative activity of the brain caused by it, as well as violations of the connections between the nervous, endocrine and immune systems [14]. The specificity of the erythron response to blood loss under vagotomy conditions studied by us can presumably be due to several causal factors. In particular, pathological stimulation from the central ends of severed vagus nerves, causing irritation of the corresponding nuclei of the hypothalamus, can disrupt the functioning of nearby nerve centers that regulate hematopoiesis. The validity of this assumption is indirectly confirmed by the results of studies in which local irritation of various nuclear structures of the hypothalamus was performed, which caused regular rearrangements of the state of red blood [15]. In addition, it should be taken into account that according to our data, the number of labrocytes in the denervation focus is significantly increasing [16]. The latter, as is known, along with other cells actively produce cytokines, some of which (interleukin-1, gamma interferon, tumor necrosis factor) are directly involved in the regulation of erythropoiesis [17]. Along with this, a certain contribution to the specifics of the development of the studied condition can be made by the "gastric" factor. It has been shown that va-gotomy is accompanied by significant changes in the state of the parietal cells of the gastric glands [18], which produce components of hydrochloric acid and produce an internal Castle factor (hydrochloric acid is necessary for the effective assimilation of iron, Cas-la factor - vitamin B12) [19]. In this regard, it can be assumed that in conditions of violation of parasympathetic innervation of the stomach, a relative deficiency of iron and cyancobalamin, important participants in erythropoiesis, develops. To this should be added the disorders of copper metabolism revealed during vagotomy [20], which also plays a certain role in the process of erythrocyte maturation [21]. In addition, vagotomy is usually accompanied by the development of gastrostasis [22], including in rats [23]. At the same time, it has been established that irritation of stomach mechanoreceptors leads to significant quantitative and qualitative changes in red blood parameters [24]. This circumstance suggests that the accumulation and prolonged stagnation of dense food masses in the stomach of vagotomized rats may affect the process of erythropoiesis by the above mechanism. Along with the "gastric" factor, the "renal" factor can also contribute to the determination of the specificity of the blood system response to cropopotomy under vagotomy conditions, since the kidneys are the main source of erythropoietins [25], and the vagus nerve participates in their innervation [26].

Based on the results obtained, the following conclusion can be reached. The transection of the vagus nerves is not accompanied by significant changes in the shape and relief of the surface of erythrocytes, as well as the microviscosity of the lipid phase of their plasma membrane. Acute massive blood loss leads to significant changes in the morphological and physico-chemical characteristics of erythrocytes in both naturally intact and vagotomized rats. At the same time, the dynamics of the erythron response in these groups of animals varies markedly. Hypoxia and its consequences are the main cause of the revealed morphofunctional changes in erythrocytes in intact and denervated animals against the background of blood loss. At the same time, the response of vagotomized rats to hypoxia has its own peculiarities. The latter may be due to the fact that the cutting of the vagus nerves leads to a violation of the interaction of integrating systems (nervous, endocrine and immune), as well as with a violation of the state of the organs involved in the denervation focus (stomach, kidneys, etc.) and, as a consequence, a violation of homeostasis. The results obtained deepen the understanding of the mechanisms of damage to red blood cells in various extreme situations, in particular, with blood loss.

References

1. Eliseeva II, Vasilyeva EK, Klupt MA et al. (2006) Demography and population statistics. M, Finance and Statistics 2006: 688.

2. Palmer K (2007) Acute upper gastrointestinal haemorrhage Br. Med. Bull 83: 307-324.

3. Yarochkin VS, Panov VP, Maksimov PI (2004) Acute blood loss. M, Medicine, 2004. 368s.22.

4. Zhukotsky AV, Yakubova NI, Kogan EM (1991) The use of personal computers in morphological diagnostics by methods of multidimensional statistics. Karaganda 1991: 34.

5. Lakin GF (1990) Biometrics. M, Higher School 7: 351s.

6. Vorobyev AI (2007) Manual of Hematology. M, 2007, Newdiamed, 1275 p.

7. Lazareva GA, Paskova IP (1999) Changes in the chemical composition and physico-chemical properties of erythrocytes after blood loss in the book: Man and his health. Kursk 1999: 67-69.

8. Kaplan OV (1995) Lipids of erythrocytes and the gas transport function of blood in acute blood loss Vopr. med. Chemistry 41: 23-25.

9. Matveev SB, Pakhomova GV, Kifus FV, Golikov PP (2005) Oxidative stress in open abdominal trauma with massive blood loss Clinic. lab. diagnostics 2005: 14-16.

10. Maryinskikh VV (2007) Investigation of the viscoelastic properties of erythrocyte membranes of mongrel white rats with different levels of motor activity in response to stresses of various etiologies and assessment of the deformability of erythrocytes of people with hypertension. Dis. Candidate of Medical Sciences, Tyumen, 2007.

11. Soloviev SV (1989) Deformability of rat erythrocytes in dynamics after blood loss Physiol. zh. USSR 75: 1780-1782.

12. Mikashinovich ZI (1984) Mechanisms of adaptive restructuring of erythrocyte energy metabolism in acute blood loss Pathol. physiol. and we will continue. therapy 1984: 12-15.

13. Radaev SM (2001) Structural and functional properties of erythrocytes in patients with severe trauma and blood loss. Dis. Candidate of Medical Sciences, M 2001.

14. Korzhura VL (2001) Neurobiological mechanisms of massive blood loss Anesthesiol reanimatol 2001: 51-53.

15. Kann EL (1976) Studies of the structural and functional organization of adaptive behavior management processes of the erythrocyte part of the blood system in the book: Problems of interoreception, regulation of physiological functions and behavior. L, Nauka 1976: 71-91.

16. Yu TA Regularities of the neurodystrophic process in the small intestine during vagotomy. Abstract of the dissertation of the Doctor of Medical Sciences. M 1999: 48.

17. Bunn H (2007)New agents that stimulate erythropoiesis Blood 109: 868-873.

18. Helander HF, Poorkhalkali N (1997) Immunohistochemical localization of gastrin/CCK-B receptors in the dog and guinea-pig stomach Scand. J. Gastroenterol 159: 313-320.

19. Aliev BM, Aruin LI, Gabunia RI (1978) Stomach. BME Soviet Encyclopedia 8: 92-141.

20. Tsibulevskiĭ AL (1981) On the role of the autonomic nervous system in the regulation of mineral metabolism. Izv. of the USSR Academy of Sciences (ser.biol) 1981: 518-525.

21. Rondanelli M, Opizzi A, Andreoni L, Trotti R (2006) Effect of treatment with a food supplement (containing: selected sea fish cartilage, vitamin C, vitamin E, folic acid, zinc, copper) in women with iron deficiency: double blind, randomized, placebo-controlled trial Minerva Med 97: 385-390.

22. Khoromsky LN, Benedict VV, Loiko IK (1990) The concept of functional gastrostasis after surgical treatment of peptic ulcer disease Klinich. Surgery 1990: 40-42.

23. Baibekov IM, Vorozheikin VM, Rizaev RM (1985) Changes in the mucous membrane of the duodenum and stomach in experimental duodenal ulcers and vagotomy Arch. Anatomy, Histology and Embryology 33: 69-72.

24. Chernihiv VN, Yu SS, Ya YA (1967) Regulation of erythropoiesis. L, Nauka 1967: 101s.

25. Macdougall IC (2008) Novel erythropoiesis-stimulating agents: a new era in anemia management Clin. J. Am. Soc. Nephrol 3: 200-207.

26. Ya PA, Ryabov SI, Timokhov VS (1983) Kidneys. BME, vol.20, M, Soviet Encyclopedia, 1983, pp.428-477.