LITERATURE CITED

1. T. T. Luk'yanov, Efficiency Suggestions and Inventions in Medicine [in Russian], Kiev (1974), pp. 89-91.
2. N. A. Plokhinskii, Mathematical Methods in Biology [in Russian], Moscow (1978).
3. A. P. Romodanov and O. V. Kop'ev, Vest. Akad. Med. Nauk SSSR, No. 12, 19 (1984).
4. A. P. Romodanov, Vopr. Neirokhir., No. 1, 13 (1986).
5. E. S. Severin and M. N. Kochetkova, Role of Phosphorylation in Regulation of Cellular Activity [in Russian], Moscow (1985).
6. V. A. Tkachuk and V. I. Baldenkov, Biokhimiya, No. 6, 1097 (1978).
7. J. R. S. Arch and E. A. Newsholme, Biochem. J., 158, No. 2, 603 (1976).
8. J. D. Corbin, P. H. Sugden, T. M. Lincoln, and S. L. Keely, J. Biol. Chem., 252, No. 11, 3854 (1977).
9. A. S. Fleischer, D. R. Rudman, N. S. Payne, and G. T. Tindall, J. Neurosurg., 49, No. 5, 650 (1978).
10. P. Greengard and R. G. G. Robison, Adv. Cycl. Nucleot. Res., 11, 131 (1979).
11. J. F. Krall, A. M. Schindler, and S. G. Korenman, Arch. Biochem., 187, No. 1 (1978).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, No. 2, 265 (1951).
13. T. Ueda and P. Greengard, J. Biol. Chem., 252, No. 14, 5155 (1977).
14. T. Ueda and P. Greengard, J. Biol. Chem., 252, No. 14, 5164 (1977).
15. A. White and T. Zenzer, Anal. Biochem., 41, No. 2, 372 (1971).

EFFECT OF ACUTE INTESTINAL OBSTRUCTION ON THE STATE OF ERYTHROCYTE MEMBRANES

V. I. Rusakov, N. A. Lukash, and M. S. Prokof'eva

UDC 616.34-007.272-036.11-07:616.155.1:576.314

KEY WORDS: erythrocyte membranes; proteins; amino acids; intestinal obstruction.

Despite much progress in the study of all aspects of acute intestinal obstruction (AIO) mortality from this disease remains high [4], due to the irreversibility of the pathological changes in the internal organs, leading to severe disturbances of activity of the internal organs. As the writers showed previously [1, 3], considerable changes in membrane permeability of erythrocytes and lysosomes take place even in the initial stages of AIO.

In order to shed light on the possible causes of these disturbances, it was decided to study the protein component of erythrocyte membranes during the development of experimental AIO.

EXPERIMENTAL METHOD

A model of AIO was creadted on noninbred albino rats under ether anesthesia. A loop of small intestine 4 cm away from Treitz' ligament was twisted through 360°C and the base of the loop was then fixed with a silk ligature. The rats were decapitated 2, 12, and 24 h after the operation. The results of the investigations were compared with an intact group and with a group of animals undergoing laparotomy only under ether anesthesia. All groups consisted of eight to 10 animals.

Erythrocyte membranes were isolated by the method described in [6]. Membrane proteins were obtained by the method of Wherret and Tower [10]. Amino-acid analysis of the proteins was carried out on a Hitachi KLA-3B automatic amino-acid analyzer after hydrolysis in 6 N HCl. Protein was determined by Lowry's method [8].

Professorial Surgical Department, Rostov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 4, pp. 435-437, April, 1989. Original article submitted March 1, 1988.

0007-4888/89/0004-0487$12.50

TABLE 1. Amino Acid Composition of Erythocyte Membrane Proteins (moles %) during Development of AIO

	Amino acids	Control	2 h after laparotomy	After AIO for 2 h	12 h after laparotomy	After AIO for 12 h	24 h after laparotomy	After AIO for 24 h
Basic	lysine	7,7±0,5	8,8±0,5	8,3±0,2	6,8±0,6	6,5±0,3*	8,5±1,3	8,8±0,6
	histidine	7,4±0,8	7,2±0,4	6,5±0,5	9,3±1,0	5,2±0,4**	7,2±1,1	7,2±1,0
	arginine	2,3±0,3	1,6±0,09	1,7±0,3	1,9±0,2	2,0±0,2	2,7±0,3	3,0±0,4
Acidic	aspartate	9,6±0,5	9,9±0,5	9,5±0,3	8,3±1,0	7,7±0,1*	9,5±0,8	8,5±0,8
	glutamate	7,4±0,1	5,8±0,6*	4,5±0,06**	5,0±0,3	5,2±0,5	7,7±0,7	8,1±0,7
Neutral	threonine	5,8±0,4	6,9±1,1	6,5±0,6	3,5±0,3	3,5±0,08*	5,0±0,4	4,7±0,3*
	serine	7,0±0,8	3,7±0,6	4,1±0,3	4,9±0,3	5,0±0,2*	7,1±0,1	6,4±0,3**
	proline	9,6±0,9	7,9±0,5	7,2±1,0	7,2±0,8	4,9±0,4**	9,8±0,8	8,3±0,7**
	glycine	10,6±0,8	10,1±1,0	11,8±0,4	12,5±1,8	13,5±0,8*	10,5±0,4	12,0±0,6**
	alanine	9,4±1,4	4,1±0,5	3,8±0,5	5,9±0,3	5,1±0,3*	11,1±1,4	8,4±0,2**
Hydrophobic	methionine	1,1±0,08	1,4±0,2	1,8±0,03*	1,5±0,3	1,5±0,2	1,3±0,3	1,8±0,5
	valine	9,8±1,6	13,5±0,7*	14,7±0,2**	10,5±0,7	11,1±0,3	7,8±0,2	5,0±0,2**
	isoleucine	2,0±0,2	1,2±0,1*	1,8±0,08**	3,7±1,1	3,9±0,8	2,3±0,2	5,1±1,8**
	leucine	12,0±1,0	8,9±0,3	9,5±0,05**	6,7±0,3*	13,1±0,8**	11,1±0,8	9,2±0,2*
	tyrosine	2,1±0,3	1,5±0,02	1,8±0,03	1,3±0,1*	1,4±0,5	2,4±0,5	2,1±0,2
	phenylalanine	6,5±0,8	4,1±0,3	4,6±0,2	3,8±0,3*	4,4±0,5	7,1±0,9	6,2±0,9

Legend. *) Changes compared with control are significant, **) changes relative to value after laparotomy are significant.

EXPERIMENTAL RESULTS

The amino acid composition of the membrane proteins is shown in Table 1. After AIO for 2 h there was a very small rise of the level of some hydrophobic amino acids (on average by 11%) and a fall in the glutamate level (by 22%) compared with the values of these parameters after laparotomy. Changes in levels of neutral amino acids were due entirely to the anesthesia and operative trauma. The ratio of hydrophilic amino acids (basic + acid + threonine + serine) to hydrophobic fell from 1.44 to 1.23, of total charged to nonpolar (valine + leucine + isoleucine + phenylalanine) from 1.21 to 1.02, and the coefficient of polarity [5] from 40.9 to 39.4. Subsequent development of the pathological process was accompanied by lowering of the level of basic amino acids on average by 24% and by an increase in concentration of hydrophobic amino acids by 29% compared with the corresponding values after laparotomy. The greatest changes were observed with histidine (a decrease of 44%) and leucine (an increase of 96%). Concentrations of neutral and acidic amino acids showed no significant change. The ratio of basic to acidic amino acids fell from 1.35 to 1.06, of total charged to nonpolar amino acids from 1.27 to 0.82, and of hydrophilic to hydrophobic from 1.44 to 0.92; the coefficient of polarity fell from 37.8 to 35.7. The concentration of neutral amino acids 24 h after creation of AIO had fallen by 8%, with the exception of histidine, whose level was raised by 14%. The concentration of hydrophobic amino acids on average did not change significantly, but the valine level fell by 36% and the isoleucine level rose by 122%. The coefficient of polarity in this case was unchanged. The ratio of total charged to nonpolar amino acids rose from 1.26 to 1.40.

The results are evidence that in the early stages of development of AIO the greatest changes are found in concentrations of hydrophobic and acidic amino acids, but in the later stages, in basic and neutral amino acids. One cause of the change in amino acid concentrations of membrane proteins could be changes in the distribution of proteins among the fractions.

The writers showed previously that the development of AIO is accompanied in time by a sharp change in the electrophoretic spectrum of erythrocyte membrane proteins: additional protein zones (29-32 in AIO compared with 20 on fractionation of erythrocyte membrane proteins from intact animals) appeared in the disk-electrophoretic spectrum. At the same time the protein concentration fell by 25-35% in the zones of migration of polypeptide with mol. wt. of over 90 kD and the protein concentration rose by 65% in zones of migration of polypeptides with mol. wt. of under 90 kD.

Thus during the development of AIO not only was the number of protein fractions increased, but so also was the fraction of low-molecular-weight proteins, and this was accompanied by a parallel decrease in the content of high-molecular-weight polypeptides.

The increase in concentrations of some amino acids (leucine, isoleucine, glycine) during AIO lasting 12 h and, more especially, 24 h may have been connected with a relative rise of

the level of low-molecular-weight membrane protein fractions, in which the concentrations of these amino acids are considerably higher than in the low-molecular-weight fraction [2], and also with an increase in the relative concentration of proteins in the membrane characterized by strong electrostatic and hydrophobic interactions with the lipid bilayer. In the later stages of development of AIO, when compensatory processes are weaker and intensification of lipid peroxidation (LPO) takes place [3], destruction of functional groups of several amino acids, sensitive to LPO products, may develop [7, 9]. The decrease in the concentrations of certain amino acids may also be connected with a disturbance of protein-lipid interactions and the more intensive solubilization of proteins from the membranes, and with destruction of the surface proteins by peptide hydrolases, whose activity is considerably increased in AIO [1]. The appearance of new protein zones and changes in amino-acid composition may be due to interaction of the surface of the erythrocyte membranes with blood plasma proteins [2]. Another cause of the observed disturbance may be changes in the relative proportions of erythrocytes of different ages, due to intensification of hemolysis, which the writers demonstrated previously during the development of AIO [4], or an increase in the fraction of damaged erythrocytes.

The changes observed in the structure of erythrocyte membrane proteins are evidently one cause of disturbance of the permeability of the membranes and, together with changes in peptide hydrolase activity, they play a pathogenetic role in the development of the irreversible disturbances associated with intestinal obstruction. The results indicate the need to use membrane protectors and antienzyme preparations for rational therapy in the postoperative period.

LITERATURE CITED

1. N. A. Lukash, M. S. Prokof'eva, and I. N. Mozharova, Izv. Sever. Kavkaz. Nauch. Tsent., 3, No. 1, 77 (1986).
2. N. A. Lukash, Izv. Sever. Kavkaz. Nauch. Tsent., 3, No. 1, 62 (1987).
3. A. H. Maddy (ed.), Biochemical Investigation of Membranes, London (1976).
4. V. I. Rusakov, É. S. Gul'yants, N. A. Lukash, et al., The Pathogenesis of Acute Intestinal Obstruction [in Russian], Rostov-on-Don (1985).
5. A. A. Sobol', V. F. Kudinov, B. G. Vetkovskii, and G. G. Miroshnichenko, Abstracts of Proceedings of the 14th Congress of Surgeons of the Ukrainian SSR [in Russian], Donetsk (1980), p. 144.
6. R. A. Capaldi and G. Vanderkooi, Proc. Natl. Acad. Sci. USA, 69, No. 4, 930 (1972).
7. M. Garvier, J. Pei, and P. Cornillet, Pathol. Biol., 24, No. 1, 31 (1972).
8. A. L. Goldberg and F. S. Boches, Science, 215, No. 4536, 1107 (1982).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall. J. Biol. Chem., 193, 256 (1951).
10. J. Wherret and D. Tower, J. Neurochem., 18, No. 6, 1027 (1971).