Structural Alterations of Rat Erythrocytes Induced by Phosphacol Poisoning

V. B. Prozorovskii, V. G. Skopichev, V. V. Petrov, and M. S. Dubrovnik

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 119, № 5, pp. 517-520, May, 1995
Original article submitted June 7, 1994

Nembutal-anesthetized rats under conditions of artificial respiration received intramuscular injections of phosphacol in doses equal to 0.5, 5, and 50 LD_{50}. The lung ventilation per se induced blood changes typical of hyperventilation alkalosis. Poisoned animals showed the development of dose-dependent tissue acidosis. At a later stage of poisoning (2 hours) a high phosphacol dose induced decompensated acidosis, which caused the death of some animals. Electron microscopy revealed that the previously described rat erythrocyte deformation coincides in time with the changes of cytoskeleton content and depends on the dose of phosphacol but not on the blood pH.

Key Words: *phosphacol; poisoning with organophosphorus compounds; erythrocyte deformation; erythrocyte cytoskeleton; acid-base state*

Earlier we showed that phosphacol poisoning induces erythrocyte deformation in mice. It was established in experiments *in vitro* that the deformation proceeds only as a result of the simultaneous effect of blood acetylcholine esterase inhibition and administration of acetylcholine in a concentration of more than 10^{-8} M [2]. A similar effect was observed after using substances that induce molecular cross-linking [1,4]. As in the case of normal erythrocyte to echinocyte transition [6], as well as in hereditary microspherocytosis [5,7], structural and quantitative alterations of the cytoskeletal proteins were detected [8]. It is also known that changes of erythrocyte shape can be observed during changes of physicochemical properties of the blood and alterations of blood cell metabolism [9].

The goal of the present work was to establish the relationship between the severity of intoxication, acid-base ratios in the blood, and structural alterations of rat erythrocytes after phosphacol poisoning.

Research Institute of Military Medicine, Russian Ministry of Defense; State University, St. Petersburg. (Presented by S. N. Golikov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats weighing 180-250 g. Animals were anesthetized with Nembutal and subjected to tracheotomy and cannulation of the left carotid artery. The heart rate (HR) was recorded on an RM 6000 polygraph (Nihon Kohden). Gas composition and alkali content in the blood were determined by a micromethod after Astrup on an ABL-330 apparatus (Radiometer). For electron microscopic study, blood was fixed with 2.5% glutaraldehyde solution, routinely dehydrated in alcohol, and dried by means of a CO_2 critical point drop. After gold dusting, scanning microscopy of specimens was performed in a Hitachi H-300 electron microscope. The content of erythrocyte cytoskeleton was estimated after Yu. S. Chentsov in our modification [3]. Preparations were analyzed on a Morfokvant apparatus (Karl Zeiss) using monochromatic light (540 nm). The cell integral optical density and size were recorded.

0007-4888/95/0005-0500$12.50

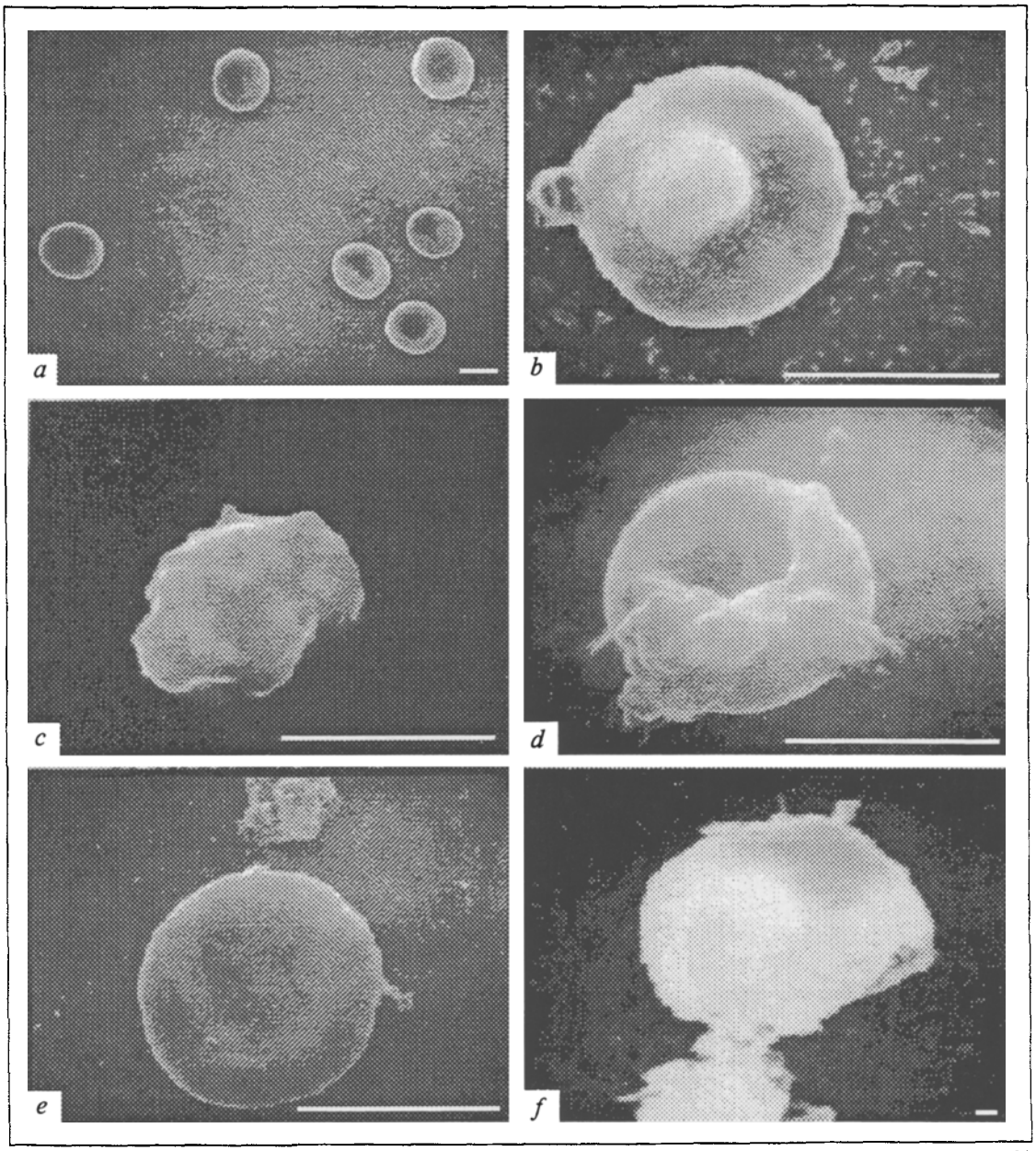

Fig. 1. Scanning electron microscopy of rat erythrocytes. *a*) control. Erythrocytes of intact rats; *b*) erythrocyte after 2-hour AR; *c*) erythrocyte 15 min after i.m. administration of phosphacol in a dose of 0.5 LD_{50} and the start of AR; *d*, *e*) erythrocytes after administration of phosphacol in a dose of 5 LD_{50} under the same conditions; *f*) erythrocyte after administration of phosphacol in a dose of 50 LD_{50} under the same conditions. Mark: 5 μ.

Before the start of the experiment the state of each animal was recorded to establish a background. Phosphacol was injected intramuscularly in doses of 0.5, 5, and 50 LD_{50}. Immediately after administration of the preparation, both injected and control rats were hooked up to the resuscitation apparatus with a respiration rate of 60/min.

The second estimation was performed 15 min after the start of artificial respiration (AR), and the 3rd after 2 hours, i.e., at times corresponding to the development of maximal intoxication and the alleviation of the acute state in rats having received phosphacol in a dose of 1 LD_{50} (0.62 mg/kg).

RESULTS

In the control animals (no phosphacol injection) all parameters underwent alterations 15 min after the start of the experiment, due to the reflex action of AR on the HR and to hyperventilation (Table 1). Hyperventilation was expressed not only in the marked drop in the partial CO_2 pressure (Pco_2), but also in the almost as rapid blood bi-

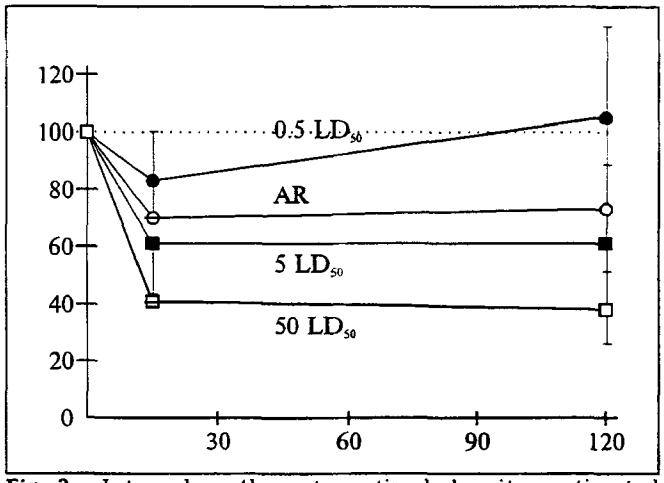

Fig. 2. Integral erythrocyte optimal density—estimated content of erythrocyte cytoskeleton (percentage of the control): dependence on the dose of phosphacol and duration of the experiment. Abscissa: time from the start of poisoning and AR; ordinate: cytoskeletal content, percentage of normal level.

carbonate increase in the control animals. This process is partially compensated by a rise of Po_2. Nevertheless, the blood pH increases from an initial level of 7.36±0.03 to 7.58±0.05 at the start of AR in the absence of poisoning.

A pronounced HR dependence on the phosphacol dose in the first 15 min of the experiment enables one to conclude that the cardiac rhythm in the first stage of poisoning is governed mostly by the tone of the parasympathetic part of the nervous system and, perhaps, by the blood acetylcholine concentration. The cholinesterase activity seems to be of no significance, since it is totally inhibited starting with the 5 LD_{50} dose.

An increase of the phosphacol dose to 5 and, especially, 50 LD_{50} leads to an increased compensation of respiratory alkalosis by a rise of tissue acidosis. As early as against the background of 5 LD_{50} the standard blood bicarbonate concentration drops to the initial level, while the standard alkaline surplus becomes negative. The blood pH meanwhile remains within the normal range. The compensatory mechanisms help maintain the blood pH at the normal level (7.33±0.03) during the first 15 min even after administration of phosphacol in a dose of 50 LD_{50}. However, 2 hours after injection of a dose of 5 LD_{50} the deficit of the standard alkaline content attains a marked level, i.e., the content drops from +5.4 to -15. Evidently, the alkaline reserves are depleted due to the appearance of abundant incompletely oxidized products of metabolism in the blood. Taking into account the high oxygen saturation of the arterial blood, the phenomenon is hardly explicable in terms other than the development of impaired tissue oxygen uptake. Besides the earlier-detected microcirculatory disorders [2], possible damage to the oxygen-binding properties of the erythrocytes should be noted. This ensues from the Po_2 decrease, despite the blood saturation with oxygen.

Two hours after the start of the experiment tissue disorders prevail, which leads to the total depletion of the alkaline reserve and near-critical drop of the blood pH (7.1±0.01) in the case of 5 LD_{50} and, perhaps, below the critical level in the case of 50 LD_{50}. In the only animal which survived the blood pH was lowered to 7.04.

Table 1. HR, Gas Content, and Acid—Base State of the Blood of Rats Poisoned with Phosphacol under Conditions of AR ($M \pm m$)

Parameter	Control	Time after phosphacol administration and start of AR							
		5 min				2 hours			
		without poisoning	phosphacol dose, LD_{50}			without poisoning	phosphacol dose, LD_{50}		
			0.5	5	50		0.5	5	50
HR, beats/min	388±20	425±2	340±20	189±31	93±20	421±2	359±22	26±11	71±12
Pco_2, mm Hg	54.7±3.5	27.4±0.4	29.6±4.6	22.6±0.8	29.2±2.3	27.1±0.5	30.3±4.6	32.7±4.9	27.9
HCO_3^-, mmol/liter	28.5±1.6	27.9±0.5	27.8±1.4	18.5±1.4	15.1±1.0	27.6±0.5	20.1±2.2	16.6±1.3	7.2
SBB, mmol/liter	26.1±1.7	31.4±0.5	30.4±1.9	22.5±1.2	17.1±1.0	31.2±0.5	22.1±3.1	16.8±1.2	8.2
SBAE, mmol/liter	5.1±0.6	5.4±0.8	5.5±1.7	−3.9±1.4	−9.8±1.2	5.1±0.8	0.8±0.5	−15.0±4.6	−21.4
pH	7.36±0.03	7.58±0.05	7.56±0.02	7.52±0.02	7.33±0.03	7.59±0.03	7.44±0.03	7.10±0.06	7.04
Po_2, mm Hg	103±4	128±1	130±4	121±3	88±10	128±1	112±4	91±11	90
$ABSO_2$, %	97±0.5	99±0.2	99±0.1	99±1.2	95±1.2	99±0.1	99±0.1	96±1.6	91

Note. Five animals were used per dose. *SBB*: standard blood bicarbonate; *SBAE*: standard blood alkaline excess; $ABSO_2$: arterial blood saturation with O_2.

Morphological investigations of the erythrocyte surface relief reveal that in the control the cells have a typical discocyte shape (Fig. 1, *a*). In long-term AR a hyperoxygenation-characteristic swelling is observed, manifested as a convexity in the central part of the discocyte (Fig. 1, *b*).

Administration of phosphacol in a dose of 0.5 LD_{50} induces a significant alteration in erythrocyte shape. Processes of considerable size (2-3 μ) and a marked reduction of cell volume are seen (Fig. 1, *c*).

Injection of a dose of 5 LD_{50} results in erythrocyte swelling, that compensates for the cell compression. Alterations of cell surface relief, the appearance of ridges (Fig. 1, *d*), and clasmatosis (Fig. 1, *e*) are observed. The maximal swelling is seen at doses of 50 LD_{50}; it is accompanied by damage to the erythrocyte stroma, followed by cell destruction (Fig. 1, *f*).

AR in the absence of phosphacol action induces an increase in the cell image area by 21% (from 747±49 to 901±28 μ). Two hours after combined AR and low doses of phosphacol the cell image area is reduced by 14%, while in cases of medium and high doses of preparation the index increases by 21 and 64%, respectively. Determination of the image area and of the integral optical density of erythrocytes makes it possible to calculate the relative content of their cytoskeleton (Fig. 2).

AR reduces the optical density of erythrocytes by 25% exclusively due to an increase of cell size. Poisoning with low doses of phosphacol practically does not affect the erythrocyte cytoskeleton. However, the doses of 5 LD_{50} and 50 LD_{50} lead to a 1.63-fold and 2.69-fold, respectively, decrease of the cytoskeleton relative density estimated at the end of the experiment, i.e., a much stronger change than the changes of the erythrocyte area. Thus, poisoning by phosphacol used in doses exceeding LD_{50} reduces the relative erythrocyte cytoskeleton content in a dose-dependent manner. The data obtained provide evidence of the probable derangement of cell integrity induced by phosphacol in doses of 5 LD_{50} and more due to the progressive cytoskeletal destruction, which inevitably results in erythrocyte destruction and the development of hemic hypoxia. The appearance of cytoskeletal alterations much earlier than the blood pH shift shows the independence of these two processes. The results suggest that the earlier-established erythrocyte deformation connected with the blood acetylcholine rise under conditions of blood cholinesterase inhibition is to a great degree realized via the contraction and destruction of the cytoskeleton. The absence of HR restoration 2 hours after phosphacol injection may be considered not as a result of the maintenance of an increased choline-positive effect, but rather as being due to the involvement of tissue metabolic disorders.

REFERENCES

1. A. Yu. Krol', M. T. Grinfel'dt, A. D. Slepl'chyavichus, *et al, Tsitologiya,* **31**, № 5, 563 (1989).
2. V. B. Prozorovskii and V. G. Skopichev, *Byull. Eksp. Biol. Med.,* **115**, № 4, 443 (1993).
3. V. I. Skopicheva, V. G. Skopichev , and N. A. Vinogradova, *Tsitologiya,* **33**, № 4, 60 (1991).
4. P. Agre, J. F. Casella, W. H. Zinkham, *et al., Nature,* **314**, 381 (1985).
5. S. Chien, *Ann. Rev. Physiol.*, **49**, 177 (1987).
6. E. Elhaty and M. P. Sheetz, *J. Cell Biol.*, **91**, 884 (1981).
7. W. Knowles, S. L. Marchesi, and V. J. Marchesi, *Semin. Hematol.*, **20**, 159 (1983).
8. P. X. La Celle, R. J. Weed, and P. A. Santillo, in: *Membrane and Disease,* New York (1976), p. 1.
9. M. P. Sheetz and S. J. Singer, *Proc. Nat. Acad. Sci USA,* **71**, 4457 (1974).