Quantitative Study of Testicular Angiotensin-Converting Enzyme on the Surface of Human Spermatozoa

M. A. Aleksinskaya, M. A. Nikolaeva*, S. M. Danilov, O. S. Elistratova, and G. T. Sukhikh***

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Expression of testicular angiotensin-converting enzyme on the surface of human spermatozoa was studied by means of flow cytometry with monoclonal antibodies. Expression of testicular angiotensin-converting enzyme on the cell surface depended on functional and morphological characteristics of spermatozoa.

Key Words: *angiotensin-converting enzyme; fractions of spermatozoa; flow cytofluorometry; monoclonal antibodies*

Angiotensin-converting enzyme (ACE, EC 3.4.15.1, CD143) is a cell membrane glycoprotein detected in various mammalian tissues and fluids and characterized by a wide range of activity. ACE plays a key role in the renin-angiotensin-aldosterone and kallikrein-kinin systems. This enzyme regulates blood pressure and electrolyte homeostasis [2,5].

There are two ACE isoforms: somatic ACE (sACE) expressed on the surface of pulmonary endothelial cells [10], epithelial cells of the intestine and kidneys, neuroepithelial cells of the brain, epithelial cells of the male genital tract, and Leydig's cells and testicular ACE (tACE) expressed in postmeiotic cells of spermatogenesis (spermatids) and spermatozoa [4].

The molecule of sACE contains two homologous domains, while tACE has only 1 homologous domain, whose peptide chain is identical to the C-terminal region of sACE (except for the first 36 amino acids) [3].

The role of ACE in reproduction is poorly understood. ACE gene knockout mice do not differ from intact animals by the number, motility, and morphological characteristics of spermatozoa. However, these cells are unable to pass through female genital tract and fertilize the oocyte [8,9].

Previous studies revealed no relationships between the concentration/morphological characteristics of spermatozoa and ACE activity [7]. A positive correlation was revealed between ACE activity and motility of human and bovine spermatozoa [6]. However, recent studies found a negative correlation between enzyme activity and motility of pig and human spermatozoa [7,11,12]. Moreover, ACE activity in normospermic patients is lower than in patients with oligospermia [11]. This discrepancy can be associated with the use of monoclonal antibodies. They interact with not only tACE, but also sACE that is present in the seminal fluid and is sorbed by the cell.

We performed a quantitative study of tACE expression on the surface of human spermatozoa using monoclonal antibodies (MAB). We isolated and characterized MAB specifically reacting with tACE. The amount of sACE sorbed on the cell surface was estimated using MAB specific to this enzyme.

MATERIALS AND METHODS

Ejaculates were obtained from healthy donors (n=10, average age 39±7 years). The sperm was collected

M. V. Lomonosov Moscow State University; *Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow; **Department of Anesthesiology, Illinois University

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in sterile containers and studied according to recommendations of the World Health Organization.

The endothelium of human umbilical vein was used to estimate the specificity of MAB to ACE. ACE concentration was measured on the surface of spermatozoa in native sperm. These motile cells were isolated by the method of flotation in M199 medium. ACE concentration was also measured in fractions separated in a Percoll gradient (fraction 1, Percoll, 90-100%; fraction 2, Percoll, 40-55%) [1].

The cell suspension (5×10^6 cells/ml) in M199 medium containing 0.5% bovine serum albumin was incubated with anti-ACE MAB at 4°C for 15 min (1E10, 4E3, i2H5, and 9B9). Nonimmune mouse IgG labeled with fluorescein isothiocyanate (FITC, Sigma Aldrich) in a concentration of 10 μg/ml served as the negative control. Polyclonal FITC-labeled goat antibodies specific to the Fc fragment of mouse IgG (Zymed, dilution 1:100) were added at 4°C for 20 min.

Cell viability was estimated in the test with propidium iodide in a concentration of 2 μg/ml. Expression was studied by means of flow cytofluorometry on a FACScan device (Becton Dickinson). The percentage of ACE-positive spermatozoa and relative fluorescence were estimated in each sample. Relative fluorescence reflects ACE concentration on the cell surface.

The results were analyzed by MadCalc software. The data are expressed as median values and standard deviations. The data were processed by nonparametric Mann—Whitney test. The differences were significant at $p<0.05$.

The data are presented in the form of a Box-and-Whisker plot. The upper and lower boundaries of rectangles correspond to the upper and lower quartiles (25-75 percentiles). The median line illustrates the median. The range of vertical line refers to the maximum and minimum noneliminated variables.

RESULTS

Specificity of antibodies to sACE and tACE was confirmed in the study of MAB binding to the surface of human umbilical vein endothelial cells and spermatozoa that expressed sACE and tACE, respectively. We showed that 1E10, i2H5+9B9, and 4E3 underwent specific binding to tACE, sACE, and both isoforms of the enzyme, respectively (Fig. 1).

Separation in a Percoll gradient allowed us to obtain two fractions of spermatozoa (Fig. 2). Fraction 1 contained a considerable number of motile cells with normal morphological characteristics. Fraction 2 contained low-motility cells presented by a considerable number of pathological forms.

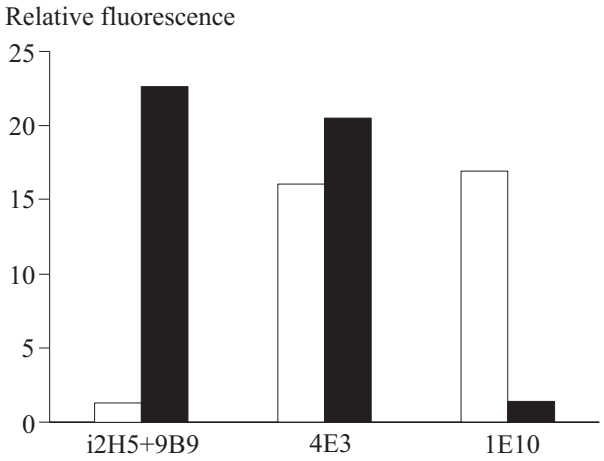

Fig. 1. Level of angiotensin-converting enzyme (ACE) on the surface of human spermatozoa (light bars) and umbilical vein endothelial cells (dark bars).

The study of fraction 1 revealed a considerable number of tACE-positive live spermatozoa (79.1%, Fig. 3). The number of tACE-positive cells in fraction 2 was 1.7-fold lower than in fraction 1 ($p<0.008$). sACE was practically undetectable.

Fixation with methanol sharply increased the percentage of sACE-positive spermatozoa in fractions 1 and 2 (35.8 and 25.7%, respectively). After fixation the percentage of tACE-positive spermatozoa increased only in fraction 2.

The study of methanol-fixed spermatozoa showed that the percentage of tACE-positive cells and concentration of tACE on the cell surface in fraction 2 were higher than in fraction 1 by 1.3 ($p<0.02$) and 1.9 times ($p<0.008$), respectively.

The results of experiments with live spermatozoa are consistent with published data on a positive

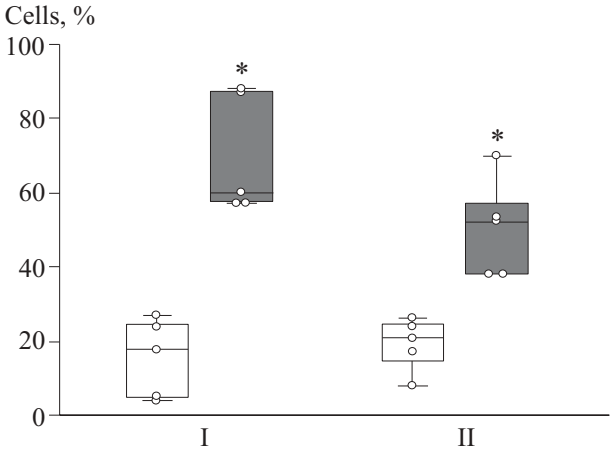

Fig. 2. Motility (I) and normal morphological characteristics (II) of spermatozoa in various fractions separated in a Percoll gradient. Fraction 1, dark bars; fraction 2, light bars. *$p<0.0001$ compared to fraction 2.

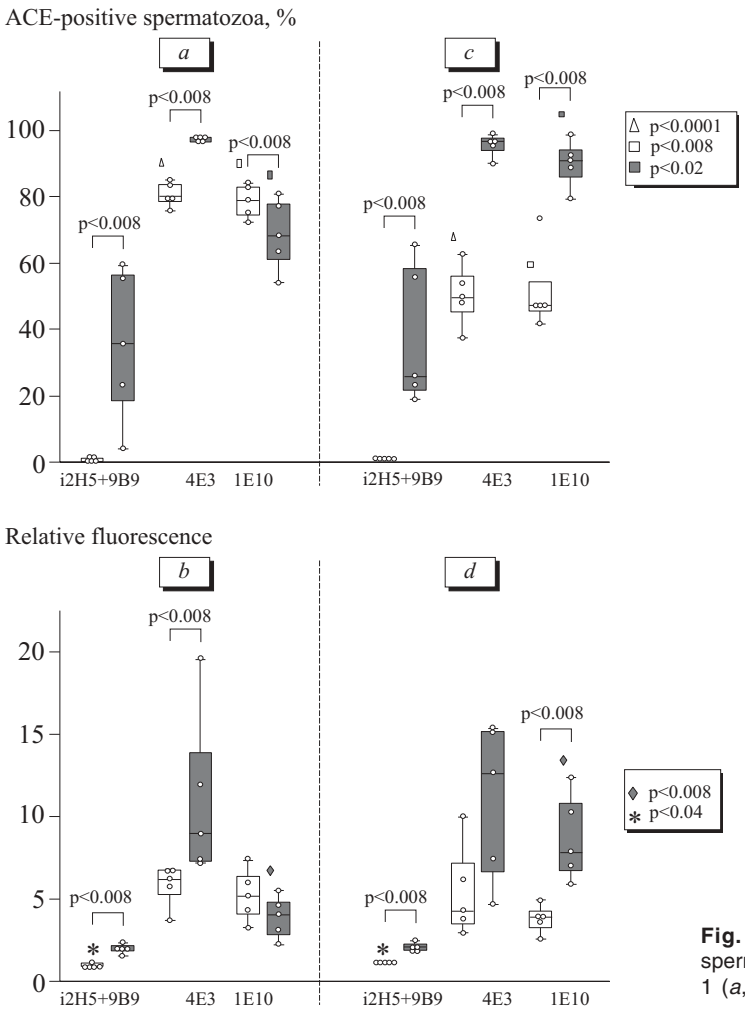

Fig. 3. ACE level on the surface of live (light bars) and fixed spermatozoa (dark bars) separated in a Percoll gradient. Fractions 1 (*a*, *b*) and 2 (*c*, *d*).

correlation between ACE activity and cell motility [6]. Analysis of fixed spermatozoa revealed high concentration of tACE on the surface of low-motility cells and/or morphologically abnormal spermatozoa, which is consistent with published data [7,12].

The lower content of tACE-positive live spermatozoa in fraction 2 is probably associated with a higher sensitivity of cells to experimental manipulations resulting in ACE removal from the cell surface. Fixation of spermatozoa with methanol prevented antibody-induced ACE removal. Enzyme concentration (MAB i2H5+9B9 and 4E3) on fixed cells was much higher than on live cells.

Our experiments were performed with MAB specific to two isoforms of the enzyme. This approach allowed us to regulate nonspecific sorption of sACE on the cell surface. The increase in sACE concentration on fixed cells (compared to live cells) can be associated with insufficient washing of spermatozoa to prevent nonspecific sorption of the enzyme. sACE was practically undetectable on the surface of live cells. This is probably related to the gradual washout away of sACE during experimental manipulations.

Our results show that the concentration of tACE is high on the surface of live motile spermatozoa with normal morphological characteristics.

Further experiments should be performed to evaluate the clinical significance of studying tACE expression on the surface of human spermatozoa for diagnostics of male sterility.

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