Yefremova U. P., Fafula R.V.

The kinetic analysis of arginase hydrolyze of L-arginine in sperm cells of infertile men

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Novadays the infertility is a common problem for most developed countries. It affects 10–15% of couples globally and approximately up to 40–50% of infertility is caused by the male factor. According to the trends observed, this problem is predicted to increase.

Elucidation of the role of NO in the development of male infertility is actively studied. Arginase is a manganese metalloenzyme which converts L-arginine to L-ornithine and urea and reciprocally regulates NO production, by competing with NO-synthase for common substarte (L-arginine).

Arginase activity has been detected in spermatozoa and this study was designed to exploring the hydrolyze of L-arginine by arginase in sperm cells of infertile men.

The studied groups involved 16 infertile men with different forms of pathospermia and 10 fertile healthy individuals. Subjects were classified into three groups as having different forms of pathospermia (oligozoospermia, asthenozoospermia, oligoasthenozoospermia). Semen samples were examined for volume, sperm concentration, pH, morphology and motility according to the World Health Organization guidelines.

Spermatozoa arginase activity was measured by determining levels of urea production, which was determined in the supernatant spectrophotometrically by measuring absorbance at 520 nm. Arginase activity was expressed as nmol urea per min per mg protein.

Kinetic analysis of the enzyme reaction was performed in a standard incubation system with modified concentration of the substrate. The apparent affinity constant for L-arginine ($K_{L\text{-}Arg}$) and maximum reaction rate (V_{max}) were determined by Lineweaver-Burk plot {1/V; 1/[S]}.

Since arginase is an enzyme that hydrolyzes L-arginine, changes in its concentration in the incubation medium affect the rate of arginase reaction. The dependence of the arginase activity on the substrate concentration in the incubation medium was determined by the apparent affinity constant to the substrate $K_{L\text{-}Arg}$. For its determination L-arginine was added to the incubation medium in concentrations ranging from 10 to 150 mM (at constant concentration of $MnCl_2$ – 2 mM). We observed a monotonic increase in the enzyme activity of sperm cells obtained from both normo- and pathozoospermic samples reaching a plateau at 100 mM. The arginase activity in pathozoospermic samples was reduced in comparison with normozoospermic samples in the whole range of L-arginine concentrations. However, the maximal arginase activity was observed in presence of 100 mM L-arginine in incubation medium for both normo- and pathozoospermic samples.

The concentration curves {1/[S]; 1/[V]} differ by angle of inclination for normo- and pathozoospermic patients. Regarding the basic kinetic parameters of arginase of sperm cells of fertile and infertile men then the maximum rate of L-arginine hydrolysis for arginase of spermatozoa obtained from men with preserved fertility was 2.0, 1.8 and 1.9 times greater than this value for oligo-, astheno- and oligoasthenozoospermic samples respectively. However, affinity constants for L-arginine were not significantly differrent between fertile and infertile men.

Consequently, in patients with oligo-, astheno- and oligoasthenozoospermia, the inhibition of arginase activity in sperm cells occurs by noncompetitive type, by reducing the reaction rate (value of V_{max} decreases). It is necessary notice that in this study we used permeabilized sperm cells in which the functioning of the enzyme corresponds to intact cells.