EFFECT OF SELECTED METAL IONS ON THE MOTILITY AND CARBOHYDRATE METABOLISM OF EJACULATED HUMAN SPERMATOZOA

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Summary : Zinc, lead and cadmium in the form of chloride salts when added to a standard assay system containing 80×10^{-6} ejaculated washed human spermatozoa caused a dose and duration-dependent inhibition of their motility. The activity of certain key enzymes of carbohydrate and energy metabolism, viz, glycogen phosphorylase, glucose-6-phosphatase, fructose-1, 6-diphosphatase, glucose-6-phosphate isomerase, amylase, Mg^{2+}— dependent ATPase and lactic and succinic acid dehydrogenases were also found to be inhibited. The order of inhibitory effects of the heavy metals were zinc<lead<cadmium. The metal chelating agent, ethylene diamine tetraacetic acid (EDTA, disodium salt) also interfered with the spermatozoal motility and inhibited the enzyme activities.

Key words : metal ions spermatozoal motility enzymes of carbohydrate metabolism

INTRODUCTION

Advent of intrauterine devices has led to the study of substances which can enhance its efficacy. Copper has been firmly established as the most effective agent. However, the possibility exists that other heavy metals in extremely low concentrations can bring about equally effective or better spermicidal result by their selective inhibition of the spermatozoal enzyme systems.

It is known that the motility of spermatozoa is regulated by the activity of the midpiece enzymes (21). An attempt has been made in this paper to study the effect of three metal ions, zinc, lead and cadmium and one metal chelating agent, ethylene diamine tetraacetic acid (EDTA, disodium salt) on the motility of ejaculated human washed spermatozoa and their enzyme systems of carbohydrate and energy metabolism. Accordingly, an *in vitro* assay system has been devised wherein the sperm cells are incubated in the presence of micromolar range concentrations of the chloride salts of these heavy metals.

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MATERIAL AND METHODS

Fresh human semen samples from the healthy donors were obtained from the Department of Obstetrics and Gynaecology, Post-Graduate Institute of Medical Education and Research, Chandigarh and the General Hospital, Sector 16, Chandigarh. Semen samples were allowed to stand for 30 min, then centrifuged at 500 rpm at room temperature for 15 min. The supernatant was discarded and the pellet reconstituted with minimal capacitation medium which contained 110 mM sodium chloride, 1 mM sodium pyruvate, 25 mM sodium bicarbonate and 1 mM calcium chloride, pH 7.4, final concentration of the sperm sample being 80×10^{-6} cells per *ml*. The reconstituted samples were incubated with indicated concentrations of the metals in the form of chloride salts and Na_2EDTA, also prepared in minimal capacitation medium. At indicated time intervals, sperm samples were withdrawn and their motility was checked by progressively forward movement as described by Seth *et al.* (16). Aliquot of sperm suspension was filled up to the mark of 0.5 in a WBC pipette and diluted with 3.8% sodium citrate solution upto 11 mark. Sperm count was done in a Neubauer's haemocytometer chamber in WBC squares. Sperm count per cm^2 was calculated as follows : $X \times 20 \times 10^4/cm^2/ml$, where X is the average mean of spermatozoa of all 4 squares. The incubation was terminated by adding chilled capacitation medium, centrifuged and the supernatant discarded. The pellet was resuspended in the medium, homogenized and an aliquot was assayed each for the glycolytic enzymes and protein content. Methods adopted for the quantitative determination of enzyme activities include glucose-6-phosphatase by Swanson (18), fructose-1, 6-diphosphatase by McGilvery (12), glycogen phosphorylase by Niemeyer *et al.* (13), glucose-6-phosphate isomerase by Slein (17), amylase by Bernfeld (2), Mg^{2+} —dependent ATPase by Kielley (8), succinic dehydrogenase by Kun and Abood (10) and lactic dehydrogenase by King (9). Inorganic phosphorus liberated in the reaction of the phosphatase enzyme systems was determined by the method of Fiske and Subbarow (4). Protein was determined in the sperm homogenates following the method of Lowry *et al* (11), using crystalline bovine serum albumin as the standard.

RESULTS

Data presented in Table I shows that the spermatozoal motility is markedly inhibited by the three metal ions used, in a time and concentration dependent manner. The extent of inhibitory effect of these metals are in increasing order, zinc<lead<cadmium. Results of quantitative estimation of the enzyme activities of carbohydrate and energy metabolism of sperm cells in the presence of 0.1 mM concentration of the three metals are presented in Table II. Activities of all the enzymes tested are greatly inhibited by these metals in the following order, zinc<lead<cadmium. EDTA also produces time- and dose-dependent inhibition of the spermatozoal motility and the activities of enzymes of carbohydrate metabolism.

TABLE I : Effect of divalent metal ions and metal-chelating agent on the motility of ejaculated human spermatozoa.

Metal salt	*mM*	*% Motile sperm count at min.*						
		0	*15*	*30*	*45*	*60*	*90*	*120*
None		82.31±1.21	80.11±4.31	76.24±6.11	72.17±5.24	69.91±7.48	65.32±3.47	62.14±2.11
$ZnCl_2$	0.05	81.13±1.23	76.11±5.61	71.44±8.92	67.87±7.81	57.01±2.18*	45.11±2.88***	35.01±4.18***
	0.10	81.13±1.23	70.19±9.42	65.42±5.42	60.00±1.00**	50.25±3.51**	35.72±5.48***	25.87±6.18***
$PbCl_2$	0.05	80.00±0.09*	73.11±8.46	70.87±6.43	66.82±7.54	60.43±6.44	43.47±8.45**	33.24±4.58***
	0.10	80.00±0.09*	67.52±4.87*	60.01±5.43*	52.28±2.89**	48.43±5.45**	31.71±6.78***	21.84±7.75***
$CdCl_2$	0.05	79.68±1.26*	70.12±3.12*	65.37±4.23*	60.11±3.12*	50.39±2.22**	41.59±8.36**	31.37±5.65***
	0.10	79.68±1.26*	65.85±4.98*	60.79±4.00*	57.29±4.56**	47.25±6.78**	29.81±4.75***	19.22±5.55***
Na_2—	0.05	78.54±0.84**	72.22±5.56	65.48±2.48*	60.03±4.85*	55.82±5.27*	49.81±6.85*	39.45±3.04***
EDTA	0.10	78.54±0.84**	70.92±8.01	63.35±2.45*	59.45±4.11*	51.43±5.43*	39.45±6.02***	29.46±7.45***

Each datum represents mean ± S. D. M. of 4 observations. *, **, and *** indicate P value <0.05, <0.01 and <0.001, respectively.

TABLE II : Effect of divalent metal ions and metal-chelating agent on certain enzymes of carbohydrate metabolism of ejaculated human spermatozoa.

Enzyme assayed (*μmol/mg protein/hr*)	*Control*	*Metal salt (0.10 mM)*			
		$ZnCl_2$	$PbCl_2$	$CdCl_2$	Na_2-EDTA
Glucose-6-phosphatase	18.63±1.12	14.99±0.76**	14.96±1.53*	13.38±1.33**	15.34±1.15*
Fructose-1, 6-diphosphatase	19.92±1.19	16.41±1.78*	16.36±1.93*	15.58±1.52**	16.54±2.56
Glycogen phosphorylase	18.83±1.12	17.35±1.71	17.20±1.19	16.73±1.60	17.90±1.80
Glucose-6-phosphate isomerase	6.23±0.95	4.20±0.75*	3.93±1.48	3.31±1.43*	4.70±1.59
Amylase	18.09±1.56	16.54±1.11	15.13±1.27*	13.09±1.87	16.65±1.14
Mg^{2+}—dependent ATPase	17.88±1.14	12.67±1.43**	11.25±1.72**	10.10±1.12***	13.29±1.46**
Lactic dehydrogenase	3.94±0.09	2.68±0.76	2.60±0.69	2.51±0.81	2.80±0.89
Succinic dehydrogenase	23.87±1.07	21.08±0.86	19.22±0.47***	16.04±1.79***	18.54±1.26**

Each datum represents the mean ± S. D. M. of 4 observations. *, ** and *** indicate P value <0.05, <0.01 and <0.001, respectively.

DISCUSSION

In the present study, metal ions and chelating agent when added to the spermatozoal suspension *in vitro* in a standard assay system cause inhibition of spermatozoal motility and all the enzymes of carbohydrate metabolism tested. The enzyme activity lowering effect is more pronounced in cadmium than lead and zinc or EDTA. Therefore, the observed inhibition of spermatozoal motility by lead, zinc and cadmium may be caused due to the interference of these metal ions with the enzymatic reactions probably by reacting with the sulphydryl groups of the enzymes, thereby inhibiting the glycolytic pathway and energy production. Mammalian spermatozoa have a high degree of glycolytic enzyme activities and oxidative phosphorylation (3) and agents causing even marginal reduction in ATP content may lead to inhibitton of motility, thus causing infertility (19). Inhibition of spermatozoal motility by added zinc in particular, in the present study is interesting, since occurrence of high concentrations of zinc in prostate semen and spermatozoa is well documented (15). Although no physiologic role is established for the high concentrations of zinc in ejaculated spermatozoa, results in animal studies suggest that zinc may not be necessary for fertility or fecundity (5). EDTA on the other hand exerts its influence probably through a different mechanism. Chelating agents have been used as microreagents in analytical chemistry, bacteriostatic and fungicidal agents. Since these agents are active in low concentrations at physiological hydrogen ion concentrations, it is suggested that they may act, in part at least, by depriving the cells of essential trace metals (20). Washed spermatozoal suspensions may offer an ideal situation as single cell entities to test the spermicidal action of these metal chelating agents. EDTA is known to have high affinity for Ca^{2+}, zinc and other metals (1). The toxic action of EDTA in spermatozoal motility and the midpiece enzyme complex may have resulted not only from the loss of certain trace metals through chelation but also actually arise from the direct toxic effects of the resultant metal-chelate compounds.

Spermatozoa possess an intracellular reservoir of glycogen (14) and since glycogen phosphorylase is the primary enzyme responsible for its breakdown, the inhibition of its activity is highly critical for the availability of glucose-6-phosphate source for further movement in the glycolytic pathway. On the other hand inhibition of glucose-6-phosphate isomerase for aldose ketose isomerization of glucose-6-phosphate into fructose-6-phosphate may shift the equilibrium towards the production of reducing equivalents via hexose monophosphate shunt pathway for fatty acid or ribose for nucleotide biosynthesis. Glucose-6-phosphatase is also the terminal hydrolytic enzyme of gluconeogenetic pathway and inhibition of it along with fructose-1, 6-diphosphatase may block the production of glucose from other sources, namely amino acids, propionate and oxaloacetate. Accumulation of fructose-1, 6-diphosphate as a result of inhibition of the enzyme, fructose-1, 6-diphosphatase,

may lead to the production of triose phosphate, dihydroxy-acetone phosphate which can particularly be utilized in the glycerol, glycerol-3-phosphate and phospholipid metabolism. Panse and Sheth (14) have confirmed the presence of the disaccharide, maltose in mammalian spermatozoa as a glycogen intermediate and so the detection of considerable amount of amylase activity in the present study is not surprising. Inhibition of amylase however, may further limit the option of maltose to be produced and converted to D-glucose and subsequent utilization in energy production via glycolytic pathway. Lactate dehydrogenase functions primarily in making the lactate present in the genital fluids made available to the spermatozoa for oxidation and energy production (6) and inhibition of it may lead to the accumulation of lactate and inhibition of glycolysis. Succinate dehydrogenase on the other hand acts on succinate which is oxidized at a very high rate as compared to pyruvate, lactate and acetate. The high rate of succinate oxidation indicates that a large portion of the respiratory chain in the spermatozoa is capable of supporting a high rate of electron flow and inhibition of succinate dehydrogenase activity will lower the electron level of glycolytic pathway and also lead to the accumulation of succinate. Since metal ions inhibit the ATPase activity, inhibition of metabolism may be a response to the increased level of ATP that should accompany decreased activity of the cation pump. Inhibition of metabolism by higher level of ATP would be expected as the metabolism is under nucleotide control (7).

It is apparent that there is a decline in all enzymatic parameters following metal ion and chelating agent treatment which brings out the interesting possibility that the flux of carbon through the process of glycolysis ceases and sufficient energy from the carbohydrate source is therefore, not available to the ejaculated spermatozoa. The inhibition of glycolysis could well be the primary site of action of metal ions and thus spermatozoa are being deprived of the much needed metabolic energy for the motility in the female tract which may ultimately render them infertile.

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