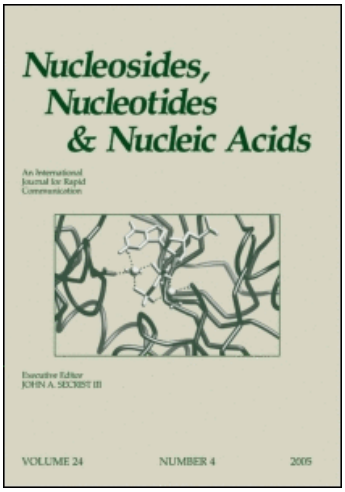

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T. Niemeyer[a]; C. Dietz[a]; L. Fairbanks[b]; I. Schroeder-Printzen[c]; R. Henkel[d]; M. Löeffler[e]
[a] Institute for Physiological Chemistry, Philipps-University, Marburg, Denmark [b] Purine Research Unit, Guy's Hospital, London, United Kingdom [c] Department of Urology, Justus-Liebig-University, Giessen, Denmark [d] Department of Medical Biosciences, University of the Western Cape, Bellville, South Africa [e] Institute for Physiological Chemistry, Philipps-University, Marburg, Germany

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EVALUATION OF URIDINE METABOLISM IN HUMAN AND ANIMAL SPERMATOZOA

T. Niemeyer and C. Dietz □ *Institute for Physiological Chemistry, Philipps-University, Marburg, Denmark*

L. Fairbanks □ *Purine Research Unit, Guy's Hospital, London, United Kingdom*

I. Schroeder-Printzen □ *Department of Urology, Justus-Liebig-University, Giessen, Denmark*

R. Henkel □ *Department of Medical Biosciences, University of the Western Cape, Bellville, South Africa*

M. Löeffler □ *Institute for Physiological Chemistry, Philipps-University, Marburg, Germany*

□ *The objective of this study was to elucidate the role of uridine for spermatozoa, since this pyrimidine nucleoside was found in millimolar concentration in human seminal plasma. Here, the degradative activity of uridine-phosphorylase [EC 2.4.2.3] and the salvage activity of uridine kinase [EC 2.7.1.48] were detected in human spermatozoa. HPLC analysis depicted the uptake of exogeneous ^{14}C-labelled adenine, but not of uridine and of hypoxanthine, into nucleotide pools of boar spermatozoa. On addition of uridine, the computer-assisted semen analysis (CASA) of human cells revealed a reduction of the percentage of motile spermatozoa in contrast to an elevation of some velocity parameters. It is concluded that exogeneous uridine could function as suppressor for early capacitation and as a substrate for phosphorolysis, if ribose is needed, rather than to satisfy a demand for intracellular pyrimidine nucleotides.*

Keywords Uridine phosphorylase; Kinase; Nucleotide pools; CASA; Spermatozoa

INTRODUCTION

Quite a lot of metabolites, enzymes and proteins have been found as normal ingredients in semen. Some of these are considered as characteristic markers that reflect the contribution made by the different accessory glands

Address correspondence to Monika Löeffler, Institute for Physiological Chemistry, Philipps-University, Karl-von-Frisch-Strasse 1, D-35033, Marburg, Germany. E-mail: löeffler@staff.uni-marburg.de

to this body fluid (e.g., fructose from seminal vesicles, zinc, citrate, and acidic phosphatase from the prostate or carnitine from the epididymis). [1] Clinically, they can be analyzed to support diagnoses of causes for male infertility. A previous report on semen containing high amounts of uridine, presumably secreted by seminal vesicles, [2] was verified by our recent HPLC analyses of samples from animal and human seminal plasma (unpublished data), which stated the millimolar conentration versus the micromolar range of uridine in human blood serum. [3] The rationale of our studies is to find an explanation for the marked presence of uridine in the ejaculate, with respect to viability and function of germ cells. Here, we make an approach to follow its uptake by cells and to look for an involvement of uridine kinase (uridine + ATP→ uridine-5'-phosphate + ADP) and of phophorylase (uridine + phosphate→ uracil + D-ribose-1-phosphate) and also to prove its effect on activity and motility features of human spermatozoa.

MATERIAL AND METHODS

Computer Assisted Semen Analysis. Semen samples of 10 healthy semen donors ($\sim$20–25 $\times$ 10^6 cells/mL, ejaculate volume >2 mL) were processed by the swim-up technique, incubated in HTF-medium, supplemented with 10 mg/mL human serum albumin, [4] and HTF-medium plus 5 mM uridine. Semen analysis was performed according to the guidelines of the World Health Organization (1996). Sperm motility and various motion parameters were measured for 60 minutes by CASA using a Strömberg-Mika-Cell Motion Analyser. For statistical evaluation the percentage of motile, immotile and hyperactivated cells were considered as main criteria. In addition, sperm velocity parameters (VSL, VCL, VAP), and linear and lateral head displacement were taken into account. The SPSS statistical programme was used for the analysis. In order to test for statistical significance, a multivariate analysis (ANOVA) was performed and $p < 0.05$ was considered significant.

Radio-Labelling Studies. To estimate the salvage of nucleosides, the incorporation of radiolabelled precursors ([2–^{14}C]-uridine; [8–^{14}C]-hypoxanthine; or [8–^{14}C]-adenine) in the nucleotide pools of boar spermatozoa was analyzed by HPLC. [5] The uptake of [5–^{3}H]-uridine into the acid soluble fraction of human spermatozoa was measured as described elsewhere. [6]

Enzyme Activities. Uridine kinase (UK) in lysates of human spermatozoa was determined in the presence of [5–^{3}H]-uridine and ATP over a period of 20 minutes, according to a method described for uridine kinase and thymidine kinase. [7,8] After separation of the phosphorylated from non-phosphorylated uridine by absorption of uridine-5'-phosphate

on DE-81 filters, these filters were eluted with 0.2 M KCl/0.1 N HCL and liquid scintillation (LSC) counting was performed. Uridine phosphorylase (UPase) activity was measured in the presence of [2–^{14}C] uridine following the method described for uridine phosphorylase and thymidine phosphorylase; [9,10] the separation of the product uracil from uridine was performed on PEI-cellulose thin layer chromatography. The label was detected with LCS after cutting and elution of spots.

RESULTS

Motility of spermatozoa is an essential criterion for fertilization. In comparison to control cells (20% motile), in the presence of 5 mM uridine motility decreased to 17% ($p = 0.499$); the percentage of hyperactivated spermatozoa (17%) in the group of the motile cells also declined (12%, $p = 0.532$). The influence of exogeneous uridine on other parameters, such as sperm velocity and different modes of movement was not quite consistent. However, the statistical evaluation of the velocity of hyperactivated cells revealed a significantly positive effect of uridine ($p = 0.017$).

The analyses of nucleotide pools in boar spermatozoa, did not reveal a specific label in the peaks of UTP, UDP, and UMP after incubation with uridine nor in GTP, GDP, GMP after addition of hypoxanthine. Since the presence of adenine resulted in labelling of ATP, ADP, NAD, and AMP (ratio 4:1:0.6:0.3), it is concluded that the salvage activity for adenine by phosphoribosyltransferase may be of great importance with respect to the function of adenosine nucleotides and NAD in the energy metabolism of spermatozoa.

With 2 samples of human semen, the uridine uptake into acid soluble fraction of cells was determined to be 0.06–0.12 pmol/10^6 cells at the beginning, but decreased during the incubation time of 25 minutes (0.03–0.08 pmol/10^6 cells) without appropriate label in the acid insoluble fraction. This could support the finding that exogeneous labelled uridine was not taken up into the nucleotide pool of boar spermatozoa for further metabolism.

Uridine kinase activity was observed in all homogenates from samples of five human donors. The variation in activity between individuals (Figure 1) reveals the problem when comparing enzyme activities in human samples with determinations of high reproducibility obtained with cells from established lines. On the average the formation of uridine-5'-phosphate was 0.097 ± 0.063 pmol/min·10^6 human spermatozoa. Uridine phosphorylase activity also was detected in cell homogenates of 5 human donors. Two of these met the expectation that the decrease in uridine was accompanied by the appropriate increase in the amount of uracil. From the spots of radiolabelled uracil obtained after 5, 10, 25, and 55 minutes of

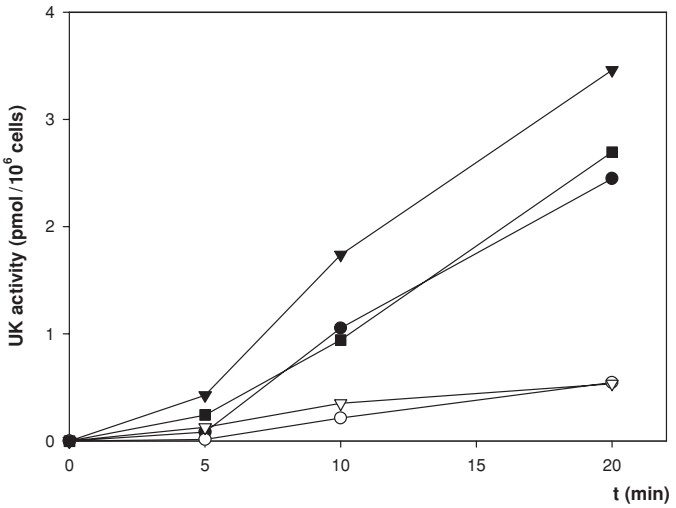

FIGURE 1 Uridine kinase activity in human spermatozoa. ●, ○,▼, ▽, ■, assay with cell homogenates of semen from 5 different healthy donors.

incubation, the activity of uridine phosphorylase in human spermatozoa was calculated as 2.412 ± 1.682 pmol/min·10^6 cells. Fluctuations of the activity observed in the others samples might be due to the critical separation technique on TLC used in this study.

DISCUSSION

The high concentration of uridine in semen can hardly be explained on the basis of an arbitrary production of UMP in the male sexual glands. Although the enzymes of UMP biosynthesis were localized in mammalian spermatozoa, [11] uridine is produced in high amounts by the glands, presumably to support the proper function of spermatozoa during and especially after ejaculation. Since spermatozoa only contain a residual amount of cytosol after differentiation and maturation, [1] activities of cytosolic enzymes—if present at all—can be expected to be low in comparison to those in normal or proliferating human cells, e.g. human colon carcinoma cells or human leucemia cells, [8–10] as was the case for uridine kinase and phosphorylase in this study. Even if uridine kinase activity as determined in cell homogenates could enable cells to produce uridine-5'-phosphate, animal and human spermatozoa used in this study appeared not to favour the salvage pathway to take up uridine and to spend ATP for its activation. Therefore, we suggest uridine phosphorylase to be an alternative candidate to catabolize the exogeneous uridine following uptake in order to produce ribose-1-phosphate, when needed. Ribose-1-phosphate could be interconverted and metabolised through the pentose-phosphate pathway. Also, it

could be a convenient source for phosphoribosyl-1-pyrophosphate, if the salvage of purine bases were necessary. [12] The more so, since metabolism of fructose, which replaces glucose in human and animal semen, [1] is different from that of glucose and may not produce ribose derivatives in spermatozoa. Salvage activites could be deduced for spermatozoa from the present finding of adenine uptake and incorporation in nucleotides. Since the hyperactivation of individual spermatozoa is assumed to be of importance for capacitation and acrosome reaction, [13] the present observation that exogeneous uridine was able to enhance the velocity of hyperactivated cells point to a modulating role of this nucleoside for the timing of capacitation while passing the female reproductive tract.

REFERENCES

1. Mann, T.; Lutwak-Mann, C. *Male Reproductive Function and Semen*. Springer Verlag, Berlin, 1981.
2. Ronquist, G.; Niklasson, F. Uridine, xanthine and urate contents in human seminal plasma. *Arch. Androl.* **1984**, 13, 63–70.
3. Traut, T.W. Physiological concentration of purines and pyrimidines. *Mol. Cell Biochem.* **1994**, 140, 1–22.
4. Quinn, P.; Kerin, J.F.; Warnes, G.M. Improved pregnancy rate in human in vitro fertilization with the use of a medium based on the composition of human tubal fluid. *Fertil. Steril* **1985**, 44, 493–498.
5. Fairbanks, L.; Bofill, M.; Rueckemann, K.; Simmonds, H.A. Importance of ribonucleotide availability to proliferating T-lymphocytes from healthy humans. *J. Biol. Chem.* **1995**, 270, 29682–29689.
6. Löffler, M. The anti-pyrimidine effect of hypoxia and brequinar is of consequence for tumor cell growth. *Biochem. Pharmacol.* **1992**, 43, 2281–2287.
7. Haugen, T.B.; Hansson, V.; Fritzon, P. Cellular distribution, developmental changes and effects of cryptorchidism on uridine kinase in the rat testis. *J. Reprod. Fert.* **1988**, 19, 193–196.
8. Jacobosson, B.; Albertioni, F.; Eriksson, S. Deoxynucleoside anabolic enzyme levels in acute myelocytic leukemia and chronic lymphocytic leukemia cells. *Cancer Lett.* **2002**, 165, 195–2000.
9. Cao, D.; Russell, R.L.; Zhang, D.; Leffert, J.J.; Pizzorno, G. Uridine phosphorylase(-/-) murine embryonic stem cells clarify the key role of this enzyme in the regulation of the pyrimidine salvage pathway and in the activation of fluoropyrimidines. *Cancer Res.* **2002**, 62, 2313–2317.
10. De Bruin, M.; van Capel, T.; van der Born, K.; Kruyt, F.A.; Fukushima, M.; Hoekman, K.; Pinedo, H.M.; Peters, G.J. Role of platelet-derived endothelial cell growth factor/thymidine phosphorylase in fluoropyrimidine sensitivity. *Brit. J. Cancer* **2003**, 88, 957–64.
11. Carrey, E.A.; Dietz, C.; Glubb, D.M.; Löffler, M.; Lecocq, J.M.; Watson, P.F. Detection and location of the enzymes of de novo pyrimidine biosynthesis in mammalian spermaozoa. *Reproduction* **2002**, 123, 757–768.
12. Mascia, L.; Cappiello, M.; Cherri, S.; Ipata, P.L. In vitro recycling of D-ribose-1-phosphate for the salvage of purine bases. *Biochem. Biophys. Acta* **2000**, 1474, 70–74.
13. Stauss, C.R.; Votta, T.J.; Suarez, S.S. Sperm motility hyperactivation facilitates penenetration of the hamster zona pellucida. *Biol. Reprod.* **1995**, 53, 1280–1285.