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Extraction Resistance of Mammalian Sperm Axonemal Microtubules

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Axonemal microtubules responsible for sperm motility are more stable than cytoplasmic microtubules but the reasons are still unclear. We subjected mouse and human spermatozoa to nuclear matrix — intermediate filaments (NM—IF) extraction including sequental detergent, high salt and nuclease treatment. This protocol has been applied to other cell types and reported to dissolve all structures except nuclear matrix and intermediate filaments. In spermatozoa, however, axoneme was retained. Electron microscopic observation of extracted sperm whole mounts showed presence of microtubules in all parts of the tail. To determine whether microtubules had preserved their appearance owing to axonemal compononts other than tubulin, we performed immunofluorescent and immunoelectron microscopic localization of tubulin in extracted cells. Tubulin labeling was not diminished in treated spermatozoa compared to untreated controls. It could be concluded that in the non-physiologic conditions of this approach, sperm microtubules showed remarkable intrinsic stability comparable to that of intermediate-type filaments.

Key words: axoneme, tubulin, sperm, cytoskeleton, microscopy.

Introduction

Progressive motility and, hence, fertilizing capacity of the spermatozoon depends on the microtubular cytoskeleton of its tail — the axoneme. Axonemal microtubules are known to be considerably more stable than their cytoplasmic counterparts but few studies have addressed this issue specifically. The difference could be partly attributed to the fact that most cytoplasmic microtubules normally undergo transition between polymerization and collapse called dynamic instability [3]. However, axonemal microtubules are also likely to be intrinsically more stable because of their composition. Unlike cytoplasmic microtubules, they contain intermediate filament-like proteins called tektins [6]. The general symmetry of axoneme is preserved even after extraction of 80% of tubulin, which leaves thin tektin-rich filaments at microtubule positions [7]. A procedure known to dissolve cytoplasmic microtubules is the nuclear matrix and intermediate filaments (NM—IF) extraction including sequental Triton X-100 treatment, high salt extraction and nuclease digestion [1, 2]. We subjected mouse and human spermatozoa to NM—IF extraction and then assessed the structure and tubulin content of their microtubules by microscopic and immunocytochemical methods.

Materials and Methods

Specimens from 4 mice and 9 normozoospermic patients were used for this study. Mouse spermatozoa were obtained from vas deferens and human spermatozoa from ejaculates. Cells were subjected to NM—IF extraction as described earlier [5] with the following more important steps: permeabilization for 10 min at 4°C with 10 mM HEPES buffer pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 0.5% Triton X-100, 1.2 mM phenylmethylsulfonylfluoride (PMSF); extraction for 10 min at 4°C with 10 mM HEPES pH 6.8, 250 mM $(NH_4)_2SO_4$, 300 mM sucrose, 3 mM $MgCl_2$, 0.5% Triton X-100, 1.2 mM PMSF; digestion at room temperature for 20-40 min in 10 mM HEPES pH 6.8, 50 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 0.5% Triton X-100, 1.2 mM PMSF, 100-200 μg/ml DNase I.

For routine whole-mount electron microscopy, cells were adhered on formvar-coated grids either before or after extraction, fixed with glutaraldehyde and OsO_4 and stained with uranyl acetate.

For immunofluorescence, extracted and unextracted spermatozoa were processed as in [9]. After fixation with methanol and acetone, they were treated for 1 h at room temperature with anti-alpha tubulin monoclonal antibody TU-01 (Prague, Inst. Mol. Genet., diluted 1:50; for detailed description of this antibody see [8]). FITC-conjugated anti-mouse polyvalent Ig (Sigma) diluted 1:100 was applied as second antibody for 30 min at room temperature.

Immunocytochemical detection of tubulin was performed also at electron microscopic level. Unextracted spermatozoa were processed for pre-embedding immunogold electron microscopy as described earlier [4] with the following more important steps: incubation with TU-01 antibody, diluted 1:50, overnight at 4°C; incubation with anti-mouse IgG-10 nm or 5 nm gold conjugate (Sigma), diluted 1:20, for 2 h at room temperature; postfixation, embedding and sectioning. Extracted spermatozoa were treated as whole mounts on grids with the same first and second antibody, fixed and observed without uranyl acetate staining. Positive and negative controls were used in all variants of the immunocytochemical reaction.

Results

Most extracted axonemes showed remarkable preservation of microtubules in all parts of the tail (middle, principal and end piece), although signs of collapse could often be seen at their ends. Virtually all mouse spermatozoa contained at least several microtubules even at end piece level. Human axonemal microtubules were slightly less stable, being present in the end piece in about 80% of tails (in the remaining cells distal fibrous sheath was "empty", devoid of microtubules). When spermatozoa were left to adhere to grids before extraction, end piece microtubules retained parallel position (Fig. 1A), while in cells extracted in suspension they generally had broom-like or more complex appearance (Fig. 1B).

Immunofluorescent staining for alpha-tubulin was observed in the entire sperm tail and its intensity was not diminished by extraction (Fig. 2). Immunoelectron microscopy showed localization of colloidal gold labelling to axonemal microtubules both in pre-embedding sections of unextracted spermatozoa and in whole-mount preparations of NM—IF extracted cells (Fig. 3).

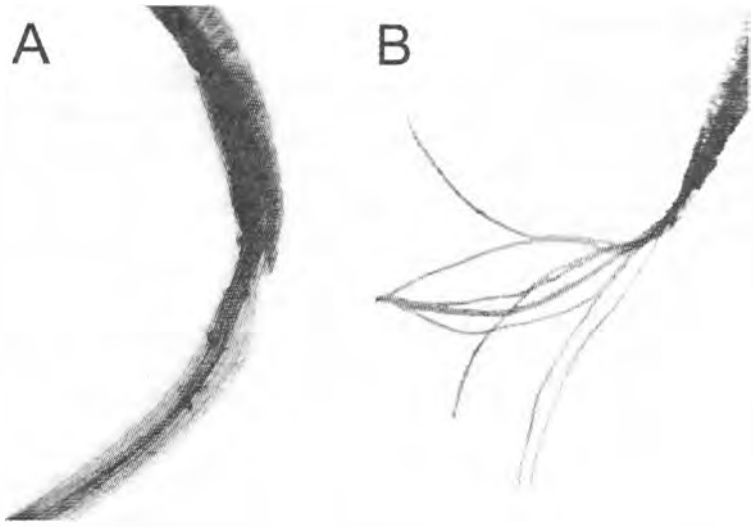

Fig. 1. End piece microtubules
A – of human spermatozoon extracted on the grid (× 22 500); B – of mouse spermatozoon extracted in suspension (× 16 000)

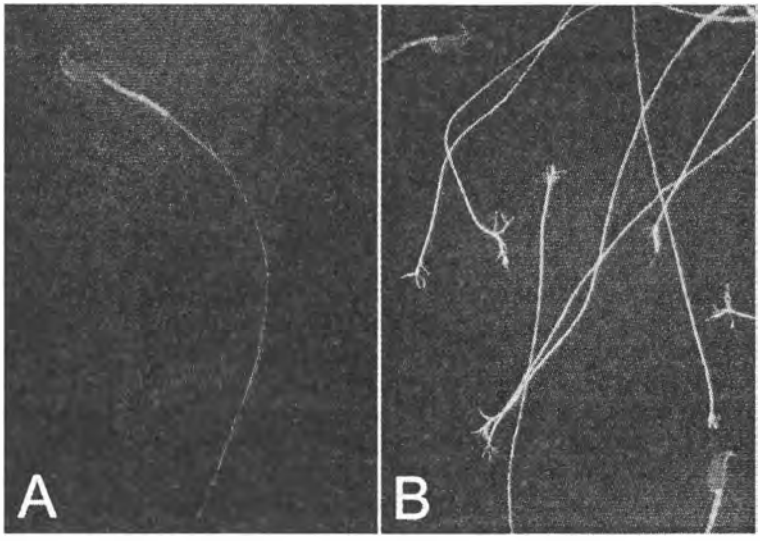

Fig. 2. Immunofluorescent detection of alpha-tubulin in mouse sperm cells
A – unextracted (× 1000); B – extracted (× 1000)

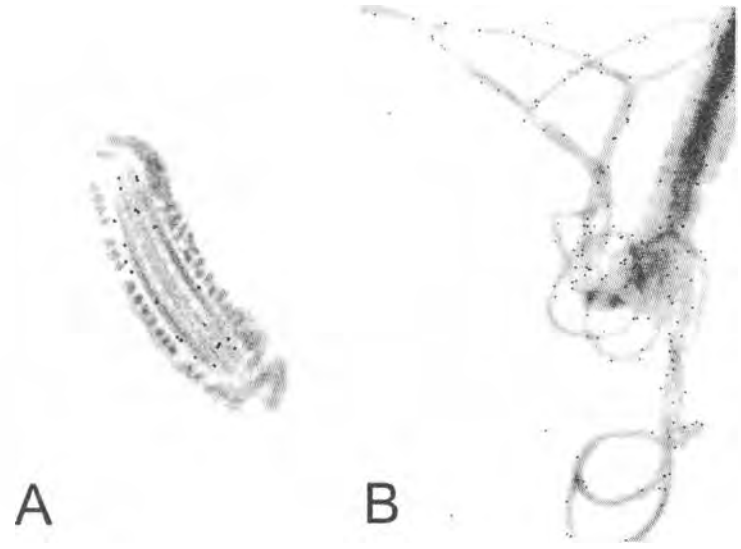

Fig. 3. Immunogold detection of alpha-tubulin in human sperm tails
A – preembedding section of unextracted cell (× 42 000); B – whole-mount of extracted cell (× 26 000)

Discussion

The NM—IF procedure had been developed to extract all cellular structures except intermediate filaments and nuclear matrix and had been shown to solubilize cytoplasmic microtubules [1, 2]. For that reason, it was surprising that in our experiments sperm axonemal microtubules were retained. Their extraction resistance was higher in mouse than in human sperm tails, consistent with the general mechanical inferiority of human spermatozoa to their rodent counterparts.

Although the microtubules appeared to be relatively well preserved, it was not clear whether they still contained most of their tubulin or had preserved their appearance owing to other extraction-resistant axonemal components such as tektins [6, 7]. To check the latter possibility, we examined the presence of tubulin in extracted cells by immunocytochemistry at light and electron microscopic level using monoclonal anti-tubulin antibody. The immunofluorescent and immunogold labelling for tubulin showed no apparent decrease in treated spermatozoa compared to untreated controls.

In conclusion, our experiment underscored the remarkable stability of sperm microtubules. Subjected to NM—IF chemical dissection, they showed resistance comparable to that of intermediate-type filaments. Because the conditions were non-physiological, this resistance could not be attributed to lack of dynamic instability but presumably was an intrinsic property of their composition and structure.

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