SHORT COMMUNICATION

UTRECHT, THE NETHERLANDS

The Effect of Colchicine Treatment on Spermatozoa: A Cytogenetic Approach

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INTRODUCTION

Colchicine is an alkaloid which exerts its main effect at the cellular level by its interference with microtubule formation, thereby affecting mitosis and other microtubule-dependent functions (1). In routine cytogenetic diagnostics, colchicine is commonly used in in vitro culture systems to block spindle formation and arrest cells undergoing mitosis at the metaphase stage (2,3). On the other hand, due to its antiinflammatory effect, colchicine is used for the treatment of several diseases, including gouty arthritis (4), familial Mediterranean fever (FMF) (5), and Behcet's disease (6). The effects of colchicine on sperm production and function in men are controversial. Recently, Haimov-Kochman and Ben-Chetrit (7) reviewed all papers published from 1966 to April 1997 regarding this effect in healthy individuals as well as in patients. The authors concluded that colchicine seems to have no significant direct adverse effect on sperm production and function as determined by routine sperm analysis. However, it is unknown whether the incidence of aneuploidy in spermatozoa of men treated with colchicine is affected, since colchicine can act directly on chromosomes by detaching chromosomes from the meiotic spindle.

Recently, two infertile couples of which the male partners were treated with colchicine attended our in vitro fertilization (IVF) center. In both couples routine sperm analyses were performed and an additional ejaculate was prepared for cytogenetic analysis by three-color fluorescence in situ hybridization (FISH) with probes specific for chromosomes 18, X, and Y (8).

PATIENTS AND METHODS

FISH

Semen samples were prepared similarly as during the routine IVF/intracytoplasmic sperm injection (ICSI) procedure, i.e., by density gradient centrifugation and two washings of the sperm pellet. Subsequently, sperm cells were fixed in fresh cold fixative (methanol:acetic acid, 3:1) and stored at −20°C. Aliquots were washed with fresh fixative and gently applied to coated glass slides immediately prior to hybridization. Interphase FISH was performed essentially according to Martini *et al.* (9), using DNA probes specific for chromosomes 18 (spectrum green), X (spectrum orange), and Y (spectrum aqua; Vysis Inc., Downers Grove, IL). Posthybridization washes were performed according to the Vysis protocol. To allow detection of the blue fluorochrome (spectrum aqua), extremely diluted DAPI (1:12,000) was used for counterstaining.

Only sperm cells exhibiting a normal morphology with well-defined margins and a tail by phase-contrast microscopy were selected for scoring. FISH analysis was carried out by switching to fluorescence illumination with a multiple filter set containing both a combination filter for simultaneous visualization of green, red, and blue signals and single-band pass filters. Signals of a particular fluorescent color separated by at least one signal diameter were considered as multiple spots. Scoring was performed by two independent observers.

Semen of a normospermic donor with proven fertility served as a control. Of 1000 sperm nuclei scored, 19 (1.9%) were classified as aneuploid (Table I): 6 sperm cells had a nullisomy for chromosome 18, and 4 for either chromosome X or chromosome Y. Three

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Table I. Semen Parameters and the Overall Aneuploidy Rate in Sperm Nuclei of a Normospermic Control and Two Patients Treated with Colchicine: Patient A Incidentally Treated for Gout and Patient B, with FMF, Treated with Colchicine Continuously for 12 Years

	Routine sperm analysis		Three-color FISH	
	Total motile sperm count ($\times 10^6$)	Normal morphology (%)	Sperm nuclei analyzed (*n*)	Aneuploidy rate (%)
Patient A	56.4	11	707	2.3
Patient B	4.7	8	351	9.1*
Control	63.0	47	1000	1.9

* Significantly different from control ($P < 0.001$; χ^2 test).

sperm cells exhibited a chromosome 18 disomy, and five sperm cells a X/Y disomy. One sperm cell was possibly diploid (disomic for both chromosome 18 and chromosome X).

Case 1

The male partner of couple A was a 45-year-old patient with borderline teratozoospermia and very mild gouty arthritis, with less than one attack per year. The couple, suffering from long-standing infertility caused mainly by a female factor, was about to start their next IVF treatment cycle when the male partner had an acute attack of gouty arthritis. After a total dosage of 8 mg colchicine the first day, 1 mg colchicine was administered daily during several weeks. The scheduled IVF attempt was postponed for several months to eliminate possible adverse effects of the colchicine treatment. About 7 weeks after the last day of colchicine administration, a fresh ejaculate was prepared for FISH. Semen parameters of this sample were similar to those of previous samples before colchicine treatment. The total motile sperm count was 56.4×10^6, and 11% of the spermatozoa displayed a normal morphology according to World Health Organization criteria. Of this patient, 707 sperm nuclei were scored after FISH, of which 691 (97.7%) appeared to be normal, i.e., either 18/X or 18/Y. Of the abnormal sperm nuclei, two (0.3%) were found to be nullisomic for chromosome 18, and nine (1.3%) for either chromosome X or Y. One (0.1%) sperm cell was found to be disomic for chromosome 18, and four (0.6%) sperm cells for the sex chromosomes. Thus 16 (2.3%) sperm cells were classified as aneuploid (Table I).

Case 2

Couple B had a duration of infertility of about 13 years. Sperm analyses of the 34-year-old proband showed severe oligoasthenoteratozoospermia (OAT). They were referred to our IVF center for ICSI after complete failure of fertilization in two previous IVF attempts. In 1982, FMF was diagnosed in the male partner, and since 1986 daily administration of 1.0–1.5 mg colchicine has been applied as a prophylaxis to suppress attacks as well as the development of amyloidosis. So far, no amyloidosis or renal failure has been diagnosed. After a pregnancy was achieved in the first ICSI treatment cycle, the patient was willing to donate a fresh semen sample for cytogenetic analysis. The semen sample contained 4.7×10^6 motile spermatozoa, and 8% of the spermatozoa were morphologically normal. For this patient, 351 sperm nuclei fulfilled the inclusion criteria and were included for scoring. In 319 (90.9%) sperm nuclei either 18/X or 18/Y signals were observed, indicating normal monosomic sperm nuclei for the chromosomes analyzed. Nine (2.6%) sperm cells were found to be nullisomic for chromosome 18, and three (0.9%) for either chromosome X or chromosome Y. Nineteen (5.4%) sperm cells were disomic for the sex chromosomes and one (0.3%) sperm cell was disomic for both chromosome 18 and chromosome X and classified as diploid. Thus for patient B, a total of 32 (9.1%) sperm cells appeared to be aneuploid (Table I).

DISCUSSION

It is generally accepted that colchicine is the drug of choice for FMF and many other rheumatic and nonrheumatic diseases. When used at appropriate therapeutic doses, colchicine is relatively safe and efficacious (1). In vitro, high dosage colchicine causes mitotic arrest, which has raised serious concerns regarding its potential effect to cause meiotic arrest as well. Controversial results have been published on the effect of colchicine on spermatozoa in humans. Sporadic reports described that colchicine affects sperm production and function (10–12). However, the overall impression is that colchicine treatment at the dosages commonly used (<2 mg daily) does not have a significant adverse effect on sperm production and function (7,13). It is assumed, though, that some men are unusually sensitive to a toxic effect of colchicine on the testis, which probably could explain the rare cases of azoospermia observed under colchicine treatment. Due to its direct effect on meiotic spindle formation, colchicine might be capable of inducing numerical chromosomal abnormalities in spermatozoa. In lymphocytes, no evidence of chromosomal

aberrations caused by (long-term) colchicine therapy has been detected (14), but no data are available on cytogenetic evaluations of spermatozoa of patients treated with colchicine. In the present study, spermatozoa of two patients were analyzed cytogenetically and the incidence of aneuploidy for chromosomes 18, X, and Y was determined. Patient A was incidentally treated with colchicine for a short period of several weeks. The incidence of aneuploidy observed in sperm nuclei of this patient was similar to that in the control semen, 2.3 and 1.9%, respectively. The therapeutic dose of colchicine used for several weeks did not seem to affect sperm parameters or to increase the aneuploidy rate in spermatozoa. In patient B, receiving long-term colchicine prophylaxis, the aneuploidy rate was significantly higher, 9.1%. This could suggest that the increased aneuploidy rate was causally related to long-term colchicine treatment. However, the incidence of aneuploidy in sperm cells of patient B matched within the range observed in 10 ICSI candidates with comparable semen parameters. We previously showed that the incidence of aneuploidy in spermatozoa of 10 ICSI candidates with severe OAT ranged from 2.6 to 11.0% (mean, 7.4%), which was significantly higher than the mean aneuploidy rate of 1.4% observed in spermatozoa of 3 normospermic donors (8). The total motile sperm count of those 10 OAT patients ranged from 0.1 to 4.6×10^6 and the percentage of spermatozoa with normal morphology ranged from 0 to 15%. In that study, no relation could be observed between the aneuploidy rate and semen parameters in the 10 OAT patients.

The results of the present study suggest that the incidence of aneuploidy observed in the two patients treated with colchicine is more likely related to sperm parameters rather than an effect of colchicine treatment. This supports the hypothesis of Haimov-Kochman and Ben-Chetrit (7) that the expression of the adverse effect of colchicine in FMF patients may depend upon other predisposing factors related to the basic disease itself.

For patient B, it is unknown whether poor sperm parameters already existed at the time FMF was diagnosed or whether sperm production and function were affected by colchicine treatment. However, in 1984 sperm parameters did not improve after the couple had expressed their child wish and colchicine administration was discontinued for several months and other treatment alternatives were tried. The recurrence and severity of attacks forced the patient to receive daily colchicine administration as continuous prophylaxis. Unfortunately, the pregnancy recently achieved after the first ICSI attempt was ectopic and no embryonic tissue could be obtained for cytogenetic analyses.

In conclusion, the cytogenetic analyses of sperm cells of males treated with colchicine seem to confirm the hypothesis that colchicine by itself may not have a significant direct adverse effect on sperm production and function. The incidence of aneuploidy observed correlates with the frequency observed in sperm cells of untreated patients with comparable semen parameters, which supports the hypothesis that underlying factors causing infertility in colchicine treated patients may determine sperm pathologies.

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Peter Kastrop,[1]
Ine Kimmel

[1] To whom correspondence should be addressed at University Hospital Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands.

Laszlo Bancsi
Sjerp Weima
Department of Obstetrics, Neonatology & Gynaecology

and

Jacques Giltay
Department of Medical Genetics
University Hospital
Utrecht, The Netherlands