Influence of sickle cell disease and treatment with hydroxyurea on sperm parameters and fertility of human males

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ABSTRACT

Background

Recent progress in the treatment of sickle cell disease, in particular the use of hydroxyurea, has considerably modified the prognosis of this disease. Many more patients now reach reproductive age. The objective of this study was to assess the potential impact of hydroxyurea on the semen of patients.

Design and Methods

In this retrospective multicenter study, we evaluated the sperm parameters and fertility of 44 patients and analyzed the potential impact of hydroxyurea.

Results

We report data from the largest series so far of semen analyses in patients with sickle cell disease: 108 samples were analyzed, of which 76 were collected before treatment. We found that at least one sperm parameter was abnormal in 91% of the patients before treatment, in agreement with published literature. All sperm parameters seemed to be affected in semen samples collected during hydroxyurea treatment, and this impairment occurred in less than 6 months, later reaching a plateau. Furthermore, after hydroxyurea cessation, while global results in 30 patients were not statistically different before and after hydroxyurea treatment, in four individuals follow-up sperm parameters did not seem to recover quickly and the total number of spermatozoa per ejaculate fell below the normal range in about half the cases.

Conclusions

The observed alterations of semen parameters due to sickle cell disease seem to be exacerbated by hydroxyurea treatment. Until prospective studies reveal reassuring findings, we suggest that a pre-treatment sperm analysis be performed and sperm cryopreservation be offered to patients before hydroxyurea treatment.

Key words: sickle cell disease, hydroxyurea, sperm, male fertility

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CECOS: Centre d'étude et de conservation des oeufs et du sperme humains.

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Introduction

Recent progress in the therapy of sickle cell disease, particularly the use of hydroxyurea, has considerably improved the prognosis of patients with this disease.[1,2] Their mean life expectancy is currently about 50 years,[3] their quality of life has improved and many more patients now reach reproductive age.

To our knowledge, only five analyses of sperm parameters in patients with sickle cell disease have been published till now, representing a total of 86 sperm samples.[4-8] All these studies described alterations of spermatozoa concentration, motility and morphology.[4-8] Some also reported a decrease in ejaculate volume[5-7] and sperm vitality.[8] Finally, 72 to 100% of the patients with sickle cell disease had an impairment of at least one sperm parameter. Patients with sickle cell disease often have moderate to severe hypogonadism,[9-11] of unknown origin, although several mechanisms have been suggested: primary hypogonadism,[9,12] hypogonadism induced by repeated testicular infarction,[13] zinc deficiency,[14,15] and puberty delay due to span-height retardation.[16-18]

Hydroxyurea itself has been reported to impair spermatogenesis in mammals, resulting in testicular atrophy,[19-21] a reversible decrease in sperm count,[19-23] and abnormal sperm morphology[20,24] and motility.[19] Furthermore the chromatin structure of germ cells is also affected, mainly in preleptotene spermatocytes[20,21] and apoptosis is increased, essentially in spermatogonia and early spermatocytes[25] while stem spermatogonia do not seem to be affected, resulting in the possibility of repopulation of seminiferous tubules.

A report of hypogonadism with testicular atrophy and gynecomastia in a 68-year old man with essential thrombocythemia treated with hydroxyurea for 8 years was the first case of a possible drug effect in humans.[26] In this case, the hormonal status pointed to the hypogonadism being of testicular origin, and testosterone treatment reversed the reported erection and ejaculation failures. No sperm analysis was available. The only other publication reports the follow-up analysis of sperm parameters in a 29-year old patient with sickle cell disease under hydroxyurea treatment.[27] After a normal semen analysis at the beginning of therapy and 1 month later, the patient became azoospermic at 6 months, and remained so 1 month later. Ten months after stopping treatment, another sperm analysis showed partial recovery of spermatogenesis. Thus an adverse effect of hydroxyurea was suspected in this case of transient and partially reversible azoospermia. We, therefore, conducted a retrospective study to evaluate sperm parameters and fertility of men suffering from sickle cell disease; to analyze the potential impact of hydroxyurea and to consider the advisability of proposing sperm cryopreservation before this treatment is started.

Design and Methods

Patients

Forty-four patients seen in two hematology centers between September 1994 and December 2004, and who agreed to a semen analysis were included in this study. The diagnosis of sickle cell disease was made using standard investigations, including blood counts, hemoglobin electrophoresis, and family studies. Forty-one men had homozygous SS, one was a compound heterozygote SC and two were compound heterozygotes for sickle β^0 thalassemia. Eleven patients originated from the West Indies, 29 from sub-Saharan Africa and three from the Maghreb. The geographic origin of one patient was not documented. The mean age of the patients was 25.8 years (range, 16-48 years). The mean age of puberty in 11 patients was 14.4 years (range, 12-15.5 years).

Twenty-five patients (57%) had a past history of genito-urinary complaints, mainly priapism (22/44 patients) (50%) and/or other risk factors for spermatogenesis (3/44 patients displayed orchiepididymitis [n=1], testicular torsion [n=1] and urinary schistosomiasis [n=1]). Twenty percent of the patients questioned about sexual intercourse had problems (6/30 patients). The mean baseline hemoglobin level in the patients was 8.8 g/dL (range, 7-11 g/dL) and the baseline fetal hemoglobin was 6% (range, 0.5% to 19%). The two main indications for hydroxyurea treatment were at least three painful crises requiring hospital admission during the preceding year or recurrent episodes of acute chest syndrome. Hydroxyurea was prescribed at the dose of 20-30 mg/kg body weight/day according to hematologic tolerance.

Methods

A total of 108 ejaculates were analyzed: 76 samples before treatment obtained from 34 patients; 6 samples during treatment obtained from 5 patients and 26 samples after treatment obtained from 8 patients, at different intervals after the end of treatment. The same patient could have produced sperm samples before, during and after treatment.

Evaluation of sperm parameters

Sperm was collected in a sterile container by masturbation and analyzed after liquefaction according to WHO criteria.[28] The parameters assessed included volume of ejaculate, sperm concentration, motility (forward movement), vitality and morphology.[29] The total sperm count was obtained by multiplying the sperm concentration by the volume of ejaculate. Normal values for volume, sperm concentration, forward motility, sperm viability and morphology are, respectively, between 2 and 6 mL, at least 20 million/mL, 50% minimum, ≥60%, and the presence of 30% or more morphologically normal spermatozoa.

Evaluation of fertility

A questionnaire, devised by the team, was completed using data from the medical file on marital status, sexual activity and whether the patient had caused at least one pregnancy.

Statistical analysis

Table 1 presents values (mean±SD) obtained for each sperm parameter in the three categories of samples (before treatment, during treatment, and after treat-

ment). The data are also expressed as the percentage of abnormal values according to the WHO reference values for each parameter. Furthermore, minimum and maximum values are indicated.

Data from the four patients who produced samples both before and after hydroxyurea treatment, (representing, respectively, 10 and 12 samples) are excluded from Table 2.

For the remaining patients (30 who provided samples before treatment and 4 after), when several samples were available for a given parameter, we averaged the values for each patient. Comparisons of averaged values before and after hydroxyurea treatment were performed using the Mann-Whitney test. A Fisher's exact test was used to compare the percentage of abnormal values. Tests were performed using Stata Software (Texas, USA). A p value of <0.05 was considered statistically significant.

Results

Sperm parameters before treatment

Seventy-six samples were collected from 34 patients before treatment with hydroxyurea. The mean of each sperm parameter ± standard deviation, minimum and maximum values and the percentage of abnormal values are reported in Table 1. The most affected parameters were forward motility and morphology. In 40.3% of the samples, the total sperm count was decreased but no case of azoospermia was observed. The volume of semen was normal in 74.3% of the samples. In only three patients (9%) were all the sperm parameters normal.

There was no correlation between mean baseline hemoglobin level or percentage of baseline fetal hemoglobin and sperm parameters (*data not shown*).

Sperm parameters during treatment

Six samples were collected from five patients during hydroxyurea treatment. The duration of treatment varied from 2 to 10 years. The results are reported in Table 1. The most affected parameters were total sperm count and forward motility.

All patients had abnormal sperm parameters but there was no case of azoospermia.

Sperm parameters after treatment

Twenty-six samples were collected from eight patients after treatment with hydroxyurea. The treatment was interrupted between 6 months to 5 years before these samples were collected. The results are reported in Table 1. All parameters appeared impaired, according to WHO normal values, except the volume of ejaculate. In all, seven patients (87.5%) had abnormal sperm parameters and one patient was azoospermic, 4 years after treatment (Table 4- patient #2).

Comparison of sperm parameters before and after hydroxyurea treatment

Table 2 presents the results of averaged individual values for 30 patients who provided samples only before treatment and for four patients whose samples were taken only after treatment. There was no statistically significant difference for any sperm parameter between the two groups.

Individual evaluation of sperm parameters in relation to hydroxyurea treatment

Sperm parameters were evaluated both before and after hydroxyurea therapy in four patients. Data concerning this evaluation are as follows: patient #1, treated for 6 months, had a drastic decrease in sperm density 4 and 5 years after treatment was stopped (Table 3);

Table 1. Sperm parameters of samples obtained from sickle cell disease patients before, during or after treatment with hydroxyurea.

	Before treatment (76 samples from 34 patients)		*During treatment (6 samples from 5 patients)*		*After treatment (26 samples from 8 patients)*	
Sperm parameter	*Mean ± standard deviation (range)*	*Percent of abnormal values*	*Mean ± standard deviation (range)*	*Percent of abnormal values*	*Mean ± standard deviation (range)*	*Percent of abnormal values*
Volume of ejaculate, mL	3.08±1.67 (0.3-8)	25.7	2.68±1.28 (1.5-4)	50	2.99±2.85 (0.4-15)	36
Spermatozoa concentration, millions/mL	38.55±43.12 (0.02-280)	38.9	2.66±3.75 (c*-8.75)	100	18.46 ± 26.86 (0-86)	76
Total sperm count, millions	114.17±124.12 (0.07-588)	40.3	7.02±10.18 (c*-21.9)	100	61.12±107.37 (0-387)	68
Initial forward motility, % of motile	28.66±18.38 (0-60)	83.6	30.00±5.77 (25-50)	80	29.46±20.13 (0-80)	87.5
Spermatozoa morphology, % of normal	21.92±14.63 (0-53)	64.1	34.50±21.92 (19-65)	66.7	19.16±16.3 (0-49)	75
Vitality, % of living	59.75±21.61 (0-95)	43.1	52.00±14.23 (40-68)	50	44.40±20.12 (0-90)	77

c: cryptozoospermia.*

Table 2. Comparison of sperm parameters obtained in sickle cell disease patients before and after treatment with hydroxyurea.

	Volume of ejaculate, mL	*Spermatozoa concentration, millions/mL*	*Total sperm count, millions*	*Initial forward motility, % of motile*	*Spermatozoa morphology, % of normal*	*Vitality, % of living*
Mean ± SD before treatment (median) (30 patients-30 samples)	2.93±1.39 (2.63)	42.13±52.94 (23.5)	124.77±124.31 (105.41)	30.14±16.15 (31.25)	22.3±14.96 (22.5)	55.99±17.39 (60)
Mean ± SD after treatment (median) (4 patients-4 samples)	2.32±1.34 (2.23)	25.37±28.25 (17.54)	83.86±119.05 (37.6)	42.62±27.91 (38.12)	18.91±15.66 (10)	58.85±21.22 (50.7)
p value	0.45	0.48	0.45	0.47	0.89	0.95
Percent of abnormal values before treatment	23	43	37	90	64	47
Percent of abnormal values after treatment	50	50	50	75	67	75
p value	0.62	1	1	0.40	1	0.60

Table 3. Individual evaluation of hydroxyurea treatment on sperm parameters of patient #1 (duration of treatment 6 months).

Chronology	*Volume of ejaculate, mL*	*Spermatozoa concentration, millions/mL*	*Total sperm count, millions*	*Initial forward motility, % of motile*	*Spermatozoa morphology, % of normal*	*Vitality, % of living*
Before HU (sample 1)	0.9	37.2	33.48	10	ND	44
Before HU (sample 2)	2	17.5	35	3	ND	63
Before HU (sample 3)	2.1	31.6	66.36	2	ND	69
After HU (4 years later)	0.9	3.3	2.97	5	2	48
After HU (5 years later)	2.4	1.2	2.88	0	ND	10

ND: not determined; HU: hydroxyurea.

patient #2, treated for 5 years, had azoospermia 4 years after the end of treatment (Table 4) but had also suffered from orchitis and epididymitis during treatment; patient #3, treated for 6 years, had alterations in all sperm parameters except morphology (Table 5); the sperm of patient #4 was not altered (Table 6).

In summary, three out of the four patients (75%) had alterations in sperm production after hydroxyurea treatment.

Evaluation of fertility

Forty-three percent of the patients stated that they were living with a partner.

Seventeen of 42 men (40%) had caused at least one pregnancy. The mean age of these patients was 37.2±8.2 years, while that of the patients without a history of pregnancy in their partner was 26.9±5.1 years ($p<0.0001$). The total number of pregnancies reached 36, including 35 natural conceptions and one resulting from assisted *in vitro* fertilization by intracytoplasmic sperm injection using sperm cryopreserved before hydroxyurea treatment had been started. The pregnancy outcomes were considered within the normal range with 29 normal births, three spontaneous miscarriages (for the same patient) and four abortions.

Two pregnancies in the partners of two patients who had been receiving hydroxyurea treatment for 2 months and 2.5 years resulted in the birth of normal babies. The partners of three patients who had received hydroxyurea for a short period (1, 6 or 12 months) conceived within a normal delay (< 2 years).

Discussion

In this retrospective study, we analyzed the sperm parameters of 44 patients with sickle cell disease. With 108 samples, 76 of which were collected before treatment, this study represents the largest series of semen analyses in this hemoglobinopathy. Furthermore, we report for the first time the potential impact of hydroxyurea and its possible long-term effect after drug cessation. Such studies are now possible given the improvement in life expectancy of patients with sickle cell disease mainly due to hydroxyurea treatment, who now reach reproductive age. The evaluation of possible side-effects of hydroxyurea on male fertility has, therefore, become a question of public health. Any deleterious impact of hydroxyurea on spermatogenesis and sperm parameters would represent a major concern necessitating advice on sperm cryopreservation as a preventive measure in order to preserve future male fertility. Furthermore, this may represent a possible obstacle to treatment compliance in young sickle cell disease patients.

In the population studied before treatment with hydroxyurea, we found an alteration of at least one sperm parameter in 91% of the patients, in agreement with published data.[4-8] This is not surprising as the patients eligible for hydroxyurea therapy are those most seriously affected by their disease, with at least three painful crises requiring hospital admission during

the preceding year or recurrent episodes of acute chest syndrome. An alteration of sperm production has been described and related to hypogonadism,[9,10,12-15] recurrent infections and fever attacks, repeated testicular infarction[13] and hypoxia.[5]

In the present series, 40% of individual values of concentration of spermatozoa per volume and per ejaculate were below normal.[28] Sperm morphology was severely impaired with abnormal values in two-thirds of the cases. Although it is difficult to compare the profile of morphological abnormalities between different studies as the authors used different morphological classifications, sperm head defects are the most common abnormality, mimicking the stress pattern seen in patients with varicocele.[5,7]

A decrease in semen volume in patients with sickle cell disease was previously described,[7] suggesting that in addition to testicular dysfunction, there may be abnormalities in the accessory sex organs, such as the seminal vesicles and the prostate gland, resulting from recurrent infections of the urinary tract. In the present study, the volume of ejaculate was in the normal range in 75% of the samples. It should be noted that Osegbe *et al.* did not find any significant difference in ejaculate volume between sickle cell disease patients and fertile male controls.[6]

In adult male subjects with only sickle cell trait, there was no detectable difference in any of the common indices of semen quality compared to those in an age-matched group of subjects with a normal hemoglobin genotype.[30]

All sperm parameters in semen samples collected during hydroxyurea treatment were affected. We did not find any case of azoospermia in our five patients who provided samples in this period, but observed a marked decrease in sperm density when comparing semen before and during treatment (total sperm count: 114.17±124.12 *vs.* 7.02±10.18 million spermatozoa, respectively). The potential adverse impact of the treatment occurred rapidly (in less than 6 months), later reaching a plateau. Only one publication has reported the kinetics of sperm values after starting hydroxyurea treatment:[27] in a 29-year patient with sickle cell disease semen analysis was normal at the beginning of treatment and 1 month later, but azoospermia was detected 6 months after starting treatment and confirmed 1 month later.

After stopping HU treatment, sperm parameters do not seem to recover to their initial levels. In four patients, semen analysis was performed before and after treatment, adding new information on the impact of hydroxyurea on spermatogenesis, after the single case report so far ever published to our knowledge.[27] Three out of the four patients still have altered sperm production after hydroxyurea treatment, one of whom is azoospermic, 4 years after the cessation of any treatment. The sperm of the fourth patient was not altered but his compliance with treatment was questioned.

Given as a single injection to mice, hydroxyurea was

Table 4. Individual evaluation of hydroxyurea treatment on sperm parameters of patient #2 (duration of treatment 5 years).

Chronology	*Volume of ejaculate, mL*	*Spermatozoa concentration, millions/mL*	*Total sperm count, millions*	*Initial forward motility, % of motile*	*Spermatozoa morphology, % of normal*	*Vitality, % of living*
Before HU (sample 1)	4.7	42.8	201.16	35	33	56
Before HU (sample 2)	5.3	36.8	195.04	30	ND	49
After HU (1 year later)	1	0	0	–	–	–
After HU (4 years later)	0.4	0	0	–	–	–

HU: hydroxyurea.

Table 5. Individual evaluation of hydroxyurea treatment on sperm parameters of patient #3 (duration of treatment 6 years).

Chronology	*Volume of ejaculate, mL*	*Spermatozoa concentration, millions/mL*	*Total sperm count, millions*	*Initial forward motility, % of motile*	*Spermatozoa morphology, % of normal*	*Vitality, % of living*
Before HU (sample 1)	5	70	350	45	ND	95
Before HU (sample 2)	8	50	400	40	ND	95
Before HU (sample 3)	3.5	40	140	30	ND	94
After HU (1 year later)	3.5	2.2	7.7	15	16	30
After HU (1 year later)	4	1.5	6	55	ND	7
After HU (1 year later)	3.2	0.67	2.13	25	ND	40
After HU (3 years later)	15	0.44	6.6	5	16	16
After HU (3 years later)	6.2	1	6.2	10	ND	33

HU: hydroxyurea.

Table 6. Individual evaluation of hydroxyurea treatment on sperm parameters of patient #4 (duration of treatment 4 years).

Chronology	*Volume of ejaculate, mL*	*Spermatozoa concentration, millions/mL*	*Total sperm count, millions*	*Initial forward motility, % of motile*	*Spermatozoa morphology, % of normal*	*Vitality, % of living*
Before HU (sample 1)	4	35	140	40	42	92
Before HU (sample 2)	4	27	108	25	ND	91
After HU (1 year later)	4	68	272	40	42	60
After HU (1 year later)	4.5	86	387	40	49	49

HU: hydroxyurea.

cytotoxic to differentiated spermatogonia whereas it had little effect on stem cells.[22] In sickle cell disease, hydroxyurea is administered at an infra-cytotoxic dose (i.e. a dose not decreasing the hemoglobin level, white blood cell and platelet counts) of 20-30 mg/kg body weight/day. Nevertheless, if one can extrapolate from rodent studies to humans, such a dose may lead to azoospermia by spermatogonia depletion during long-term treatment, possibly followed later by a repopulation of seminiferous tubules by stem cells.

Despite alterations of sperm parameters, fertility seems to be conserved in this population of young men with young partners. The rate of miscarriage is in the normal range and pregnancies resulted in normal births. In a multicenter study on hydroxyurea, two pregnancies established while the male partners were under treatment led to the birth of healthy babies.[1] Such data are important in order to provide reliable information and counselling to couples consulting in the future.

However, despite these reassuring fertility data and our finding of no changes pre- and post-hydroxyurea treatment, we observed alterations of most semen parameters in our patients. These alterations may be due to sickle cell disease, but they could potentially be exacerbated by hydroxyurea treatment. It cannot, therefore, be guaranteed that patients who take hydroxyurea will maintain normal fertility during their whole reproductive life. We suggest that a pre-treatment sperm analysis be performed and sperm cryopreservation be offered prior to hydroxyurea treatment to patients wishing to preserve their fertility potential, until prospective studies yield definitely reassuring data. Furthermore, these uncertainties highlight the need for more long-term studies of sperm alterations and fertility in a larger number of sickle cell disease patients, ideally analyzing the same patients before, during and after treatment leading to an individual follow-up.

Authorship and Disclosures

JM was the principal investigator and takes primary responsibility for the paper. FKN, DB, FG, and RG recruited the patients. IB, VDL, CR, JMK, LL, and PJ performed the laboratory work for this study. PYA participated in the statistical analysis, IB, GG, and JM coordinated the research. IB, JM, and RG wrote the paper. The authors reported no potential conflicts of interest.

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