SPERM ABNORMALITIES AND HISTOPATHOLOGICAL CHANGES IN THE TESTES IN Crj:CD(SD)IGS RATS

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ABSTRACT — In this study, morphological examination and computer-assisted sperm analysis (CASA) of epididymal spermatozoa in non-treated Crj:CD(SD)IGS rats were performed, and the relationship between the data obtained and the retention of step 19 spermatids in Stage IX to XI seminiferous tubules was examined.

Retention of step 19 spermatids in Stage IX to XI seminiferous tubules was observed in all 50 untreated males, and the incidence ranged from 3.3% to 100%. Eighteen animals showed a high incidence of retention ($74.7 \pm 14.2\%$, HIR for short), and the others showed a low incidence ($24.9 \pm 11.0\%$, LIR for short). Although the incidence of retention in Stage X and XI seminiferous tubules was very low in LIR males, it was high in HIR males ($1.8 \pm 3.0\%$ vs $58.6 \pm 23.2\%$). Morphological abnormalities of sperms in the caudal region of the epididymis, mainly amorphous head and no head, were more frequently observed in HIR males than in LIR males ($36.2 \pm 28.5\%$ vs $1.8 \pm 1.2\%$). Sperm analysis also revealed some differences between HIR and LIR males: sperm motility in HIR males was severely lower than that in LIR males, and sperm velocity, beat/cross frequency and amplitude of lateral head displacement in HIR males were lower than the corresponding values in LIR males.

In summation, retention of step 19 spermatids frequently occurred in the non-treated Crj:CD(SD)IGS males, and a relationship between the retention of these spermatids and sperm abnormalities, such as morphologically abnormal sperms, low motility and other items revealed by sperm analysis (CASA), was suggested.

KEY WORDS: Retention of step 19 spermatids, Sperm morphological abnormalities, Sperm analysis by CASA

INTRODUCTION

Sprague-Dawley rats have been widely used in toxicological studies. However, intrastrain differences in fetal weight and body weight gain after birth have arisen because this strain has been used for such a long time, and high mortality within 2 years has also been noted. An International Gold Standard system has therefore been proposed by the Charles River groups, and Crj:CD(SD)IGS rats have been named as the strain to be used around the world.

Recently, analysis of sperm characteristics such as sperm count, sperm motility and sperm morphology

has been recommended as an optional procedure for confirmation or characterization of the effects of compounds on male fertility, and histopathologic examination of the testes and epididymides has also been recommended on case-by-case basis (ICH guideline).

In the present study, to collect historical data on sperm characteristics and histopathology of the testes and to investigate the relationship between sperm abnormalities and histopathologic changes in the testes of Crj:CD(SD)IGS rats, morphological features of sperms collected from the caput, corpus and caudal areas of the epididymides were examined microscopically; a sperm motility analysis was performed by com-

puter-assisted sperm analysis (CASA); and the testes were examined histopathologically.

MATERIALS AND METHODS

Animals

Fifty sexually mature (18-week-old) Crj:CD (SD) IGS male rats (Charles River Japan, Inc.) were used in this study. They were acclimatized to the environmental conditions for about 6 weeks. Animals were housed individually in metal cages in a clean booth which was placed in an animal room with a temperature of 20–26°C, relative humidity of 40–70%, air exchange about 10 times/hr and a 12-hr light/dark cycle. The animals were allowed free access to tap water and a laboratory animal diet (CRF-LPF, Oriental Yeast Co. Ltd. γ -ray irradiated). The animals weighed 474–628 g at necropsy.

Morphological examination of sperms

All males were weighed and were then exsanguinated under ether anesthesia. Their reproductive organs were examined macroscopically. The testes and epididymides were weighed. After weighing, the right epididymis was cut to divide the caput, corpus and caudal regions, and the 3 regions were placed in Petri dishes containing 5 or 10 ml of medium (37°C, M-199 containing 0.5% BSA) and cut with scissors to diffuse the sperms. Sperms were smeared on a slide glass, and sperm smear samples were fixed in 10% neutral buffered formalin and were stained with eosin. Four hundred sperms in each sample were examined microscopically for the following morphological abnormalities: 1) no head, 2) no hook, 3) amorphous head, 4) flexion at the cervical region and 5) others.

Sperm analysis

Before the cutting of the right epididymis, the caudal region was pricked with the tip of a surgical knife, and a small amount of the sperm fluid which leaked out was collected using a glass rod. The rod with the sperm fluid was carefully put in medium to diffuse the sperms. These sperm suspensions were examined microscopically, and the movements of the sperms were recorded on video tape. Two hundred or more sperms per each suspension were analyzed by CellSoft-4000 to obtain data on sperm motility, curvilinear velocity, straight line velocity, linearity, beat/cross frequency and amplitude of lateral head displacement (ALH for short).

Histopathologic examination of the testes

The right testes from all males were fixed in Bouin's solution for a few days and then were fixed in 10% neutral buffered formalin. They were embedded in paraffin, sectioned and stained with periodic acid-schiff (PAS). The number of seminiferous tubules which showed retention of step 19 spermatids was counted in about 30 Stage IX to XI seminiferous tubules in each male.

Statistical analysis

The data were tested by the F-test (Snedecor and Cochran, 1980) for homogeneity of variance. If the variances were homogeneous, Student's *t*-test (Snedecor and Cochran, 1980) was applied. If the variances were heterogeneous, Aspin-Welch's *t*-test (Snedecor and Cochran, 1980) was applied to compare the LIR (low incidence of retention of step 19 spermatids) group and the HIR (high incidence of retention of step 19 spermatids) group. Both *t*-tests were conducted at the two-tailed significance levels of 0.05 and 0.01, and the F-test was conducted at the significance level of 0.2.

RESULTS

Sexually mature Crj:CD(SD)IGS males were examined for variations in sperm morphology and motion. Table 1 shows body weight and the testis and epididymis weights. The mean testis weight was 1.72 g for both the right and left side, but the ranges were wide (1.22 to 2.14 g for the right testis and 1.06 to 2.07 g for the left testis). The mean testis weight in the undernourished HIR group (1.69 g for the right side and 1.71 g for the left side) was comparable to that in the LIR group (1.74 g for both the right and left sides). A similar wide deviation was observed with the epididymides. However, there was no relationship between these deviations and the results of histopathologic analysis of the testes or sperm examination described below.

Table 1. Body weight and the testis and epididymis weights in untreated Crj:CD(SD)IGS males.

	Body weight (g)	Testis weight (g)		Epididymis weight (mg)	
		Right	Left	Right	Left
Mean	559	1.72	1.72	595	586
S.D.	31	0.16	0.17	83	59
Range Max	628	2.14	2.07	794	699
Minimum	474	1.22	1.06	403	377

Sperm abnormalities in Crj:CD(SD)IGS rats.

Photo 1, 3 and 5 depict normal Stage IX, X and XI seminiferous tubules, respectively. In these tubules, step 19 mature spermatids had been released.

Photo 2, 4 and 6 depict abnormal Stage IX, X and

XI seminiferous tubules, respectively. In these tubules, retention of step 19 spermatids was observed. This abnormal finding was frequently observed in Stage IX seminiferous tubules in this strain but not in Stage X or

Photo 1~6. Histopathology of seminiferous tubules in Crj:CD(SD)IGS males. Photos 1 and 2 show Stage IX of seminiferous tubules, Photo 2 and 4 Stage X, and Photo 3 and 6 Stage XI, respectively. Photo 1, 3 and 5 show normal features and Photo 2, 4 and 6 show retention of step 19 spermatids in Stages IX, X and XI seminiferous tubules, respectively.

XI seminiferous tubules.

Table 2 shows the incidence of retention of step 19 spermatids in Stage IX, X and XI seminiferous tubules of which approximately 30 were examined for each male. Data from 1 of the 50 males were excluded from this table because this male showed atrophy in almost all seminiferous tubules. The remaining 49 males were divided into two groups according to the incidence of retention of step 19 spermatids in Stage X and XI seminiferous tubules; 32 males showed an incidence of less than 10% and were allocated to the low incidence of retention (LIR for short) group, and the other 17 males, which showed an incidence of 15% or more, were allocated to the high incidence of retention (HIR for short) group. In the LIR group, retention was frequently observed in Stage IX seminiferous tubules (mean: 60.4%, range: 8.3 to 100%); however, the mean incidence was 3.3% and 0% in Stage X and Stage XI tubules, respectively. On the other hand, all Stage IX seminiferous tubules showed retention of step 19 spermatids in 12 of the 17 males in the HIR group, and the mean incidence in this group was higher than that in the LIR group (94.6% vs 60.4%). Moreover, the incidence of retention in Stage X and XI seminiferous tubules in the HIR group was severely high when compared with that in the LIR group (58.6% vs 1.8%).

Table 3 shows morphological examination data of sperms from the caput, corpus and caudal regions of the epididymides. The HIR group contained 1 male which showed atrophy of almost all seminiferous tubules. The frequency of total abnormalities in the HIR group was high in all 3 regions when compared with that in the LIR group (11.1% vs 2.2% in caput region, 10.9% vs 1.7% in corpus region and 36.2% vs 1.8% in caudal region). The main type of abnormalities was sperms with an amorphous head in the caput and corpus regions and sperms with no head in the caudal region. Sperms with tail abnormalities were not seen in any region.

Table 4 shows data from the computer-assisted sperm analysis (CASA) using sperm suspensions collected from the caudal region of the epididymides. Sperm motility in the HIR group was lower than that in the LIR group (40.6% vs 83.9%). Straight-line velocity in the HIR group was 83 μ m/s and was also low when compared with the value in the LIR group (101 μ m/s). Beat/cross frequency and ALH (mean and max.) values were slightly low in the HIR group. There were no significant differences in curvilinear velocity or linearity values between the HIR group and the LIR group.

Table 2. Incidence of retention of step 19 spermatids in Stage IX-XI seminiferous tubules in untreated Crj:CD(SD)IGS males.

	No. of males	Total incidence (%)	Stage IX (%)	Stage X+XI (%)
LIR group	32	24.9 $\pm$ 11.0	60.4 $\pm$ 24.2	1.8 $\pm$ 3.0
HIR group	17	74.7 $\pm$ 14.2 **	94.6 $\pm$ 9.8 **	58.6 $\pm$ 23.2 **

LIR: Low incidence of retention of step 19 spermatids

HIR: High incidence of retention of step 19 spermatids

**: Significantly different from the LIR group value ($p < 0.01$)

Data are expressed as mean $\pm$ S.D.

Table 3. Morphological abnormalities of sperms from the caput, corpus and caudal regions of the epididymides in Crj:CD(SD)IGS males.

	Total abnormalities (%)	No head (%)	No hook (%)	Amorphous (%)	Flection (%)
Caput LIR	2.2 $\pm$ 1.3	0.6 $\pm$ 0.4	0.3 $\pm$ 0.3	1.2 $\pm$ 1.0	0.1 $\pm$ 0.2
HIR	11.1 $\pm$ 9.4**	2.6 $\pm$ 2.5	1.1 $\pm$ 1.4	6.8 $\pm$ 6.2	0.6 $\pm$ 1.0
Corpus LIR	1.7 $\pm$ 1.0	0.5 $\pm$ 0.4	0.2 $\pm$ 0.3	0.9 $\pm$ 0.6	0.1 $\pm$ 0.2
HIR	10.9 $\pm$ 11.0**	3.9 $\pm$ 5.0	0.8 $\pm$ 1.0	5.5 $\pm$ 5.2	0.7 $\pm$ 1.0
Caudal LIR	1.8 $\pm$ 1.2	1.0 $\pm$ 0.9	0.2 $\pm$ 0.3	0.6 $\pm$ 0.5	0.0 $\pm$ 0.1
HIR	36.2 $\pm$ 28.5**	32.7 $\pm$ 27.1	0.4 $\pm$ 0.4	2.8 $\pm$ 2.5	0.3 $\pm$ 0.5

LIR: Low incidence of retention of step 19 spermatids

HIR: High incidence of retention of step 19 spermatids

**: Significantly different from the LIR group value ($p < 0.01$)

Data are expressed as mean $\pm$ S.D.

Table 4. Characteristics of sperms from the caudal region of the epididymis in Crj:CD(SD)IGS rats.

Item	LIR group	HIR group
Motility (%)	83.9 $\pm$ 4.4	40.6 $\pm$ 27.0**
Curvilinear velocity (μ m/s)	409 $\pm$ 37	376 $\pm$ 103
Straight line velocity (μ m/s)	101 $\pm$ 11	83 $\pm$ 27*
Linearity	2.6 $\pm$ 0.2	2.5 $\pm$ 0.6
Beat/Cross frequency (Hz)	10.9 $\pm$ 1.8	9.3 $\pm$ 2.1**
ALH mean (μ m)	24.4 $\pm$ 2.7	20.3 $\pm$ 6.2*
ALH max (μ m)	29.7 $\pm$ 2.8	24.9 $\pm$ 8.2*

LIR: Low incidence of retention of step 19 spermatids

HIR: High incidence of retention of step 19 spermatids

ALH: Amplitude of lateral head displacement

*, **: Significantly different from the LIR group value ($p < 0.05$, $p < 0.01$)

Data are expressed as mean $\pm$ S.D.

DISCUSSION

When male rats are treated with testicular toxicants, necrotic changes in germ cells or exfoliation of these cells into the tubular lumen occurs, and prolonged dosing will almost invariably result in severe germ cell loss and atrophy of the seminiferous tubules (Creasy, D.M., 1997, Jezek, D., *et al.*, 1993, Hess, R.A., *et al.*, 1991). The retention of step 19 spermatids in Stage IX to XI seminiferous tubules also occurs when the testes are only mildly affected by treatment with boric acid (Linder, R.E., *et al.*, 1990, Ku, W.W., *et al.*, 1993, Chapin, R.E. and Ku, W.W., 1994, Fail, P.A., *et al.*, 1998), bromoacetic acids (Linder, R.E., *et al.*, 1994), sodium dichloroacetate (Toth, G.P., *et al.*, 1992) and cadmium (Hew, K., *et al.*, 1993). Although the cause of spermatid retention has not been determined, hormonal changes are considered to affect the function of Sertoli cells. Some compounds such as reserpine (Kishimoto, K., *et al.*, 1995), nefiracetam (Harada, S., *et al.*, 1995) and bromoacetic acid (Linder, R.E., *et al.*, 1994) have been reported to affect production/secretion of testosterone and to induce retention of step 19 spermatids in Stage IX to XI seminiferous tubules in rats. In the present study, 17 out of 50 males (HIR rats) showed severe retention of step 19 spermatids in Stage IX to XI seminiferous tubules, but these males were not treated with any testicular toxicant. Lee, K.-P., *et al.* (1993) reported an increased incidence in spermatid retention in control animals used for inhalation toxicity studies when compared with control animals used for oral toxicity studies. The reason for this change has been considered to be the stress associated with immobilization in the restrainer used for nose-only exposure conditions. Lee and his colleagues used CrI:CD^R/BR rats, and we used Crj:CD(SD)IGS rats. Since these two strains are genetically similar, the retention of step 19 spermatids observed in the HIR males in this study may be related to hormonal changes caused by some type of stress associated with animal husbandry. However, further investigation will be needed to clarify the exact reason for this change.

Toth, G.P., *et al.* (1992) reported that in rats treated with sodium dichloroacetate inhibited spermiation was observed upon histopathological examination of the testes, and sperms with loose heads were also observed upon epididymal sperm examination. Similar changes such as retention of step 19 spermatids and morphological abnormalities of epididymal sperms were seen in rats treated with boric acid (Linder, R.E., *et al.*, 1990), dibromoacetic acid (Linder, R.E., *et al.*,

1994), methoxychlor (Linder, R.E., *et al.*, 1992) and dinitrobenzene (Hess, R.A., *et al.*, 1988). In this study, sperm head abnormalities, amorphous head and no head, were observed in the caput, corpus and caudal regions of the epididymides, and retention of spermatids was also observed in the HIR rats. Therefore, sperm head abnormalities are considered to be closely related to retention of step 19 spermatids in Stage IX to XI seminiferous tubules because release of mature spermatids is a function of the Sertoli cells mediated by specialized cell-to-cell junctions between these cells and the spermatids.

Decreases in motility, straight-line velocity, beat/cross frequency and ALH values were noted in the computer-assisted sperm analysis results in the HIR rats in this study. Similar results in rats treated with sodium dichloroacetate (Toth, G.P., *et al.*, 1992) and dibromoacetic acid (Linder, R.E., *et al.*, 1994) have been reported, and morphological sperm head abnormalities were also observed in these rats.

However, we reported that these changes in histopathology of the testes, sperm morphology and sperm analyses using the CASA system were not observed in untreated Jcl:Wistar or F344/DuCrj rats (Matsumoto, K., *et al.*, 1998).

In summation, failure of sperm release in Stage IX seminiferous tubules can easily occur in Crj:CD(SD)IGS rats, and sperm morphological abnormalities and decreases in sperm motility and velocity are considered to be associated with failure of mature sperm release from the Sertoli cells.

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