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Effects of the Chemotherapeutic Agents for Non-Hodgkin Lymphoma, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (CHOP), on the Male Rat Reproductive System and Progeny Outcome

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Abstract

Chemotherapy of non-Hodgkin lymphoma (NHL) with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) is associated with significant gonadal damage. Our goal was to determine the impact of CHOP chemotherapy on the male reproductive system, fertility, and progeny outcome in the rat model. Adult male Sprague-Dawley rats received saline or CHOP, 4 cycles of 3 weeks each, at doses analogous to 1/3x, 2/3x, or 1x the human dose; males were mated to evaluate effects on progeny outcome. Reproductive organ weights were significantly decreased in the 1x CHOP–exposed group. The spermatozoal contents of the testes and epididymides were decreased in 1x CHOP–treated males; the 1/3x and 2/3x doses also affected testicular sperm contents. Seminiferous tubule diameters were decreased by 20% in 1x CHOP–treated males. Damage ranged from the presence of small vacuoles in the epithelium to tubules deprived of spermatocytes and spermatids and was accompanied by an increased incidence of germ cell apoptosis. The acridine orange assay revealed a significant increase in sperm with abnormal DNA integrity profiles in the 1x CHOP group. Despite effects on germ cell number and quality, CHOP-exposed rats remained fertile. However, a 50% decrease in live fetuses was observed in litters sired by 1x CHOP–treated males due to a significant increase in both pre-implantation and postimplantation losses;

postimplantation loss was also elevated among litters sired by 2/3x CHOP–treated males. Thus, CHOP treatment affected both the quantity and quality of male germ cells; conceptal loss is a sensitive measure of the integrity of the male genome.

Key words: Anticancer drugs, spermatogenesis, male germ cell apoptosis, adverse effects, developmental toxicity

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Non-Hodgkin lymphoma (NHL) is the fifth most common cancer in Canada (Canadian Cancer Statistics, 2006); survival rates have improved dramatically in recent years because of sophisticated diagnostic tools and better treatment modalities using surgery, chemotherapy, and radiation or a combination. Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in combination are currently the gold standard in chemotherapy for the treatment of NHL (McKelvey et al, 1976). CHOP high-dose therapy has brought the 5-year survival rate up to 80% for patients with high-grade NHL (Bernard et al, 2005) and 40% for patients with aggressive NHL (Gregory and Trumper, 2005). Unfortunately, treatment with cytotoxic chemotherapy is associated with significant gonadal damage in men. Fertility after treatment is of concern because of the gonadotoxic effects of the chemotherapeutics and the young age of many of the patients. At lower doses of alkylating agents, recovery of spermatogenesis may occur within 1 to 3 years; however, at higher doses, infertility may be prolonged or even permanent (Shetty and Meistrich, 2005). Several authors have reported that CHOP chemotherapy results in azoospermia in all men during the treatment period; 67% recovered to normospermic levels within 5 years, whereas 5% remained oligospermic (Meistrich, 1999; Howell and Shalet, 2005). Patients experience prolonged azoospermia because the majority of the germ stem cells are killed by the cytotoxic agents (Van Thiel et al, 1972). Surviving cells are arrested during spermatogenesis at the spermatogonial (Kreuser et al, 1989) or spermatocyte (Meistrich et al, 1990) stage. Although the individuals who will be most affected cannot be predicted, reports have suggested that cumulative cyclophosphamide dosages >9.5 g/m^2 are associated with a high risk of permanent sterility (Pryzant et al, 1993).

All 4 of the drugs in the CHOP cocktail have been reported to be sterilizing as single agents. Previous studies from our laboratories using a rat model have shown that chronic cyclophosphamide treatment significantly increases DNA single- and double-strand breaks and cross-links in spermatozoa and elevates preimplantation and postimplantation embryo loss and fetal growth retardation and malformations (Trasler et al, 1985, 1986, 1987; Codrington et al, 2004). A number of studies have shown that male rats exposed to chronic treatment with doxorubicin have significant decreases in the number of spermatozoa in the cauda epididymides, sperm motility, the percentage of progressive sperm, and sperm velocity (Kato et al, 2001); a decrease in the numbers of implantations and live embryos and an increase in pre-implantation loss have also been observed. Adachi et al (2000) reported a suppression of body weight gain and decreased testis weight in rats treated for 2 weeks with doxorubicin (1 mg/kg/wk); histologic examination revealed that the number of spermatogonia was dramatically decreased. Kinkead et al (1992) and Stanley and Akbarsha (1994) showed that vincristine treatment of rats resulted in Leydig cells that were necrotic and swollen, with severe damage to the steroidogenic machinery. Chronic vincristine treatment of male mice resulted in decreases in testis weight and sperm count, effects on sperm morphology, and increases in DNA damage (Dobrzynska et al, 2005). However, the impact of exposure to a drug combination may be different than that of each alone. The goal of this study was to determine the consequences of exposure to the combination of drugs used in the CHOP chemotherapeutic regimen on male reproduction, fertility, and progeny outcome using the rat model.

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Animals, Treatment of Males, and Mating Schedule

Adult Sprague-Dawley male (400–425 g) and female rats (200–225 g) were purchased from Charles River Canada (St Constant, Canada) and maintained on a 14:10-hour light:dark cycle in the Animal Resources Centre of McGill University. Control and treated rats were provided with food and water ad libitum; there were no differences in food intake. All animal studies were conducted in accordance with the procedures and principles outlined in the Guide to the Care and Use of Experimental Animals prepared by the Canadian Council on Animal Care (McGill Animal Research Centre protocol 4687).

One week after arrival males were randomly divided into 4 treatment groups, each composed of 8 rats. Rats were treated with the combination chemotherapy most often used for NHL, which involves the administration of CHOP. Cyclophosphamide (Sigma-Aldrich, Oakville, Canada), doxorubicin and vincristine (LKT Laboratories, St Paul, Minn), and prednisone (Sigma-Aldrich) were administered for 1 week out of 3, in 4 cycles. The doses of the drugs administered to rats were analogous to human doses after adjustment for the differences in surface area to weight ratio. Thus, in rats, the 1x dose regimen was 27 mg/kg cyclophosphamide, 1.8 mg/kg doxorubicin, and 0.05 mg/kg vincristine, given intraperitoneally (1 injection per cycle on the first day of the first week of each cycle), and 1.47 mg/kg prednisone by gavage (5 times per week during the first week of each cycle) (Figure 1). The rats received 1/3x, 2/3x, or 1x, with 1x representing the analogous human dose; control rats received saline. Males were weighed every second day. During the 10th week each male was mated with 2 females in pro-estrus; the females were euthanized and pregnancy outcome was assessed, as described below, 20 days later. Males were anaesthetized, and the left testes and epididymides, seminal vesicles, and ventral prostates were removed, trimmed of fat, and weighed in week 11 of the treatment protocol. The epididymides were divided into caput-corpus and cauda regions. The caput-corpus epididymides and testes were frozen in liquid nitrogen for the determination of spermatozoal head counts. Blood samples were collected in plastic syringes and transferred to plastic tubes for the blood cell counts. The right testes and epididymides were perfuse-fixed through the abdominal aortas with Bouin fluid (BDH Inc, Toronto, Canada) for 15 minutes.

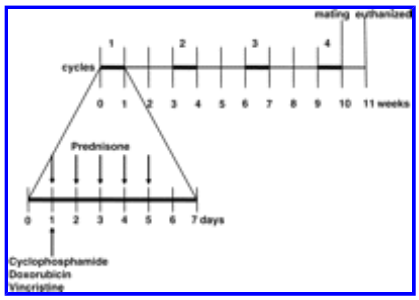

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Figure 1. Cyclophosphamide, doxorubicin, vincristine, and prednisone treatment regimen. Male rats were given prednisone daily by gavage for the first 5 days in every 3 weeks and were injected intraperitoneally with cyclophosphamide, doxorubicin, and vincristine once every 3 weeks on day 1 of the first week. Rats received 4 cycles of 3 weeks each. At the end of the treatment, each male was mated with 2 females in pro-estrus and euthanized 1 week later.

Enzyme-Linked Immunosorbent Assay Measurement of Testosterone

Serum testosterone concentrations were evaluated with a testosterone enzyme-linked immunosorbent assay (ELISA) kit (IBL Immuno-Biological Laboratories, Hamburg, Germany) following the

manufacturer's recommendations.

Spermatozoal Head Counts

The frozen testis and caput-corpus epididymidis from each of the 8 rats per group were homogenized (Polytron PTA7, setting 5; Brinkman Instruments, Westbury, NY), for two 15-second periods separated by a 30-second interval in 5 mL of 0.9% NaCl, 0.1% thimerosal, and 0.5% Triton X-100. To assess tissue content of spermatozoa, the heads of spermatozoa were counted hemocytometrically (Robb et al, 1978).

Testicular Histology

The perfuse-fixed testes were cut in 2 parts, placed in Bouin fluid overnight, and processed for routine paraffin embedding. The testes were cut into 5-µm sections. Three serial sections per testis were mounted on slides, deparaffinized, rehydrated, and stained with periodic acid Schiff (Sigma-Aldrich) following the manufacturer's recommendations. Sections of the seminiferous tubules at all stages were examined by light microscopy; tubule diameters were assessed in each testis (≥200 random determinations per testis) using a previously calibrated micrometer eyepiece.

In Situ Detection of Apoptosis

Sperm DNA fragmentation was evaluated with the terminal deoxynucleotidyl transferase (TdT)–mediated dUTP-biotin nick end labeling (TUNEL) assay (Gavrieli et al, 1992), which detects apoptotic cells (ApopTag *In Situ* Apoptosis Detection Kit; CHEMICON International Inc, Temecula, Calif). Testes embedded in paraffin were cut into 5-µm sections. After deparaffinization and rehydration, tissue sections were incubated with proteinase K (20 µg/mL) for 15 minutes at room temperature, washed in distilled water, treated with 3.0% hydrogen peroxide in phosphate-buffered saline (PBS) for 5 minutes at room temperature to quench endogenous peroxidase activity, and washed twice with water for 5 minutes each. Sections were treated with equilibration buffer and then incubated with working-strength TdT enzyme in a humidified chamber for 1 hour at 37 °C. Following this incubation, slides were immersed for 10 minutes at room temperature in working-strength stop/wash buffer and washed in 3 changes of PBS for 1 minute each. Antidigoxigenin conjugate was applied to the slides for 30 minutes at room temperature. After incubation, the slides were washed in 4 changes of PBS for 2 minutes per wash at room temperature. To determine the optimal staining time, color development was monitored by looking at the slides under the microscope. Specimens were counterstained, rehydrated, and mounted following the recommendations of the manufacturer. At least 200 tubules per slide were randomly examined with the light microscope.

Acridine Orange Assay for Sperm DNA Denaturation

To measure the susceptibility of sperm nuclear DNA to denaturation in situ, the acridine orange sperm chromatin structure assay (AO, SCSA®) was completed as described (Stanley and Akbarsha, 1994; Evenson et al, 2002). Sperm samples were thawed and sonicated on ice to remove the tails. A 200-µL aliquot of spermatozoa in PBS was mixed with 400 µL of denaturation buffer containing 0.08 N HCl, 0.15 M NaCl, and 0.1% Triton X-100 (pH 1.4) and incubated for 30 seconds at room temperature to denature uncondensed sperm DNA. After 30 seconds spermatozoa were stained with 1.2 mL of AO staining solution (0.2 M N_2HPO_4, 0.1 M citric acid buffer, 1 mM EDTA, 0.15 M NaCl [pH 6.0], and 5–10 µg of AO).

Stained spermatozoa were analyzed using a FACSCAN flow cytometer (BD Biosciences, San Jose, Calif) fitted with an argon ion laser (488-nm line excitation). Fluorescence of individual cells was measured at wavelengths of green (515- to 530-nm band pass filter) and red (630- to 650-nm long pass filter) in the peak mode. A total of 10 000 sperm were analyzed for each sample. The data were

processed using WinList Software (Verity Software House; Verity Software, Topsham, Me). The AO parameters obtained from this analysis included DNA fragmentation index (DFI), the standard deviation of DFI, and mean DFI ± SD.

Analysis of Pregnancy Outcome

The vaginas of all mated females were flushed with saline the morning after mating to assess the presence of spermatozoa. On day 20 of gestation (day 0 = morning of the day when sperm were found in the vagina), females were euthanized with CO_2 asphyxiation, decapitated, and sectioned by caesarean incision. The ovaries were removed and the numbers of corpora lutea counted. The uteri were removed and opened, and the numbers of implantations, resorption moles, and live fetuses were recorded. Preimplantation loss per litter was determined as the number of corpora lutea minus the number of implantation sites divided by the number of corpora lutea for each female. Postimplantation loss was calculated as the number of implantation sites minus the number of live fetuses divided by the total number of implantations. Each fetus was examined for any external malformations, weighed, sexed, killed by hypothermia, and fixed in ethanol. Fetuses weighing less than 75% of the mean weight for each group were considered as growth-retarded fetuses (Kirk and Lyon, 1984).

Statistical Evaluation

Data were analyzed by 1-way analysis of variance with Dunnet's multiple range test. Data are presented as means ± SEM. The level of significance was $P < .05$.

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Weights of Male Rats and Reproductive Organs

After 2 cycles of the treatment, most of the rats had oral mucositis that continued during the treatment. Because this caused eating difficulties and to avoid tooth fractures, animals were provided with soft, smooth food (5057 Rodent Diet; Charles River) during the rest of the experiment. The effects of CHOP treatment on body weights are shown in Figure 2. All CHOP-treated males gained weight during the treatment period, but the weight gain was less than that in the control group. There was a significant dose-dependent decrease in the weights of CHOP-treated rats by the end of the treatment.

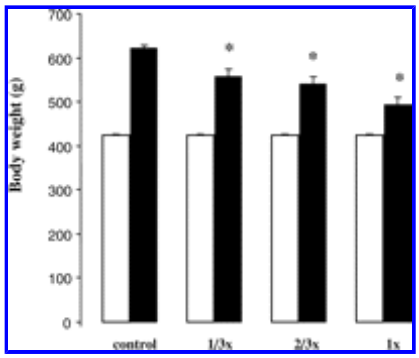

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Figure 2. Effects of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment on the body weights of male rats. Male rats were randomly divided into 4 treatment groups: control, 1/3x, 2/3x, and 1x. Animals were weighed before (white bars) and at the end (black bars) of the 11-week treatment period. Bars represent the means ± SEM (n = 8/group, except for the 1x group, in which there were 6 rats by the end of the experiment). The weight gain was significantly lower after CHOP treatment in all dose groups, compared with controls. *$P < .05$.

The weights of sex accessory tissues and concentrations of serum testosterone are presented in Figure 3. There was a significant decrease in ventral prostate weights in the 1x CHOP treatment group (Figure 3A). In contrast, CHOP treatment had no effect on seminal vesicle weights (Figure 3B) or on serum testosterone concentrations (Figure 3C). 1x CHOP treatment resulted in significant decreases in the weights of the testes and epididymides (Figure 4A and B), which were accompanied by decreases in testicular and epididymal spermatozoal head counts (Figure 4C and D). Interestingly, the total numbers of spermatozoa per testis were also decreased in the rats exposed to lower doses of CHOP (1/3x and 2/3x).

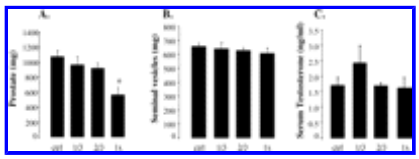

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Figure 3. Effects of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment on sex accessory tissue weights and serum testosterone concentrations. At the end of the CHOP treatment period, **(A)** prostate and **(B)** seminal vesicles were weighed. Blood was collected, and **(C)** serum testosterone concentrations were measured by enzyme-linked immunosorbent assay. Bars represent the means ± SEM (n = 6–8/group). *P < .05 compared with controls.

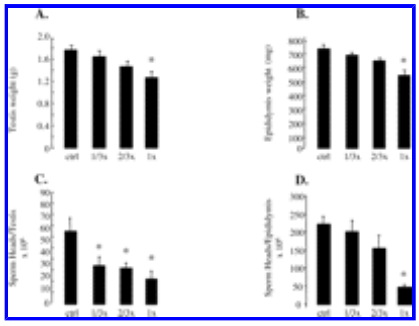

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Figure 4. Effects of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment on testis and epididymis weights and their spermatozoal head contents. At the end of the CHOP treatment, the **(A)** testes and **(B)** epididymides were weighed. Tissues were homogenized and spermatozoal heads were counted in the **(C)** testis and **(D)** epididymis. Bars represent the means ± SEM (n = 6–8/group). *P < .05 compared with controls.

Effects of CHOP Treatment on Testicular Histology

CHOP treatment–induced disturbances in the histology of the seminiferous tubules varied from small vacuoles in the epithelium to total loss of germ cells (Figure 5). Staging could not be evaluated due to the dramatic damage to tubules. Among the 5 animals analyzed for each treatment group, the greatest damage occurred in the 1x CHOP–treated group. Although the extent of damage was variable, a mean of 10% of the seminiferous tubules were heavily damaged; the range was 3% to 23%. Although various tubules contained round spermatids with the acrosomic head caps of spermatogenesis stage VI, some did not have the second generation of round or elongated spermatids (Figure 5C). In some tubules, germ cells were completely absent (Figure 5D); in others, the number of germ cells was greatly decreased (Figure 5E). Seminiferous tubule diameters were significantly reduced in the 1x CHOP treatment group compared with controls (Figure 5F); this effect was more pronounced in the periphery of the testis compared with central tubules (data not shown).

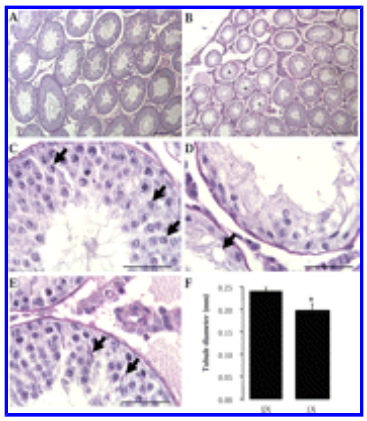

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Figure 5. Effects of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment on testicular histology. Testis sections (5 µm) were stained with periodic acid Schiff and observed under the light microscope. Sections from **(A)** control rats showed different stages of spermatogenesis, whereas sections from **(B)** 1x CHOP–treated rats showed alterations in spermatogenesis (+) and vacuolization (arrowhead in D) were observed. **(C)** Section from 1x CHOP–treated rat, stage VI of spermatogenesis with round spermatids (star) with an acrosomic zone (arrows) and no elongated spermatids. **(D)** Section from 1x CHOP–treated rat with no germ cells. **(E)** Section in which the number of elongated spermatids is dramatically decreased. **(F)** The measurements of seminiferous tubule diameters in control and 1x CHOP–treated groups. Bars represent the means ± SEM (n = 5). *P < .05. Bar = 200 µm in A–B and 50 µm in C–E. Color figure available online at www.androlgyjournal.org.

In Situ Detection of Apoptosis in Testicular Germ Cells of CHOP-Treated Rats

The TUNEL assay was completed to evaluate whether the CHOP regimen induced apoptosis (Figure 6). The incidence of apoptosis was significantly increased in 1x CHOP–treated rats compared with controls (Figure 6C). Interestingly, a low incidence of spontaneous apoptosis was observed in normal rat testes in stages I to IV and XI to XIV; in the 1x CHOP–treated rat testes, the incidence of apoptotic cells per 200 tubules was elevated in all stages of spermatogenesis (data not shown).

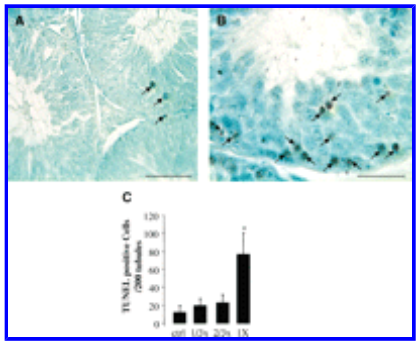

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Figure 6. Effects of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment on the number of terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling (TUNEL)–positive germ cells. TUNEL-positive cells (arrows) were stained in sections from **(A)** control and **(B)** 1x CHOP–treated rats. Bar = 50 µm. **(C)** Quantification of TUNEL-positive germ cells per 200 tubules in the different CHOP-treated groups. Bars represent the means ± SEM (n = 4). *P < .05. Color figure available online at www.androlgyjournal.org.

AO SCSA for Sperm DNA Denaturation

Results from the AO SCSA clearly showed a significant increase in the percentage of cells with abnormal DNA denaturation in the rats treated with 1x CHOP (Figure 7). The mean DFI, representing the mean fluorescence observed in the population, was higher in 1x CHOP–treated rats compared with controls (Figure 7A), as was the SD of DFI, a reflection of the width of the sample population (Figure 7B). The percent DFI, an assessment of the percent of cells outside the main population, was elevated more than fivefold in the rats exposed to the highest dose of CHOP relative to controls (Figure 7C).

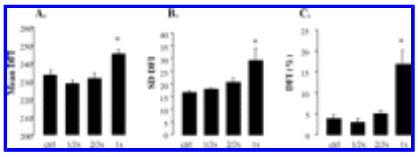

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Figure 7. Effects of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) treatment on sperm DNA fragmentation index (DFI) as determined by the acridine orange (AO) assay. Cauda sperm were collected at the end of the treatment period, and the AO assay was completed. The **(A)** mean DFI, **(B)** SD of DFI, and **(C)** percent of cells outside the main population (percent DFI) were determined by flow cytometric analysis. Bars represent the means ± SEM (n = 6–8). *P < .05 compared with controls.

Effects of CHOP Treatment on Pregnancy and Progeny Outcome

A significant, dose-dependent reduction in the number of pregnant females per sperm-positive females was observed among the females mated to the 2/3x and 1x CHOP–treated males: 100% of the sperm-positive female rats were pregnant in the control group, 91.7% in the 2/3x CHOP group, and 66.7% in the 1x CHOP group. A significant increase in pre-implantation loss was observed among the females mated to 1x CHOP–exposed males (Figure 8A). Postimplantation loss increased in a dose-dependant manner to reach almost 40% among the females mated to the 1x CHOP–treated males (Figure 8B). A consequence of these elevations in pre-implantation and postimplantation loss in the 1x CHOP treatment group was a twofold reduction in the average number of live offspring per litter among females mated to 1x CHOP–treated males (Figure 8C). Paternal CHOP treatment did not affect fetal weights (control, 3.5 ± 0.03 g; 1x CHOP, 3.5 ± 0.05 g) or sex ratios (data not shown). There were no fetuses with external malformations in this study, although 2 fetuses with significant growth retardation were observed among those sired by 1x CHOP–treated males.

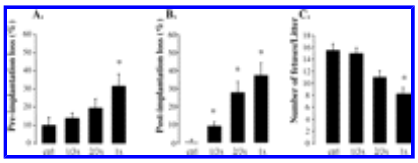

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Figure 8. The effects of paternal cyclophosphamide, doxorubicin, vincristine, and prednisone treatment on progeny outcome. At the end of the treatment, each male was mated with 2 females in pro-estrus. On day 20 of gestation females were euthanized, and the **(A)** preimplantation losses, **(B)** postimplantation losses, and **(C)** litter sizes were determined. Bars represent the means ± SEM (n: control, 12; 1/3x, 14; 2/3x, 9; 1x, 9 pregnant females). *P < .05 compared with controls.

Discussion

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The present study is one of the first to examine the effects of combination treatment with CHOP on the male reproductive system, fertility, and progeny outcome in a rat model. Because patients treated with CHOP chemotherapy are reported to have decreased posttreatment sperm concentrations (Bahadur et al, 2005), we assessed spermatozoal numbers in the testes and epididymides of treated rats. We report that CHOP chemotherapy resulted in decreases in testicular and epididymal posttreatment sperm concentrations. Interestingly, the numbers of spermatozoa per testis were significantly lower in rats from all CHOP treatment groups, despite the lack of effect of the lower dose treatments (1/3x and 2/3x) on testicular organ weights. CHOP chemotherapy kills proliferating cells so it may affect spermatogonia undergoing mitosis. In addition, CHOP exposure may affect differentiating cells, such as spermatocytes, spermatids, and mature spermatozoa. Although the

numbers of sperm heads per testis were significantly decreased in all CHOP-treated groups, testicular weights were lower, but not significantly decreased, in the 1/3x and 2/3x dose groups. Based on these results, it is tempting to speculate that the ability of sperm heads to resist homogenization is reduced with the lower dose regimens. This would imply a decreased rate of compaction of the chromatin, making it more amenable to dispersion upon homogenization.

We found that the CHOP drug regimen produced oral mucositis, also called stomatitis, in most of the rats; this is the most debilitating side effect of this cancer therapy seen in patients. Stomatitis is a common dose-limiting toxicity of cancer therapy (Sonis and Clark, 1991; Peterson, 1999) and occurs in approximately 40% of patients treated with chemotherapy (Sonis and Clark, 1991). Jones et al (2006) reported that approximately 15% of patients treated with CHOP chemotherapy develop severe oral or gastrointestinal complications during the treatment.

Alterations in the light microscopic appearance of the seminiferous tubules were observed mostly at the periphery of the testis sections. The vasculature in the rat testis has been described to be denser in the periphery (Chubb and Desjardins, 1982), suggesting that the drugs may be more concentrated and have a greater effect in this region of the testis. The general decrease in seminiferous tubule diameters observed after 1x CHOP treatment supports the observation that the number of germ cells per tubule is reduced. Previous studies showed that antitumor drugs such as cisplatin, etoposide, and cyclophosphamide induce apoptosis in male germ calls (Huddart et al, 1995; Chresta et al, 1996; Cai et al, 1997). High-dose CHOP treatment did induce a significant increase in apoptosis, which would contribute to the decrease in sperm count.

The AO SCSA was used to investigate if CHOP treatment alters DNA chromatin structure in spermatozoa. AO fluoresces green when bound to condensed or double-stranded DNA and red when intercalated into denatured DNA. Only sperm derived from the cauda epididymides from the 1x CHOP–treated males had higher mean DFI, SD of DFI, and percent DFI values than control rats, indicating an effect on DNA integrity. Previous studies have shown that there is a significant inverse correlation between SCSA parameters and fertility (Evenson et al, 1980; Ballachey et al, 1987; Huddart et al, 1995); the SD of DFI was more strongly correlated with infertility than percent DFI (Evenson and Wixon, 2005).

Our data suggested that AO parameters correlate with the number of DNA strand breaks as measured by the TUNEL assay (Evenson et al, 2002; Delbes et al, 2007).

The effects of CHOP treatment on both sperm count and sperm chromatin quality/DNA susceptibility to low pH denaturation suggest that fertility may be affected. Indeed, the fertility of 2/3x and 1x CHOP–treated male rats was decreased significantly. In addition, we observed dramatic increases in both pre-implantation and postimplantation loss among the progeny sired by 1x CHOP–treated rats. Interestingly, postimplantation loss was also higher among the progeny sired by males exposed to 2/3x CHOP; in the absence of effects on AO assay parameters of sperm quality, the only other parameter affected in this treatment group was the spermatozoal count per testis. These data suggest that early embryo failure is a sensitive measure of male genome integrity.

In men, the spontaneous abortion rate is twofold higher if >30% of the sperm have fragmented DNA, as measured by SCSA (Evenson and Wixon, 2005). Previous animal studies have shown that sperm with fragmented DNA are capable of fertilization (Marchetti et al, 2004); the chromosomal aberrations in the zygotes that were produced were highly predictive of subsequent abnormal embryonic development. Ahmadi and Ng (1999) noted that epididymal mouse sperm exposed to different doses of radiation fertilized mouse eggs with equal efficiency; however, the incidences of pre-implantation and postimplantation loss increased dramatically in a dose-response fashion. Kinkead et al (1992)

reported a significant increase in pre-implantation loss among the progeny of male rats exposed to cis-platinum. In previous studies from our laboratories, we demonstrated that male germ cells exposed to chronic low doses of cyclophosphamide throughout spermatogenesis had more DNA damage, as assessed by the comet assay, and transmitted this damage to the conceptus (Harrouk et al, 2000; Codrington et al, 2004). DNA repair does not occur during the later stages of spermiogenesis (Codrington et al, 2004; Olsen et al, 2005); higher pre-implantation and early postimplantation losses most likely represent a failure on the part of the conceptus to respond to the genotoxic stress introduced by a damaged male genome.

In conclusion, CHOP treatment affected male reproductive tissue weights, testicular histology, and sperm counts. The impact of CHOP exposure on male germ cells was translated into increases in pre-implantation and postimplantation loss with a resulting decrease in the number of live fetuses per litter. While early embryo loss clearly represents a sensitive measure of the adverse effects of chemotherapeutics on spermatozoal quality, new end points are needed to elucidate how drug exposures impact male germ cell genomic structure and function to further our understanding of the mechanisms underlying male-mediated developmental toxicity.

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Adachi T, Nishimura T, Imahie H, Yamamura T. Collaborative work to evaluate toxicity on male reproductive organs by repeated dose studies in rats. Testicular toxicity in male rats given adriamycin for two or four weeks. *J Toxicol Sci*. 2000; 25: 95 –101.[Medline]

Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. *J Exp Zool*. 1999; 284: 696 –704.[CrossRef][Medline]

Bahadur G, Ozturk O, Muneer A, Wafa R, Ashraf A, Jaman N, Patel S, Oyede AW, Ralph DJ. Semen quality before and after gonadotoxic treatment. *Hum Reprod*. 2005; 20: 774 –781.[Abstract/Free Full Text]

Ballachey BE, Hohenboken WD, Evenson DP. Heterogeneity of sperm nuclear chromatin structure and its relationship to bull fertility. *Biol Reprod*. 1987; 36: 915 –925.[Abstract]

Bernard M, Cartron G, Rachieru P, LeMevel A, Branger B, Le Maignan C, Berthou C, Ghandour C, Delwail V, Milpied N, Cassasus P, Celigny PS, Guyotat D, Lamy T, Desablens B. Long-term outcome of localized high-grade non-Hodgkin's lymphoma treated with high dose CHOP regimen and involved field radiotherapy: results of a GOELAMS study 1. *Haematologica*. 2005; 90: 802 –809. [Abstract/Free Full Text]

Cai L, Hales BF, Robaire B. Induction of apoptosis in the germ cells of adult male rats after exposure to cyclophosphamide. *Biol Reprod*. 1997;56: 1490 –1497.[Abstract]

Canadian Cancer Statistics 2006. Available at: http://www.cancer.ca/vgn/images/portal/cit_86751114/31/21/935505792cw_2006stats_en.pdf.pdf. Accessed April 9, 2007.

Chresta CM, Masters JR, Hickman JA. Hypersensitivity of human testicular tumors to etoposide-induced apoptosis is associated with functional p53 and a high Bax:Bcl-2 ratio. *Cancer Res*. 1996; 56: 1834 –1841.[Abstract/Free Full Text]

Chubb C, Desjardins C. Vasculature of the mouse, rat, and rabbit testis-epididymis. *Am J Anat*. 1982; 165: 357 –372.[CrossRef][Medline]

Codrington AM, Hales BF, Robaire B. Spermiogenic germ cell phase-specific DNA damage following cyclophosphamide exposure. *J Androl*. 2004;25: 354 –362.[Abstract/Free Full Text]

Delbes G, Hales BF, Robaire B. Effects of the chemotherapy cocktail used to treat testicular cancer on sperm chromatin integrity. *J Androl*. 2007;28: 241 –249.[Abstract/Free Full Text]

Dobrzynska MM, Czajka U, Slowikowska MG. Reproductive effects after exposure of male mice to vincristine and to a combination of X-rays and vincristine. *Reprod Fertil Dev*. 2005; 17: 759 –767. [CrossRef][Medline]

Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science*. 1980; 210: 1131 –1133.[Abstract/Free Full Text]

Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. *J Androl*. 2002; 23: 25 –43.[Medline]

Evenson DP, Wixon R. Environmental toxicants cause sperm DNA fragmentation as detected by the sperm chromatin structure assay (SCSA). *Toxicol Appl Pharmacol*. 2005; 207: 532 –537.[CrossRef][Medline]

Gavrieli Y, Sherman Y, Ben Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J Cell Biol*. 1992; 119: 493 –501.[Abstract/Free Full Text]

Gregory SA, Trumper L. Chemotherapy dose intensity in non-Hodgkin's lymphoma: is dose intensity an emerging paradigm for better outcomes? *Ann Oncol*. 2005; 16: 1413 –1424.[Abstract/Free Full Text]

Harrouk W, Codrington A, Vinson R, Robaire B, Hales BF. Paternal exposure to cyclophosphamide induces DNA damage and alters the expression of DNA repair genes in the rat preimplantation embryo. *Mutat Res*. 2000;461: 229 –241.[Medline]

Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. *J Natl Cancer Inst Monogr*, 2005; 12–17.[Abstract/Free Full Text]

Huddart RA, Titley J, Robertson D, Williams GT, Horwich A, Cooper CS. Programmed cell death in response to chemotherapeutic agents in human germ cell tumour lines. *Eur J Cancer*. 1995; 31A: 739 –746.[CrossRef]

Jones JA, Avritscher EB, Cooksley CD, Michelet M, Bekele BN, Elting LS. Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. *Support Care Cancer*. 2006; 14: 505 –515.[CrossRef][Medline]

Kato M, Makino S, Kimura H, Ota T, Furuhashi T, Nagamura Y. Sperm motion analysis in rats treated with adriamycin and its applicability to male reproductive toxicity studies. *J Toxicol Sci*. 2001;

26: 51 –59.[CrossRef][Medline]

Kinkead T, Flores C, Carboni AA, Menon M, Seethalakshmi L. Short term effects of cis-platinum on male reproduction, fertility and pregnancy outcome. *J Urol*. 1992; 147: 201 –206.[Medline]

Kirk KM, Lyon MF. Induction of congenital malformations in the offspring of male mice treated with X-rays at pre-meiotic and postmeiotic stages. *Mutat Res*. 1984; 125: 75 –85.[Medline]

Kreuser ED, Kurrle E, Hetzel WD, Heymer B, Porzsolt F, Hautmann R, Gaus W, Schlipf U, Pfeiffer EF, Heimpel H. Reversible germ cell toxicity following aggressive chemotherapy in patients with testicular tumors: results of a prospective study [in German]. *Klin Wochenschr*. 1989; 67: 367 –378. [CrossRef][Medline]

Marchetti F, Bishop JB, Cosentino L, Moore D, Wyrobek AJ. Paternally transmitted chromosomal aberrations in mouse zygotes determine their embryonic fate. *Biol Reprod*. 2004; 70: 616 –624. [Abstract/Free Full Text]

McKelvey EM, Gottlieb JA, Wilson HE, Haut A, Talley RW, Stephens R, Lane M, Gamble JF, Jones SE, Grozea PN, Gutterman J, Coltman C, Moon TE. Hydroxyldaunomycin (Adriamycin) combination chemotherapy in malignant lymphoma. *Cancer*. 1976; 38: 1484 –1493.[CrossRef][Medline]

Meistrich ML. Restoration of spermatogenesis by hormone treatment after cytotoxic therapy. *Acta Paediatr Suppl*. 1999; 88: 19 –22.[Medline]

Meistrich ML, Van Beek ME, Liang JC, Johnson SL, Lu J. Low levels of chromosomal mutations in germ cells derived from doxorubicin-treated stem spermatogonia in the mouse. *Cancer Res*. 1990; 50: 370 –374.[Abstract/Free Full Text]

Olsen AK, Lindeman B, Wiger R, Duale N, Brunborg G. How do male germ cells handle DNA damage? *Toxicol Appl Pharmacol*. 2005; 207: 521 –531.[CrossRef][Medline]

Peterson DE. Research advances in oral mucositis. *Curr Opin Oncol*. 1999;11: 261 –266.[CrossRef] [Medline]

Pryzant RM, Meistrich ML, Wilson G, Brown B, McLaughlin P. Long-term reduction in sperm count after chemotherapy with and without radiation therapy for non-Hodgkin's lymphomas. *J Clin Oncol*. 1993;11: 239 –247.[Abstract/Free Full Text]

Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J Reprod Fertil*. 1978;54: 103 –107.[Abstract/Free Full Text]

Shetty G, Meistrich ML. Hormonal approaches to preservation and restoration of male fertility after cancer treatment. *J Natl Cancer Inst Monogr*, 2005; 36 –39.[Abstract/Free Full Text]

Sonis S, Clark J. Prevention and management of oral mucositis induced by antineoplastic therapy. *Oncology (Williston Park)*. 1991;5: 11 –18.[Medline]

Stanley A, Akbarsha MA. Ultrastructural changes in the Leydig cell on treatment with vincristine. *Cytobios*. 1994; 79: 51 –58.[Medline]

Trasler JM, Hales BF, Robaire B. Chronic low dose cyclophosphamide treatment of adult male rats: effect on fertility, pregnancy outcome and progeny. *Biol Reprod*. 1986; 34: 275 –283.[Abstract]

Trasler JM, Hales BF, Robaire B. Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting male fertility. *Nature*. 1985; 316: 144 –146.[CrossRef][Medline]

Trasler JM, Hales BF, Robaire B. A time-course study of chronic paternal cyclophosphamide treatment

in rats: effects on pregnancy outcome and the male reproductive and hematologic systems. *Biol Reprod*. 1987;37: 317 –326.[Abstract]

Van Thiel DH, Sherins RJ, Myers GH Jr, De Vita VT Jr. Evidence for a specific seminiferous tubular factor affecting follicle-stimulating hormone secretion in man. *J Clin Invest*. 1972; 51: 1009 –1019.[Medline]

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