# Effect of Carboplatin on the Functional Integrity of the Human Sperm Membrane In Vitro

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**ABSTRACT:** Although it is well known that carboplatin is a drug that binds directly to DNA, causing DNA-DNA and DNA-protein cross-links, which is the presumptive method for killing cells, the whole mechanism of action of carboplatin on spermatozoa is unclear. There are no published data in peer-reviewed journals focused on the interaction between carboplatin and cell membranes. Therefore, the purpose of this study was to investigate the minimal concentration of carboplatin that would affect the functional integrity of the human sperm membrane in an in vitro model. Human-ejaculated spermatozoa were obtained from 20 healthy normozoospermic donors. Solutions (SOL) of 0.5 mL of the semen samples and 0.5 mL NaCl (0.9%) containing increasing concentrations (7.5, 15, 30, and 60 ng) of carboplatin per 1 mL of SOL were prepared. Then, the hypoosmotic-swelling (HOS) test and the eosin test were performed on these samples and compared with the control (no carboplatin) group. Significant damage to the plasma membrane in the head region (eosin test positive) and in the tail region of spermatozoa, as assessed by the HOS test, was observed in concentrations of 30 and 60 ng carboplatin per 1 mL of SOL in comparison to the values evaluated in the control group. The results demonstrate that a minimal carboplatin concentration of 30 ng/mL causes significant damage to membrane integrity of spermatozoa in healthy volunteers.

Key words: Testicular cancer, fertility, seminoma.

**J Androl 2002;23:338–340**

Over the last 3 decades, the survival of patients with testicular cancer has increased significantly due to new treatment options and the appropriate integration of cisplatin-based polychemotherapy, tumor-reductive surgery, and radiotherapy. Seminoma is the most frequent type of tumor to arise in the testis. Histologically, pure seminomas account for at least 40% of testicular tumors, with another 20% arising as mixed tumors containing both seminoma and teratoma (Horwich, 1991). The testicular germ cell tumor represents a highly curable tumor even in the presence of metastases, with an overall survival rate of approximately 90%–95% considering all stages. This high disease-free, long-term survival rate and the relatively young age of the involved patients place particular importance on the long-term sequelae of the diagnostic and treatment procedures used. It has to be kept in mind that the majority of patients with testicular tumors are men in their child-rearing years; thus, reproductive toxicity is an important concern.

Adjuvant single-agent carboplatin therapy for clinical stage I seminoma showed excellent results in multiple short-term studies (Oliver et al, 1990; Dieckmann et al, 1996, 2000; Krege et al, 1997) as well as in one recently published long-term study (Reiter et al, 2001). Although it is well known that carboplatin is a drug that binds directly to DNA, causing DNA-DNA and DNA-protein cross-links, which is the presumptive method for killing cells, the mechanism of action of carboplatin on spermatozoa is unclear. Is there a toxic influence of carboplatin on the sperm membrane besides the toxic influence on DNA? Because of the well-known fact that the functional integrity of the sperm membrane is very important for fertility (Chan et al, 1985), we tried to investigate the influence of carboplatin on isolated sperm when administered in vitro. Therefore, the purpose of this study was to investigate the minimal concentration of carboplatin that would affect the functional integrity of the human sperm membrane in an in vitro model.

## Materials and Methods

Human-ejaculated spermatozoa were obtained by masturbation from 20 healthy normozoospermic donors. Informed consent was obtained from all volunteers according to the declaration of Helsinki. The period of sexual abstinence was 4 days prior to ejaculation. Semen samples were allowed to liquefy at room temperature within 20–30 minutes of delivery. Then, semen volume, sperm concentration, motility, and viability were measured according to World Health Organization (1992) recommendations. Afterward, sperm morphology was assessed according to strict criteria (Krüger et al, 1986). All 20 samples fulfilled these requirements. Solutions (SOL) of 0.5 mL of the semen samples and 0.5 mL NaCl (0.9%) containing increasing concentrations of 7.5, 15, 30, and 60 ng carboplatin per 1 mL of SOL were
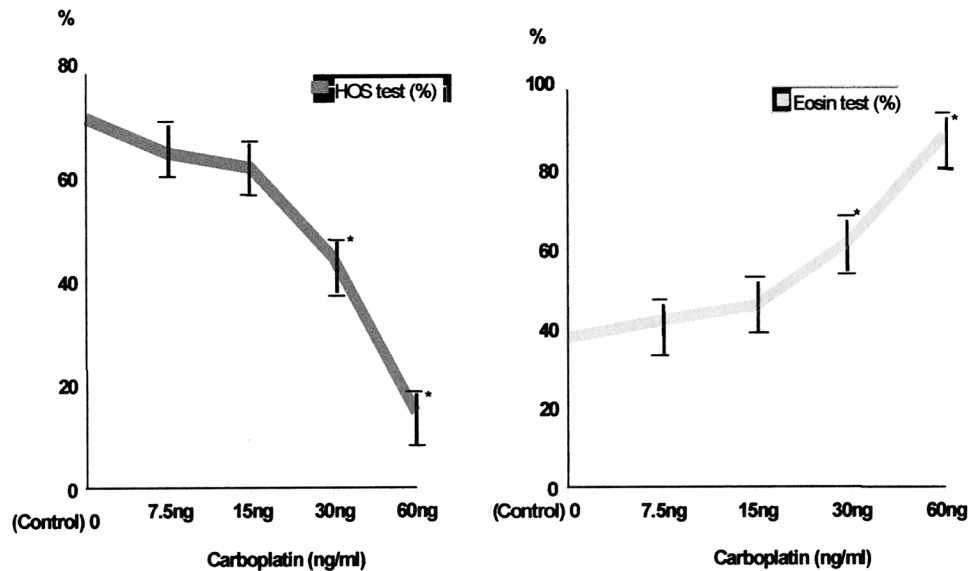
*p < 0.05 compared with control (analysis of variance)

Effects of carboplatin on the percentage of the hypoosmotic-swelling (HOS) and eosin tests.

prepared. The HOS test and the eosin test were then performed on these samples and compared with controls (no carboplatin).

### The Hypoosmotic-Swelling Test

The hypoosmotic-swelling (HOS) test was performed according to the method described by Jeyendran et al (1984). Briefly, it was performed by mixing 0.1 mL sperm suspension with 1 mL hypoosmotic solution (equal parts of 150 mOsm/kg fructose and 150 mOsm/kg sodium citrate), followed by incubation for 30 minutes at 37°C. After incubation, 200–300 spermatozoa were examined by phase-contrast microscopy at 400×. Gametes presenting a clear ballooning of their tail membranes were counted without subdividing them into swelling subclasses.

### Eosin Test

The eosin test was performed by mixing 0.1 mL sperm suspension with 0.1 mL eosin solution (0.5% [wt/vol] eosin in saline), followed by incubation at room temperature for 5 minutes. This mixture was then smeared on a slide, and a minimum of 200 spermatozoa were scored in a light microscope. Spermatozoa that exhibited abnormal membrane structure and that thus permitted eosin to enter the cell stained positive, while spermatozoa with normal membrane structure remained unstained (75% or more live spermatozoa was considered a normal count).

### Statistical Analysis

All results are expressed as mean plus or minus standard deviation. After testing for normal distribution, an analysis of variance for repeated measurements was performed. A Newman-Keuls test was then performed to test for differences between the groups. *P* values less than .05 were considered significant.

## Results

There was a statistically significant increase in the percentage of functional membrane damage (HOS test) to spermatozoa in concentrations of 30 and 60 ng carboplatin per 1 mL of SOL in comparison to the values evaluated in the control group. The number of HOS-positive spermatozoa (swollen) decreased from 71.4% plus or minus 4.3% (median, 70.8%) to 14.6% plus or minus 2.8% (median, 17.4%). Similarly, a statistically significant difference was observed in the eosin test results (sperm membrane structure in the head region) between the control samples and the spermatozoa in concentrations of 30 and 60 ng carboplatin per 1 mL of SOL. The number of eosin-positive spermatozoa increased from 37.6% plus or minus 12.9% (median, 35.9%) to 88.4% plus or minus 7.2% (median, 90.0%). The *P* values of both tests were less than .05 and were therefore considered statistically significant. All data are presented in the Figure.

## Discussion

Although it is well known that a minimum of 12 months is recommended for contraception after each kind of cytotoxic treatment, the exact time of restoration of spermatogenesis after treatment and the exact mechanism of carboplatin on spermatozoa are unknown. Koepf-Maier (1992), who investigated histologically the influence of carboplatin on the testicular morphology of male CF1 mice, demonstrated that

following treatment with carboplatin, early structural lesions of Sertoli cells occurred and were first manifested by the disruption of their tight junctions to neighboring cells. As a consequence of this violation of the blood-testis barrier, necrotization of spermatic cells at diverse levels of differentiation was seen. Additionally, Dieckmann and Loy (1995) revealed that the efficacy of cisplatin-based chemotherapy on invasive intratesticular tumors is not compromised by the blood-testis barrier. Therefore, the effect of this drug treatment seems to be primarily on the rapidly dividing spermatogonia and spermatocytes. Regarding the well-known fact that carboplatin also penetrates into ascites (Shea et al, 1989) and red blood cells (Elferink et al, 1987), we followed the hypothesis that carboplatin could penetrate in seminal fluid. It was possible to prove the evidence of carboplatin in seminal fluid after adjuvant carboplatin chemotherapy in a small number of 4 patients (unpublished data). The range of carboplatin concentrations in seminal fluids after adjuvant carboplatin chemotherapy was between 2.7 and 64 ng/mL measured the first day after treatment. Because of the statistically insignificant number of patients (4) and the wide range of carboplatin concentrations, we believe that these data are too preliminary to be published, but the important issue for this manuscript was the proven evidence of carboplatin in seminal fluid after adjuvant carboplatin treatment. Therefore, we decided to investigate the minimal concentration of carboplatin that would affect the functional integrity of the human sperm membrane in an in vitro model.

There are no published data in peer-reviewed journals focused on the interaction between carboplatin and cell membranes. Therefore, it is unknown whether carboplatin has an additional influence on the human sperm membrane besides its toxic mechanism on DNA. Because of the well-known fact that the functional integrity of the sperm membrane is very important for the fertility, we investigated the influence of carboplatin on isolated sperm when administered in vitro. The concentrations of carboplatin were chosen according to the data of van der Vijgh (1991), who was able to demonstrate that the concentration time curves of carboplatin in plasma are quite stable over the first 6 hours and that carboplatin penetrates into tissue. In the present study, significant damage to the plasma membrane in the head region (eosin test positive) was observed in the 30 and 60 ng/mL carboplatin in comparison to the values evaluated in the control group. In addition, injury to the plasma membrane in the tail region of spermatozoa, as assessed by the HOS test, correlated significantly to increasing concentrations of carboplatin in comparison to the control group. The extent of this spermatocidal effect of a minimal carboplatin concentration of 30 ng/mL may be related to the amount of platinum-DNA adducts formed in human spermatozoa. The tests applied give evidence only for the integrity of sperm membranes; they do not give evidence for the mechanism leading to a damage of membranes. This might be due not only to a direct effect, but also to an induction of apoptosis or other metabolic effects causing cell death. This question can not be answered with our data and could be an interesting topic for further research projects.

In conclusion, the present study demonstrates that a minimal carboplatin concentration of 30 ng/mL causes significant damage to membrane integrity of spermatozoa in healthy volunteers, which could be an additional toxic factor on the sperm quality in patients with testicular tumors during carboplatin chemotherapy. The results of an ongoing study of our research group, which is focused on the measurement of carboplatin levels in seminal fluid after adjuvant carboplatin chemotherapy in a significant number of patients, are needed to estimate the outcome of this in vitro investigation.

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