Reproductive Biology Insights

ORIGINAL RESEARCH

Reproductive Toxic Effects of Cisplatin and Its Modulation by the Antioxidant Sodium 2-Mercaptoethanesulfonate (Mesna) in Female Rats

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Abstract

Study Objective: Chemical protection against cisplatin, which is a commonly used cancer chemotherapeutic agent, is not well defined. We tested the hypothesis that the antioxidant mesna might protect against the cisplatin-induced repoductive effects in female rats.

Design & Setting: Adult female rats were injected with saline, cisplatin alone, or mesna + cisplatin, mated with males, and euthanized on gestational day 17.

Patients: Animal Model.

Interventions: The administration of either cisplatin or mesna + cisplatin (two injections one week apart, mesna 30 minute pretreatment) followed by mating one week after treatment.

Main Outcomes Measured: The number corpora lutea, implantation and resorptions sites, viable and non-viable fetuses, fetal weights, and the level of progesterone per corpus luteum.

Results: The administration of cisplatin caused an increase in pre- and post- implantation loss, an increase in the number of resorptions and a decrease in the number of viable fetuses. Mesna administered prior to cisplatin resulted in a decrease in the rate of the pre- and post implantation loss, along with a decrease in the number of resorptions and an increase in the number of live fetuses.

Conclusions: Prior exposure to cisplatin caused significant adverse effects on fertility as evidenced by the decreased implantation due to increased fetal loss. The administration of mesna appeared to temper cisplatin damage by lessening the cisplatin effects on fetal resorption.

Keywords: ovary, pregnancy, chemotherapy, cisplatin, mesna, antioxidant

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Background

Cisplatin is a commonly used chemotherapeutic agent for the treatment of a wide variety of cancers. In humans, cisplatin and other chemotherapeutics have been known to cause damage to the ovary as well, leading to premature ovarian failure in approximately 40% of female patients who undergo chemotherapy.[1] The effects of cisplatin on reproductive function subsequent to treatment are less understood. Studies have shown that women who have undergone cisplatin chemotherapy either intravenously or intraperitoneally were able to become pregnant spontaneously while other females failed to conceive.[2–4] In some pregnancies following cisplatin administration, spontaneous abortions have been reported.[4] Cisplatin treatment during pregnancy in humans has been demonstrated to have very few to no known effects on fetuses in postnatal or early life.[5] Long term effects of cisplatin on such offspring have not been investigated. In rats, cisplatin has previously been shown to cause damage to the ovaries by increasing the percentage of follicular apoptosis and follicular cyst formation in rats.[6,7]

In animal models, several studies have been performed on the effects of cisplatin administration during pregnancy. Administration of cisplatin during pregnancy in mice and rats resulted in fetal mitochondrial toxicity, DNA adduct formation, and increased incidence of tumors of kidney, skin, lung and other organs.[8–12] Cisplatin administered during pregnancy has also been shown to cause skeletal abnormalities in fetal mice.[13]

Only a few laboratory studies have addressed the effects of cisplatin on future reproductive capability. One such study investigated the effects of a single intravenous injection of cisplatin prior to pregnancy on the reproductive capabilities of female rats.[14] The study demonstrated that a single dose of cisplatin caused a significant increase in the pre-implantation and post-implantation loss 3 months and 6 months after administration. One month following cisplatin exposure, there were not significant increases in the pre- and post-implantation indices. In this study, the authors demonstrated also minor increases in external abnormalities and a delay in the ossification process of the fetuses, and minor alterations in postnatal development.[14] An additional study conducted using a similar protocol demonstrated comparable results.[15]

The antioxidant sodium 2-mercaptoethanesulfonate (mesna) is an FDA approved drug used clinically to reduce the systemic side effects of chemotherapy administration.[16–18] In the clinical setting, mesna is administered to patients who are receiving the chemotherapeutic agent cisplatin alone or in combination.[19] Studies of mesna using animal models have demonstrated that mesna offers protection against cytotoxic damage to the liver, bladder, and the intestines.[20–23] We have reported that mesna offers protection to the ovary by reducing ovarian damage due to cisplatin exposure.[23] Mesna appears to provide protection against cisplatin toxicity by reducing apoptosis and free radicals. Importantly, mesna does not interfere with the pharmacokinetics of cisplatin.[19,24,25]

We sought to address the effects of multiple exposures to cisplatin on the future reproductive capabilities by studying adverse effects of prior injections of cisplatin on the reproductive function of female rats. In addition, we sought to determine the degree of protection against the adverse reproductive effects of cisplatin by using mesna. The purpose of this study, thus, was to identify adverse effects of the prior administration of multiple doses of cisplatin on fetal outcomes. In addition, the purpose was to demonstrate that the administration of mesna in conjunction with cisplatin offered protection from the future loss of fertility induced by cisplatin administration prior to pregnancy.

Methods

Animals

Adult virgin female Sprague-Dawley rats aged 65–75 days were purchased from Harlan (Indianapolis, IN). Proven male breeders were also purchased from Harlan. All animals were allowed a 1-week acclimation period before the start of experiments. All procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee (protocol #GYN01055Y). The female rats used in this study were divided into two groups. Group 1 served as the cisplatin mating group and group 2 served as the mesna + cisplatin mating group.

Experiment 1: Cisplatin reproductive toxicity

The rats were divided into two treatment sub-groups, the saline control sub-group and the cisplatin treated sub-group. This protocol has been used previously by our laboratory and has been demonstrated to adversely affect the ovarian function of female rats.[6,22] The

animals were given one IP injection of saline or cisplatin (4.5 mg/kg) once a week for two weeks. Ten milliliters (mls) of sterile 0.9% NaCl was administered subcutaneous (SQ) once a week over multiple injection sites and additionally as needed to prevent dehydration. Animals were monitored and weighed daily.

Experiment 2: Effect of mesna on cisplatin induced reproductive toxicity

For the second set of mating studies, the rats were divided into four sub-groups, the control (saline) sub-group, the mesna + saline control subgroup, mesna + cisplatin treatment sub-group, and cisplatin only treatment sub-group.[23] Cisplatin was given at a dosage of 4.5 mg/kg. Mesna was given as a 200 mg/kg dosage 30 minutes prior to the administration of saline or cisplatin. Each animal received two IP injections one week apart as determined by the assigned treatment group. Each rat also received 10 mls of sterile 0.9% NaCl SQ over multiple injection sites following the administration of cisplatin and as needed throughout the course of the study to prevent dehydration. Animals were monitored and weighed daily.

Mating studies

One week following the final saline, mesna + saline, mesna + cisplatin, or cisplatin injection in one of the two protocols described above, the females were housed with proven male breeders in ratios of 3 females to one male. The females were housed with the males for up to ten days, or the length of two full estrous cycles. The estrous cycle is 4–5 days in the female rat. Each female was checked daily for a copulatory plug and vaginal lavages were performed daily to check for the presence of sperm. The day that a copulatory plug was found and/or sperm was found in the vaginal lavage was termed pregnancy day zero (d0). The female was then removed from the cage and housed singly until day 17 (d17) of gestation.

The pregnant rats were euthanized on d17 of gestation by an overdose of carbon dioxide. After euthanasia, each uterine horn was exposed and the number of implantation sites, resorption sites, the number of viable fetuses, and non-viable fetuses were counted. A resorption site was defined as an implantation site resembling a brown to greenish blood clot, with just placental tissue (early resorption) or placental and embryonic tissue (late resorption).[21] A non-viable fetus was described as a fetus that does not react to stimuli, has a pale color, stemming from a lack of blood flow, and is smaller in size compared to the viable fetuses. Both ovaries were excised, trimmed of excess tissue, and the number of corpora lutea was counted. The fecundity index was calculated as described by Griffiths et al.[20] The percentage of pre- and post-implantation losses were calculated as described by Chung et al[26] and Griffiths et al.[20]

The fecundity index was calculated as follows:

$$\text{Fecundity index} = \frac{\text{number of pregnant females}}{\text{number of mated females}} \times 100$$

In order to determine the percentage of pre-implantation loss, the following calculation/formula was used:

$$\text{Pre-implantation loss} = \frac{(\text{number of corpora lutea}) - (\text{number of implantation sites})}{\text{number of corpora lutea}} \times 100$$

In order to determine the percentage of post-implantation loss, the following calculation/formula was used:

$$\text{Post-implantation loss} = \frac{(\text{number of implantation sites}) - (\text{number of viable fetuses})}{\text{number of implantation sites}} \times 100$$

Each fetus was removed from the placenta and weighed.

Maternal blood was collected by a terminal cardiac puncture and allowed to clot overnight at 4 °C. The following morning, the blood was centrifuged, serum collected and stored at −80 °C until assayed.

Progesterone EIA

A commercially available progesterone EIA kit was purchased from Cayman Chemical (Ann Arbor, MI).

This kit was used according to the manufacturer's recommendation and a 1:40 serum dilution was used. Analysis was performed using the data analysis spreadsheet provided by the company. The inter- and intra-assay coefficients of variation were 9.6% and 13.8%, respectively. The kit was highly specific for progesterone and has a minimum detection of 7.8 pg/ml. To determine the level of progesterone level per corpus luteum (CL), the level of progesterone from each individual animal was divided by the total number of CL from both ovaries of the same animal:

$$\frac{\text{Progesterone}}{\text{CL}} = \frac{\text{total serum progesterone level}}{\text{total number of corpora lutea of both ovaries}}$$

Statistical analysis

All data are presented as mean +/− standard error of the mean (SEM). Experiments were performed at least three times and the results are presented as the combined data from all experiments. The students' t test was used to determine statistical difference between the saline and cisplatin groups from the Experiment 1 mating studies. For the Experiment 2 mesna + cisplatin mating studies, analysis of variation (ANOVA) followed by linear trend contrast was used to determine statistical difference. All statistical calculations were carried out using SPSS for Windows, version 11.0. A $P < 0.05$ was considered statistically different.

Results

Experiment 1: Effect of cisplatin on reproductive performance of females

This experimental protocol was carried out three separate times and all three data sets demonstrated similar results for all outcomes measured. The results presented here represent the combined results from all three data sets. While cisplatin caused a decrease in the percentage of females that mated to males (77.3% +/− 6.0% vs. 58.8% +/− 5.9% for saline and cisplatin, respectively), however that decrease was not statistically different (P = NS). Of the females in both sub-groups that mated with males, there was no change in the fecundity index (95.2% +/− 4.8% vs. 82.3% +/− 2.3% for saline and cisplatin respectively, P = NS). Figure 1 demonstrates the adverse effects

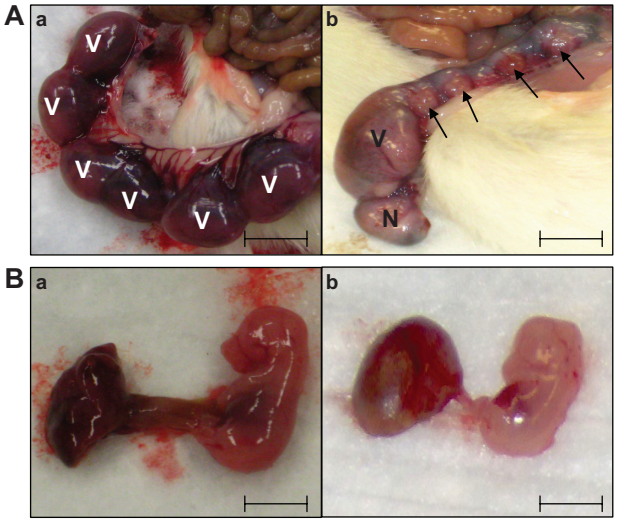

Figure 1. Representative photographs of d17 rat uterine horns and d17 fetuses in pregnant rats in which cisplatin was administered to the animals prior to mating to show the phenotypic effects of the drug. (**A**) The photographs show uterine horns at pregnancy day 17 of (a) the saline injected control animals and (b) cisplatin injected animals. There are differences in the gross morphology of the uterine horns of the cisplatin injected animals, resulting from a difference in the number of viable fetuses. The arrows in Figure 1Ab point to the sites of resorption of the fetuses in the cisplatin injected animals. The letter "V" denotes a viable fetus, and the letter "N" indicates a non-viable fetus. Scale bars indicate scale for photographs Aa and Ab. (**B**) Photograph showing a comparison in pregnancy day 17 of the cisplatin treated animals of (a) viable fetuses, fetuses that react to stimuli, and (b) non-viable fetuses, fetuses that do not react to stimuli and have a pale color, stemming from a lack of blood flow. The non-viable fetuses were also smaller in size compared to the viable fetuses. Scale bars are indicative of relative sizes for photographs in Ba and Bb.

of prior cisplatin administration on future pregnancy outcomes. Figure 1Aa is a photograph of the uterine horn from a saline control animal. Figure 1Ab shows the uterine horn from a cisplatin treated animal that had an increase in the number of resorption sites and the presence of a non-viable fetus. In Figure 1Ba and b, the photographs from a cisplatin treated female depict the differences between a viable fetus and a nonviable fetus.

Cisplatin administration prior to mating resulted in an increase in the percentage of pre-implantation loss in comparison to the control animals (Fig. 2Aa; 14.5% +/− 2.9% vs. 40.7% +/− 6.5%, $P < 0.01$). Cisplatin administration increased the percentage of post implantation loss from 2.2% +/− 1.0% in the saline controls to 52.6% +/− 7.4% in the cisplatin treated animals (Fig. 2Ba; $P < 0.001$). The total number of resorptions per pregnant animal was increased to 3.8 +/− 0.8 in the cisplatin treated animals from

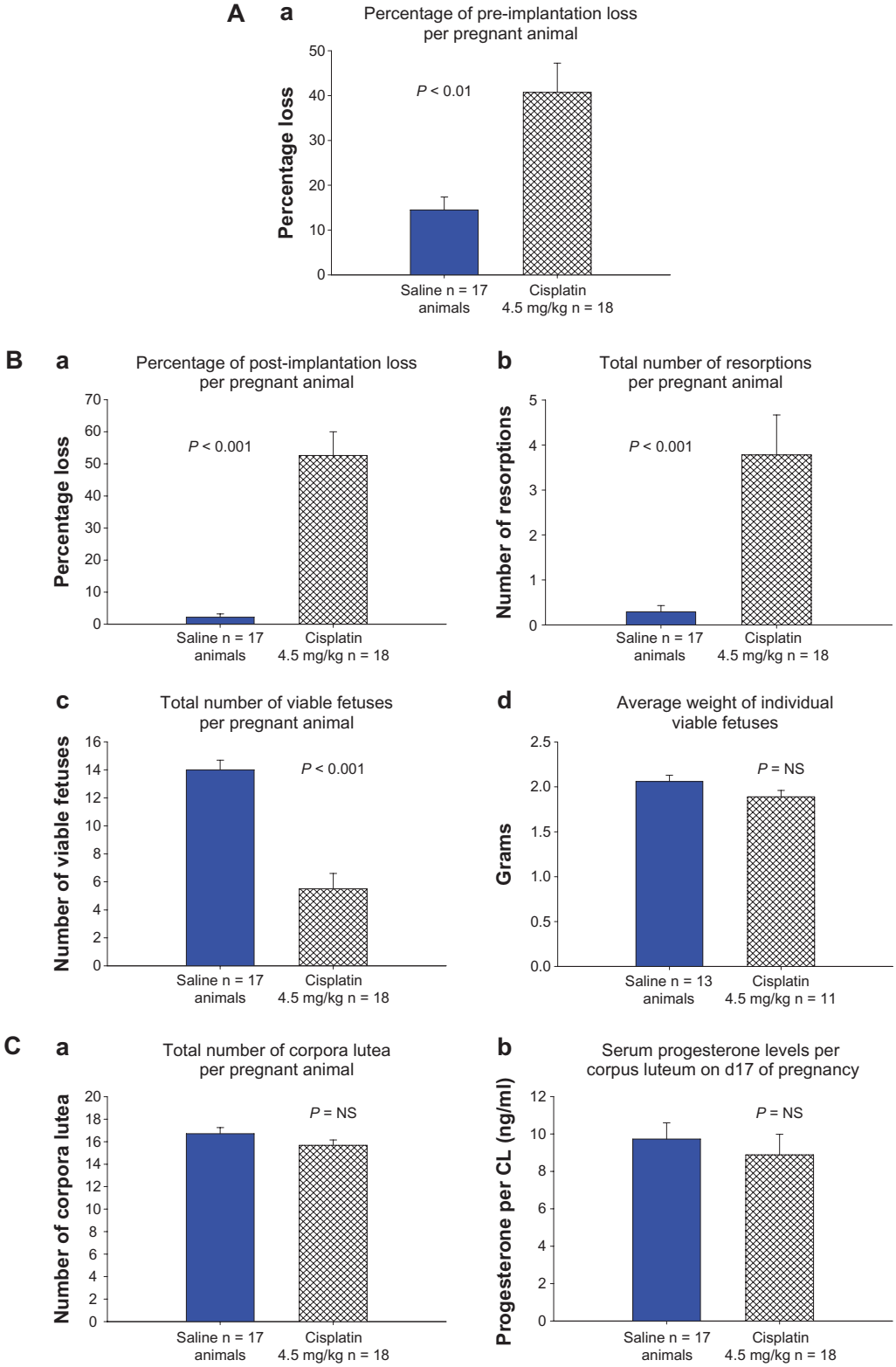

Figure 2. The subsequent reproductive function of rats previously treated with cisplatin. (**A**) Comparison of the previously treated saline injected versus cisplatin injected animals for the percentage of pre-implantation loss. a) There was a significantly higher rate of pre-implantation loss in the cisplatin pregnant animals ($P < 0.01$). (**B**) Comparison of the post-implantation effects of prior cisplatin exposure. a and b) There was a higher percentage of post-implantation loss and a higher number of resorptions in the cisplatin animals ($P < 0.001$ for both). c) The number of viable fetuses was lower in the cisplatin treated group ($P < 0.001$). d) Comparison of the fetal weights of the viable fetuses in the two treatment groups did not reveal a difference ($P = NS$). (**C**) a) The total number of corpora lutea present in both ovaries was no different in the two groups ($P = NS$). b) Serum progesterone levels per corpus luteum for the saline and cisplatin injected animals was measured and there was no difference in the two groups ($P = NS$).

0.3 +/– 0.1 in the saline treated controls (Fig. 2Bb; $P < 0.001$). In the cisplatin treated animals, there was a significant decrease in the number of viable fetuses, 5.5 +/– 1.1 compared to 14.0 +/– 1.1 in the control animals (Fig. 2Bc, $P < 0.001$). There was no difference in the fetal weight between the saline controls and the cisplatin treated sub-group (Fig. 2Bd, 2.1 +/– 0.1 grams vs. 1.9 +/– 0.1 grams for saline and cisplatin fetuses, respectively, P = NS).

After cisplatin administration, there was no difference in the total number of corpora lutea present between the saline and cisplatin sub-groups (Fig. 2Ca; 16.7 +/– 0.5 corpora lutea vs. 15.7 +/– 0.5 corpora lutea for the saline and cisplatin sub-groups, respectively, P = NS). The progesterone level per CL (Fig. 2Ca) between control and cisplatin sub-groups were not statistically different (9.7 +/– 0.9 ng/ml/CL; saline vs. 8.9 +/– 1.1 ng/ml/CL; cisplatin, P = NS). This suggests that the fetal loss was not directly associated with corpus luteum progesterone production.

Experiment 2: Effect of mesna on cisplatin induced reproductive toxicity

As a separate control experiment, we first analyzed the effects of administering mesna with saline in place of cisplatin to determine the effects of mesna on the different reproductive outcomes. The administration of mesna + saline did not effect any of the reproductive outcomes compared to the saline control (P = NS, data not shown).

For the experimental studies, the experimental protocol was carried out four separate times and all four data sets showed similar results for all outcomes measured. The combined results of all four data sets are presented. For the mesna + cisplatin set of experiments, we found that there was a non-significant change in the percentage of females that mated to a male (86.1% +/– 5.8%, 75.0% +/– 5.9%, 66.8% +/– 12.4% for saline, mesna + cisplatin, and cisplatin, respectively, P = NS). Of the females that mated with a male, there was no difference in the fecundity index for all sub-three groups (91.0% +/– 5.9%, 86.2% +/– 2.2%, 91.0% +/– 5.9% for saline, mesna + cisplatin, and cisplatin, respectively, P = NS).

Mesna protected against the pre-implantation loss of the fetuses (Fig. 3Aa; 16.8% +/– 2.9%, 26.3% +/– 3.0%, 30.0% +/– 4.1% for saline, mesna + cisplatin, and cisplatin sub-groups, respectively, $P < 0.01$). In addition, mesna treatment decreased the percentage of post-implantation loss compared to the cisplatin treated sub-group (Fig. 3Ba; 4.5% +/– 1.0%, 27.6% +/– 4.5%, and 37.7% +/– 4.5% for the saline, mesna + cisplatin, and cisplatin sub-groups, respectively, $P < 0.001$). There was a statistically significant difference in the number of resorptions seen among the control, mesna + cisplatin, and cisplatin sub-groups (Fig. 3Bb; 0.6 +/– 0.1 resorptions, 3.3 +/– 0.5 resorptions, and 3.8 +/– 0.4 resorptions for the saline, mesna + cisplatin, and cisplatin sub-groups, respectively, $P < 0.001$). Treatment with mesna leads to an increase in the total number of viable fetuses in the mesna + cisplatin compared to cisplatin (Fig. 3Bc; 13.9 +/– 0.5 fetuses, 8.9 +/– 0.6 fetuses and 7.8 +/– 0.8 fetuses for the saline, mesna + cisplatin, and cisplatin sub-groups, respectively, $P < 0.001$). The weight of the viable fetuses was not statistically different among the three sub-groups (Fig. 3Bd; 2.0 +/– 0.03 grams, 1.9 +/– 0.04 grams, 2.0 +/– 0.06 grams for the saline, mesna + cisplatin, and cisplatin sub-groups, respectively, P = NS).

After mesna and cisplatin administration, there was no difference in the total number of corpora lutea found in the saline (17.7 +/– 0.0 corpora lutea), mesna + cisplatin (16.3 +/– 0.4 corpora lutea) and cisplatin (17.0 +/– 0.4 corpora lutea) sub-groups (Fig. 3Ca; P = NS). The measurement of progesterone revealed that there was no difference in the progesterone level per corpus luteum in any of the three sub-groups (Fig. 3Cb; 14.7 +/– 1.2 ng/ml/CL, 15.3 +/– 1.7 ng/ml/CL, 14.2 +/– 1.9 ng/ml/CL for the saline, mesna + cisplatin, and cisplatin sub-groups, respectively, P = NS).

Discussion

This study provides evidence demonstrating the effects of prior administration of cisplatin on the reproductive function of female rats. The data presented here defines one specific biological locus for the loss of fertility after prior cisplatin administration. This study also demonstrated that the administration of mesna immediately prior to treatment with cisplatin modulated the reproductive loss in animals exposed to cisplatin. To the best of our knowledge, the study presented here was the

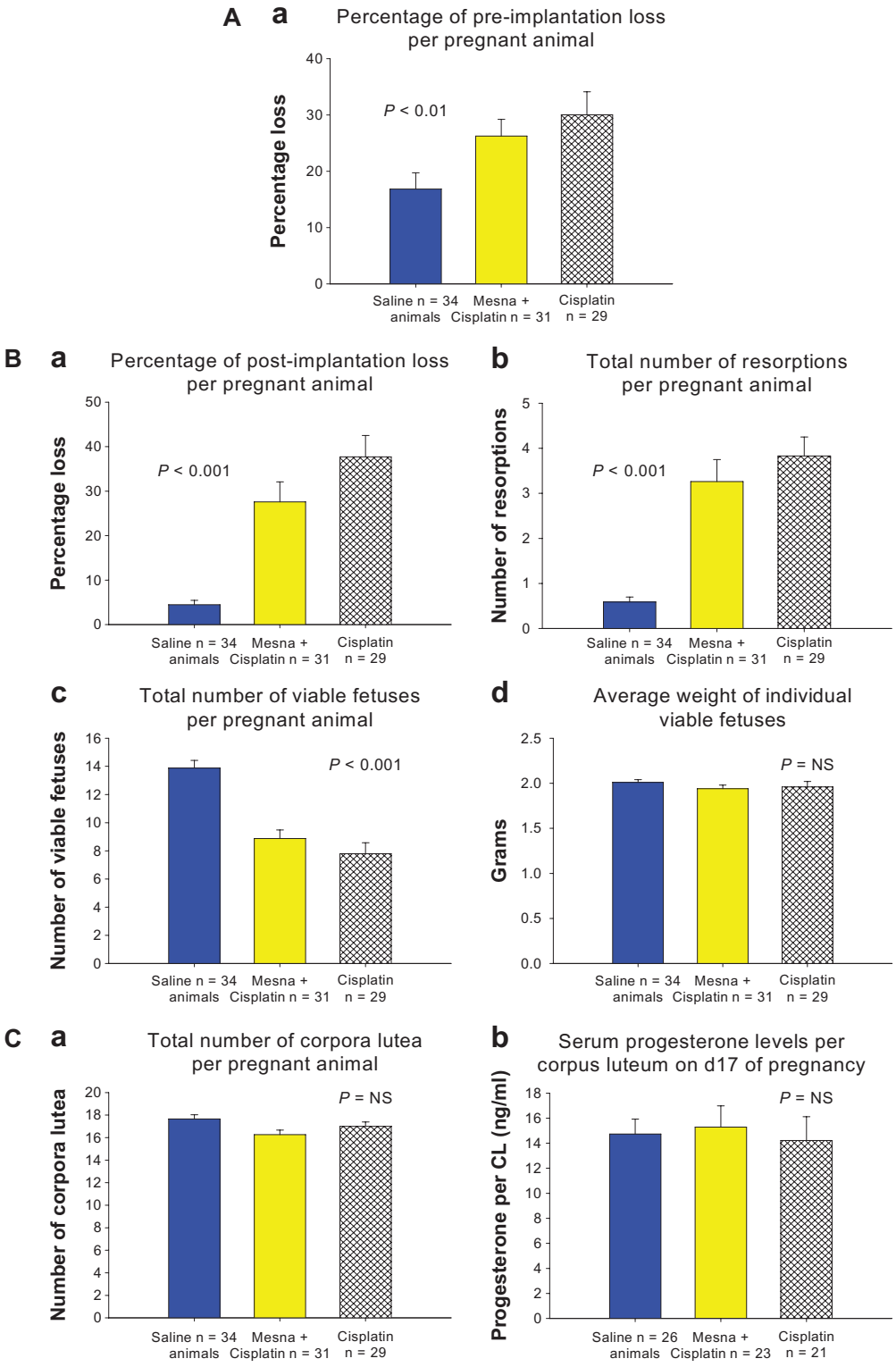

Figure 3. The results of mesna + cisplatin protection experiments, with the p values shown for the linear trend analysis for the different groups. (**A**) Comparison of the following groups for pre-implantation loss: (1) saline; (2) cisplatin + mesna; and (3) cisplatin alone. The animals were given the drugs prior to mating. a) Mesna administration protected against pre-implantation loss ($P < 0.01$). (**B**) Comparison of the post-implantation effects of mesna use during cisplatin administration. a and b) Mesna administration protected the percentage of post-implantation loss and the total number of resorptions ($P < 0.001$ for both). c) The number of viable fetuses was higher in the mesna + cisplatin treated group compared to the cisplatin group ($P < 0.001$). d) There was no difference in the fetal weights of the viable fetuses in the three groups (P = NS). (**C**) a) The total number of corpora lutea present in both ovaries in the three groups were similar (P = NS). b) Serum progesterone level per corpus luteum for the three groups were measured and there was no difference in the three groups (P = NS).

first to investigate the effects of multiple doses of cisplatin on reproductive function in female rats. We demonstrated that our cisplatin protocol adversely affected the reproductive capabilities of the female rat. Furthermore, the antioxidant mesna was able to partially reduce the toxic effects of cisplatin on reproductive function by protecting against the loss of fertility by reducing the percentage of pre-implantation loss, percentage of post-implantation loss, and number of resorptions, and by increasing the number of viable fetuses. This is similar to other studies conducted using mesna, which demonstrate partial, but not full, protection from the toxic effects of cisplatin.[24,25] While there was a non-significant decrease in the percentage of females in the cisplatin treated group that mated with the males, this may have been due to the fact that cisplatin causes minor alterations in the estrous cycle.[7] Mesna may increase the rate of females mating with males by preventing ovarian damage that would lead to an increase in estrous cycle length.

Studies conducted previously on the effects of cisplatin on reproductive outcomes have focused mainly on the effects of cisplatin given during pregnancy. In these studies, the administration of cisplatin caused significant damage to the fetus by increasing fetal abnormalities and fetal loss.[13,27–29] However, we sought to determine the effects of cisplatin on reproductive outcomes when pregnancy occurred following the cessation of cisplatin. Studies conducted on the effects of a single cisplatin dose given to female rats prior to pregnancy demonstrated that there was no change in fetal loss at one month, but moderate to severe loss at three months and six months after exposure.[14,15] The work presented here investigated the effects of multiple doses of cisplatin on reproductive function in the female rat, which more closely mimics the human clinical treatment situations. Our data suggest that multiple doses of cisplatin caused a significantly higher increase in fetal loss at an earlier time point than a single dose of cisplatin. Further studies will be needed to determine if with our rat model that there is a permanent change in the rate of fetal loss after cisplatin exposure or if it is reversible.

The data from this study suggested that cisplatin did not alter the number of oocytes ovulated, as evidenced by the same number of corpora lutea found in all three treatment groups. This is in agreement with our previous studies where we demonstrated that cisplatin did not alter the number of follicles; rather it affected the function of the follicle.[1,26] Furthermore, the present study showed that the function of the corpora lutea was not affected by prior cisplatin exposure, as the levels of progesterone did not differ between the treatment sub-groups. This suggested that prior cisplatin administration did not affect corpora lutea function and that corpora lutea dysfunction was not the cause of the increased fetal loss after ovulation.

Cisplatin and carboplatin are reported to be distributed in the uterine tissues in humans, rats, and rabbits.[30,33] Furthermore, there are persistent DNA adducts in the uterine tissue of rats.[32] Cisplatin has also been shown to cause apoptosis in primary endometrial cell cultures.[34] It is possible that the administration of cisplatin causes uterine damage, and that damage to the uterus will either prevent implantation, leading to the increased pre-implantation loss, or will lead to damage that prevents the implanted fetuses from developing, leading to the increased post-implantation loss, or a combination of both. Mesna might prevent fetal loss by reducing the levels of apoptosis in the uterus. Mesna has been demonstrated to reduce ovarian damage in the rat model by reducing apoptosis in follicles and, in parallel, may lead to decreases in apoptosis in the uterine tissue.[23] Mesna may prevent apoptosis by reducing oxidative stress or through alternative pathways not yet identified. Mesna has been shown to inhibit the activation of NF-kB pathway in an ischemia-reperfusion model of the intestine, which leads to decreased oxidative stress.[35] To determine the exact methods of reproductive toxicity caused by cisplatin exposure, future studies need to be conducted to determine the effects of cisplatin and mesna plus cisplatin on the uterus and if uterine dysfunction plays a role in the loss of reproductive function.

An alternative reason for the increased fetal loss may be from cisplatin-induced damage to the oocyte. In male rats there is evidence that cisplatin induces apoptosis in the germ cells, early spermatocytes, and spermatogonia.[36,37] Amifostine, another FDA approved drug used to reduce systemic side effects

of chemotherapy, has been demonstrated to reduce the levels of apoptosis in the rat testis.[38] While it is difficult to relate the effects of cisplatin on male germ cells to female germ cells, it is conceivable that cisplatin will cause apoptosis in female germ cells. Cisplatin has been demonstrated to cause apoptosis in a variety of healthy tissues, including ovarian follicles, so it is possible that cisplatin can cause apoptosis in the oocytes.[6,39,40] Cisplatin has also been shown to create chromatin abnormalities in the oocytes, such as disorganization, minute fragments, or chromatin bridges.[31] Higher doses of cisplatin have also been shown to cause hypohaploidy in oocytes.[30] Taxol, or paclitaxol, another commonly used chemotherapeutic agent, has also been demonstrated to cause meitotic maturation delays and chromatin abnormalities in oocytes.[32] The same study demonstrated that one-cell zygotes have an increased incidence of hyperploidy after taxol administration.[32] It is possible that the administration of cisplatin may lead to abnormalities similar to those caused by taxol. These possible effects on the oocytes may lead to the increased fetal loss both before and after implantation. Mesna may prevent fetal loss through reduction or prevention of apoptosis in the oocyte. As mesna reduces the levels of apoptosis following cisplatin administration in ovarian follicles, it may be postulated that mesna may reduce apoptosis in the oocytes. Mesna may also work through an alternative pathway to prevent the chromatin abnormalities and hypohaploidy that cisplatin causes.

One previously conducted study investigated the use of mesna to prevent cyclophosphamide induced fetal malformation.[33] Mesna was administered with cyclophosphamide during pregnancy leading to a small, but not clinically relevant degree, the rate of fetal malformations when cyclophosphamide alone was administered during pregnancy.[33] These results are different from those presented here, as our data suggest a higher degree of protection, though we did not investigate the rate of fetal abnormalities. The doses used in the cited cyclophosphamide study are significantly lower than those used here, which could account for the fact that there was less protection. Furthermore, it is possible that, in this model, mesna given during pregnancy is not as effective in preventing fetal loss as when mesna is given with the chemotherapy agent prior to pregnancy. An alternative hypothesis may be that cyclophosphamide induces damage through different pathways than cisplatin induces damage and that mesna does not work through those pathways.

Conclusion

In conclusion, this is the first study to address the affects of multiple doses of cisplatin administered prior to pregnancy on reproductive outcomes. The administration of cisplatin prior to pregnancy caused significant reductions in fertility and fetal outcomes. The addition of mesna to cisplatin administration decreased the rate of fetal loss. Prior cisplatin administration did not alter the ovulation rate or lead to corpora lutea dysfunction. The data presented here can be used to further define the toxic effects of cisplatin on fertility loss. In addition to ovarian damage, additional mechanisms leading to increased adverse fertility loss include the uterus and the oocyte. Future studies need to address the effects of cisplatin on these structures to identify how cisplatin reduces fertility and also define potential mechanisms for offering protection against the loss of reproductive function.

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Author's Contributions

JY conceived of the study, participated in the design of the study and analysis of the data and helped to draft the manuscript. BSK participated in the design of the study, performed the experiments, analyzed the data and helped to draft the manuscript. JP participated in the design of the study, performed the experiments, analyzed the data, performed the statistical analysis and helped to draft the manuscript.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements

in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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