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The Effect of Kisspeptin-10 on Cisplatin-Induced Testicular Oxidative Stress

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ABSTRACT
Objective: Cisplatin, one of the anticarcinogenic drugs, causes damage to spermatogenic cells, sertoli cells and leydig cells. This study aimed to investigate the kisspeptin-10 effect against oxidative stress caused by cisplatin in male reproductive organs and its negative effects on spermatological parameters. **Materials and Methods:** In the experiment, 34 male Sprague Dawley rats were used. Rats were divided into 4 groups as control, cisplatin, kisspeptin-10 and cisplatin+kisspeptin-10. Cisplatin 5 mg/kg/single dose and kisspeptin-10 50 nmol/kg dose were administered intraperitoneally for 7 days. **Results:** In the cisplatin group, testicular malondialdehyde level increased ($p<0.001$), catalase enzyme activity ($p<0.001$) and glutathione level ($p<0.01$) decreased, sperm motility ($p<0.05$) and testicular ($p<0.01$) and seminal vesicle ($p<0.001$) weights decreased. In the cisplatin group administered kisspeptin-10, it was determined that testicular malondialdehyde level decreased ($p<0.001$), glutathione level increased ($p<0.01$), sperm motility increased ($p<0.05$), and testicular ($p<0.01$) and seminal vesicle ($p<0.001$) weights increased compared to the cisplatin group. **Conclusion:** As a result, it was revealed that Kisspeptin-10 showed improving effects on spermatological parameters by reducing oxidative stress that is effective in cisplatin-induced testicular damage.
Keywords: Cisplatin, Testes, Kisspeptin-10, Oxidative Stress.

Sisplatinin Neden Olduğu Testikular Oksidatif Stres Üzerine Kisspeptin-10'un Etkisi

ÖZ
Amaç: Antikanserojen ilaçlardan biri olan sisplatin, spermatogenik hücrelerde, sertoli hücrelerinde, leydig hücrelerinde hasara yol açmaktadır. Bu çalışmada, sisplatinin erkek üreme organlarında neden olduğu oksidatif stres ve akabinde gelişen spermatolojik parametreler üzerindeki olumsuz etkilere karşı kisspeptin-10'un etkisinin araştırılması amaçlandı. **Gereç ve Yöntem:** Deneyde 34 adet erkek Sprague Dawley ırkı sıçan kullanıldı. Ratlar kontrol, sisplatin, kisspeptin-10 ve sisplatin+kisspeptin-10 olarak 4 gruba ayrıldı. Sisplatin 5 mg/kg/tek doz, kisspeptin-10 ise 50 nmol/kg dozda 7 gün boyunca intraperitoneal uygulandı. **Bulgular:** Sisplatin grubunda testikular malondialdehit düzeyinin arttığı ($p<0.001$), katalaz enzim aktivitesinin ($p<0.001$) ve glutatyon düzeyinin ($p<0.01$) azaldığı, sperm motilitesinin ($p<0.05$) ve testis ($p<0.01$) ile vezikula seminalis ($p<0.001$) ağırlıklarının azaldığı belirlendi. Sisplatin grubu ile kıyaslandığında Kiss-10 uygulanan sisplatin grubunda testikular malondialdehit düzeyinin azaldığı ($p<0.001$), glutatyon düzeyinin arttığı ($p<0.01$), sperm motilitesinin ($p<0.05$) ve testis ($p<0.01$) ile vezikula seminalis ($p<0.001$) ağırlıklarının arttığı belirlendi. **Sonuç:** Kisspeptin-10'un sisplatin kaynaklı testikular hasarda etkili olan oksidatif stresi azaltarak, spermatolojik parametreler üzerinde iyileştirici etkiler gösterdiği kanısına varılmıştır.
Anahtar Kelimeler: Sisplatin, Testis, Kisspeptin-10, Oksidatif Stres.

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INTRODUCTION

One of the main causes of testicular toxicity leading to permanent or temporary infertility is chemotherapeutic agents used in cancer treatment. One of the important chemotherapeutic agents used in the treatment of various cancers is cisplatin (CP) (Kostova, 2006). However, CP has side effects such as ototoxicity, nephrotoxicity, cardiotoxicity, gastrointestinal, hepatotoxicity and reproductive dysfunction (Atessahin et al., 2006; Ilbey, Ozbek, Cekmen, et al., 2009) It was reported that testicular toxicity due to CP administration is an important problem in men due to the high mitotic activity of spermatogenic cells. It has been shown that cisplatin exposure can cause azoospermia, abnormal sperm, impaired spermatogenesis and a decreased testosterone levels in rats (Ilbey et al., 2009), as well as oxidative stress characterized by histological changes in testicular tissue, increased lipid peroxidation and decreased antioxidant system (Ilbey et al., 2009). It has been suggested that testicular redox balance is disrupted by CP exposure (Antunes et al., 2001; Ilbey et al., 2009).

Kisspeptin neuropeptides are transcribed by the Kiss-1 gene (1q32) (Lee et al., 1996). Since the products of the Kiss-1 gene suppress metastasis of breast cancer and melanoma, the 54 amino acid product of the Kiss-1 gene has been named "metastin" (Gottsch et al., 2006). In later studies, shorter fragments of kisspeptin-54 were also identified and all of these were called "kisspeptins". All of these products are activated by binding to the GPR54 receptor (Shahed & Young, 2009). Known derivatives of kisspeptin are kisspeptin-10, 13, 14, 52 and 54. Kisspeptin-10 (KISS-10) is secreted from cultured human trophoblast cells and contains the C-terminal decapeptide-10. Kisspeptins are known to play an important role in puberty and fertility (Mikkelsen et al., 2009). Kisspeptin administration has been reported to stimulate the hypothalamic-pituitary-gonadal axis (HPG) (Dhillo et al., 2005). Signals generated by the binding of kisspeptins to GPR54 receptors on gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus enable the release of GnRH into the portal pituitary circulation. Studies have reported that peripheral kisspeptin-10 administration is effective on LH and FSH concentrations (Shahab et al., 2005; Thompson et al., 2004)

Reactive oxygen species (ROS) play important roles in normal physiology. However, their excessive production causes oxidative stress. Various mechanisms, including antioxidant enzyme systems such as catalase and glutathione peroxidase, protect cells from damage caused by ROS. The relationship between gonadotropins and oxidative stress has been shown in many studies. (Hurtado de Catalfo et al., 2007; Muthuvel et al., 2006). Studies show that kisspeptin provides protection against oxidative damage (Akkaya et al., 2017; Güvenç & Aksakal, 2018). The purpose of this study was to examine the effect of kisspeptin-10 against oxidative stress caused by CP in male reproductive organs and the resulting negative effects on spermatological parameters.

MATERIALS AND METHODS

Experimental animals

The study was conducted in accordance with applicable rules regarding animal experimentation and animal welfare. Sprague Dawley male rats were obtained from Fırat University Experimental Research and Application Center. A total number of 34 male rats with an average weight of 200-250 g and 10-12 weeks old were used.

Experimental design

Experimental procedures were carried out in accordance with the conditions for the care and use of laboratory animals (12 hours of light: 12 hours of darkness and 24±3°C). Standard commercial rat pellet feed and tap water were provided ad libitum to the rats during the experimental procedure. After a one-week adaptation period, the rats were divided into 4 groups as fallows. Control group (n=7): A single dose of saline was administered intraperitoneally for 7 days. Kisspeptin-10 Group (KISS-10: n=7): Kisspeptin 50 (Novopro 316777) nmol/kg/single dose (Güvenç & Aksakal, 2018) was dissolved in saline and administered intraperitoneally for 7 days. Cisplatin Group (CP: n=10): Cisplatin (Koçak Farma) 5 mg/kg/single dose (Mercantepe et al., 2018) was administered intraperitoneally at the beginning of the study. Cisplatin+ Kisspeptin-10 Group (CP+KISS-10: n=10) At the beginning of the study, cisplatin 5 mg/kg/single dose was administered intraperitoneally and then Kisspeptin 50 nmol/kg/single dose was dissolved in saline and administered intraperitoneally for 7 days.

Oxidative stress analyses

Testicular tissues were weighed and transferred to glass tubes while maintaining their cold temperatures. 1/10 lysis buffer was added to the tissues. The tissues were kept cold and homogenized in a homogenizer. This homogenate was centrifuged at 4000 rpm for 60 minutes in at +4 °C cooled centrifuge to obtain the supernatant (Arkali et al., 2021). Malondialdehyde (MDA), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSH-Px) levels were determined from the supernatants. Lipid peroxide (MDA) was determined according to the spectrophotometric method described by Placer et al (Placer ZA, . 1966). MDA levels were reported in nmol g^{-1} of tissue. Catalase activity in the testicle was measured using the spectrophotometric method described by Goth (Goth, 1991) Catalase enzyme activity was expressed as kU g^{-1} protein. The GSH level in the tissue was measured using the spectrophotometric method described by Sedlak and Lindsay (Sedlak & Lindsay, 1968). GSH levels were reported in nmol g^{-1} of tissue. GSH-Px enzyme activity in in the testicle was determined according to the spectrophotometric method described by

Lawrence et al (Lawrence & Burk, 1976). GSH-Px enzyme activity was expressed as IU g^{-1} protein. The Lowry method was used to measure the total protein content in the testis (OH, 1951).

Spermatological analyses

Sperm motility was assessed using the method outlined described by Sönmez et al. and Türk et al. after reconstitution of spermatozoa obtained by obtaining sections from the left cauda epididymis with Tris buffer solution [Tris (hydroxymethyl) aminomethane 3.63 g, glucose 0.50 g, citric acid 1.99 g and distilled water 100 ml] (Sönmez et al., 2005; Türk et al., 2007). The same method was used to determine sperm density in the cauda epididymis tissue (Sönmez et al., 2005; Türk et al., 2007). Abnormal spermatozoon ratio was determined by examining a total of 200 spermatozoa in frotis and expressing head, tail and total abnormal spermatozoon ratio as percentage according to the method described by Sönmez et al. and Türk et al. (Sönmez et al., 2005; Türk et al., 2007)

Statistical analyses

Shapiro-Wilk normality analysis was used to determine whether the values obtained as a result of the study were normally distributed. Shapiro-Wilk normality analysis was performed. Accordingly, group means of normally distributed data were tested with one-way ANOVA. Differences between groups were determined with Duncan test. Significance level $p<0.05$ was accepted. IBM SPSS Statistics 22 package program was used for statistical analyses. Data were given as Mean ± Standard Deviation (X±SD).

Ethics committee approval: This study was carried out at Fırat University Experimental Research Center with the "permission dated 16.04.2022 and numbered 2022/06" of the Fırat University Animal Experiments Local Ethics Committee

Study limitations and strengths

Because of the budget constraint, we were not able evaluate the pathology of testes and Johnsenscore**.**

RESULTS

Body Weight Results

Compared to the control group, final body weight decreased in the CP and CP+KISS-10 groups ($p<0.001$). There was no significant difference in CP+KISS-10 group compared to CP group ($p>0.05$) (Figure 1).

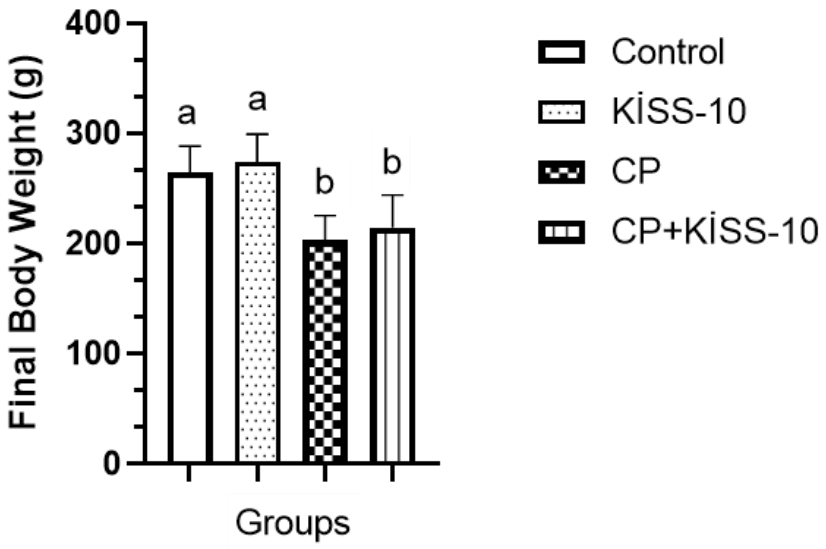

Figure 1. Final body weight (g) values at the end of the study. Data are given as mean and standard deviation. [a,b]: The difference between the groups with different letters is statistically significant.

Oxidative Stress Results

Compared to the control group, MDA level increased ($p<0.001$), GSH level and CAT enzyme activity decreased ($p<0.001$) in the CP group. Compared to the CP group, MDA level decreased ($p<0.001$), GSH level increased ($p<0.001$) and CAT enzyme activity did not differ statistically in the CP+KISS-10 group ($p>0.05$). Compared to the control group, MDA level decreased in KISS-10 group ($p<0.001$). It was determined that there was no statistically significant in GSH-Px activity between the groups ($p>0.05$) (Table 1).

Spermatologic Parameter Results

When compared with the control group, motility decreased in the CP group ($p<0.05$). Compared to the CP group, motility increased in CP+CISS-10 group ($p<0.05$). There was no difference between the groups in sperm concentration, head-tail and total abnormal spermatozoon ratios ($p>0.05$) (Table 2). When the absolute reproductive organ weights were analyzed, testis ($p<0.01$) and vesicula seminalis ($p<0.001$) weights decreased in the CP group compared to the control group. Compared to the CP group, testis increased in the CP+KISS-10 group. Compared to the

control group, there was an increase in the ventral prostate weight in the KISS-10 group (p<0.01). There was no difference between the groups in epididymis and right cauda epididymis weights (p>0.05) (Table 3).

Table 1. Testicular tissue malondialdehyde (MDA) and glutathione (GSH) levels and glutathione peroxidase (GSH-Px) and catalase (CAT) enzyme activity levels.

	MDA (nmol g^{-1} tissue)	GSH (nmol g^{-1} tissue)	GSH-Px (IU g^{-1} prot)	Katalaz (kU g^{-1} prot)
Control	35.19±3.06 [b]	1.97±0.19[a]	17.68±1.47	2.92±0.54[a]
KİSS-10	25.64±5.36[c]	2.16±0.29[a]	19.59±1.47	3.51±1.15[a]
CP	40.68±2.90[a]	1.67±0.07[b]	18.44±1.74	2.05±0.34[b]
CP+KİSS-10	35.79±2.27[b]	1.95±0.13[a]	17.73±1.83	1.93±0.30[b]
Significant	**p<0.001**	**p<0.01**	**p>0.05**	**P<0.001**

Data are presented as mean ± standard deviation. [a,b,c]: The difference between groups with different letters in the same column is statistically significant means.

Table 2. Spermatologic parameters.

Groups	Motility (%)	Sperm Concentration (million/right cauda epididymis)	Abnormal Spermatozoon Rate (%)		
			Head	Tail	Total
Control	70.95±18.12[a]	91.14±14.91	8.20±3.38	6.40±2.04	14.60±1.87
KİSS-10	69.99±16.77[a]	106.66±24.04	6.33±2.28	7.20±1.21	14.36±3.27
CP	45.55±18.18[b]	78.44±24.04	9.72±3.75	8.14±2.59	17.86±4.73
CP+KİSS-10	65.55±13.84[a]	89.33±31.98	7.77±3.45	7.66±3.75	15.44±4.17
Significant	**p<0.05**	**p>0.05**	**p>0.05**	**p>0.05**	**p>0.05**

Data are presented as mean ± standard deviation. [a,b]: The difference between groups with different letters in the same column is statistically significant means.

Table 3. Absolute reproductive organ weights (g).

Groups	Testes (Right +Left)/2	Epididymis (Right +Left)/2	Right Cauda Epididymis	Vesicula Seminalis	Ventral Prostate
Control	1.31±0.10[a]	0.45±0.05	0.18±0.03	1.08±0.10[a]	0.15±0.05[b]
KİSS-10	1.37±0.13[a]	0.48±0.06	0.19±0.05	1.19±0.12[a]	0.24±0.07[a]
CP	1.10±0.09[b]	0.40±0.03	0,17±0.01	0.52±0.16[c]	0.12±0.03[b]
CP+KİSS-10	1.26±0.18[a]	0.42±0.06	0,18±0.03	0.72±0.15[b]	0.14±0.04[b]
Significant	**p<0.01**	**p>0.05**	**p>0.05**	**p<0.001**	**p<0.01**

Data are given as mean ± standard deviation. [a,b,c]: The difference between groups with different letters in the same column is statistically significant.

DISCUSSION

The use of chemotherapy drugs causes harmful side effects and organ toxicity in the body. One of the tissues damaged by chemotherapeutic drug use is the testicular tissue (Azarbarz et al., 2020). Cisplatin causes severe dysfunction in male reproductive function, limiting the clinical use of CP. Cisplatin toxicity triggers oxidative stress and activates inflammation cascade in testicular tissue (Türk et al., 2008). The purpose of this study was to examine the effect of Kisspeptin-10 administration, which has been increasingly studied due to its antioxidant properties, against gonadotoxic side effects that may occur with cisplatin administration in male rats.

In clinical trials, azoospermia, impaired fertility and decreased male reproductive activity have been observed in men receiving cisplatin-based chemotherapy (Brenner et al., 1985; Hansen et al., 1990). Previous animal studies have also shown that cisplatin has harmful effects on spermatogenesis, especially on spermatocytes and spermatozoa (Kinkead et al., 1992; Oshio et al., 1990). In rats, a dose-dependent decrease in spermatogenesis, sperm count, sperm motility and fertility was found after cisplatin treatment (Kinkead et al., 1992). In experimental studies, low doses of cisplatin such as 1.1-2.5 mg/kg were reported to be selectively toxic to spermatogonia, while higher doses such as 10-20 mg/kg were reported to have toxic effects on spermatocytes and spermatids (Cherry et al., 2004; Mercantepe et al., 2018). Therefore, in order to reduce the side effects of these high doses, a single intraperitoneal dose of cisplatin at 5 mg/kg was used in this study. In this study, only the decrease in motility was statistically significant in the CP group compared to the control group (Table 2). The decrease in sperm concentration and increase in abnormal spermatozoon rates were not statistically significant (Table 2). Türk et al. reported that a single dose of 7 mg/kg i.p. cisplatin decreased epididymal sperm concentration and motility and increased sperm morphology abnormalities (Türk et al., 2008). Çiftci et al. reported a decrease in sperm concentration and sperm motility, no difference in head and tail abnormal spermatozoon, but an increase in total abnormal spermatozoon ratio after a single dose of 5 mg/kg cisplatin (Ciftci et al., 2011). These different results in sperm parameters after cisplatin administration are thought to be related to the dose of cisplatin used and the time elapsed after administration.

Reproductive organ weights play an important role in determining CP-induced male reproductive toxicity (Soni et al., 2016). In this study, testicular and vesicular seminalis weights in the CP group were found to be significantly lower than in the control group (Table 3) and these data are consistent with the data of many studies (Abdel-Wahab et al., 2021; Azarbarz et al., 2020). The decrease in testicular weights at the end of the study is thought to be due to severe testicular parenchymal atrophy and spermatogenic injuries after CP administration. The significant decrease in testicular weight of CP-treated rats compared to the control group suggests that CP has a detrimental effect on the function and structure of the testes (Wang et al., 2021). In some additional studies, researchers reported that CP administration caused a significant increase in the testicular weight of rats. This is contrary to the results of the present study. It is thought that this may vary depending on the extent to which the histological structure of the testis is affected and the dose and duration of cisplatin (Yucel et al., 2019).

Although there are many factors in CP-induced gonadal toxicity, oxidative stress has been reported to be one of the main causes. CP-induced oxidative stress causes male infertility by reducing sperm function (Ateşşahin et al., 2006). It has been reported that cisplatin treatment induced excessive amounts of ROS in rat testes, which decreased antioxidant activities and increased lipid peroxidation (Casares et al., 2012; Kohsaka et al., 2020). In this study, in accordance with previous studies (Casares et al., 2012; Kohsaka et al., 2020), there was a deterioration in the oxidative status in the CP-treated group due to a decrease in GSH and catalase enzyme levels parallel to an increase in testicular malondialdehyde levels (Table 1), and this is thought to be related to decreased sperm quality in this group.

Studies on the effects of kisspeptin on the HPG axis and the reproductive system are increasing day by day (Dhillo et al., 2007; George et al., 2011). In recent years, studies have been conducted on whether this peptide has antioxidant effects (Akkaya et al., 2014; Aslan et al., 2017). In a study, it was suggested that kisspeptin may reduce testicular lipid peroxidation and may have an antioxidant effect (Akkaya et al., 2017). In another study, it was reported that kisspeptin-10 administration showed antioxidant properties in methotrexate-induced testicular damage (Güvenç & Aksakal, 2018). In this study, Kisspeptin-10 administration was shown to have antioxidant effect by decreasing the CP-induced increase in MDA level and increasing GSH level in testicular tissue (Table 1). In this respect, the study is in parallel with the previous studies (Akkaya et al., 2017; Güvenç & Aksakal, 2018). The HPG axis is stimulated by Kisspeptin-10 or kisspeptin-54 (Dhillo et al., 2005; George et al., 2011). In a study, Kisspeptin and its receptor were reported to be localized in epididymal sperm (Mumtaz et al., 2017). A positive correlation between overall kisspeptin levels and sperm concentration, sperm count and motility was first demonstrated in a study by Zou et al. (Zou et al., 2019). As a result of the studies, Kisspeptin has been reported to have a positive effect on testicular function, but some studies also indicate that Kisspeptin administration has an inhibitory effect (Mumtaz et al., 2017; Pinilla et al., 2012). Administration of Kisspeptin for more than 30 days

has been reported to cause testicular degeneration (Abbara et al., 2015). This is thought to be due to desensitization of the hypothalamic pituitary axis due to desensitization of the GPR54 receptor (KISS1R) by chronic use of Kisspeptin. Studies investigating the effects of exogenous administration of Kisspeptin-10 on the male reproductive system are increasing (Aytürk et al., 2017; Feng et al., 2019). In this study, increased sperm motility was observed in the CP+Kiss-10 group compared to the CP group (Table 2).

In conclusion, when Kisspeptin-10 hormone was administered exogenously, it was found to reduce CP-induced testicular damage through its anti-antioxidant activity. This peptide may be effective in male fertility by reducing oxidative stress. However, further examination of the antioxidant effect of different forms and analogs of kisspeptin on the male reproductive system will provide more specific data and thus allow for more data to be obtained.

Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: GA, **Material, methods and data collection:** GA, TCA, MA, AY, MÇ; **Data analysis and comments:** GA, EGE, MS, TCA, FBK **Writing and corrections:** GA, MÇ, MA, FBK.

Ethical Approval

Institution: Fırat University Experimental Research Center
Date: 16.04.2022
Approval no: 2022/06.

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