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Investigation the Anti-Sterility Role of Ubiquinone-10 Against Procarbazine-Induced Infertility in Male Rats

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Abstract

Procarbazineis cytotoxic chemotherapy drug with obvious deleterious effects on male fertility. The present study aimed to investigate protective method via co-administration of Ubiquinone-10 with procarbazine to reduce harmful effects of procarbazine. Twenty- eight adult healthy male rats (3 months in age and weighing 250-300g) were randomly divided into four equal groups as following: animals of first group were received propylene glycol 20% orally and phosphate-buffered saline solution (PBS) intraperitoneally as control group while of second , third and fourth group were received procarbazine (30mg/ kg), Ubiqunone-10 (10 mg/kg) and combination of Ubiqunone-10 with procarbazine respectively, all treatments were lasted for 9 weeks. Results revealed that procarbazine induces significant increase in serum FSH and LH level while Testosterone level was significantly decreased .Similar decrease were also noticed in levels of glutathione (GSH) ,as well as activity of catalase (CAT) and superoxide dismutase (SOD) in testis . Moreover, procarbazine caused significant decrease in sperm count, motility and viability and significant increase in sperm abnormality. On the other hand, Co-administration of Ubiqunone-10 with procarbazine lead to ameliorate levels of hormone as well as to improve testicular catalase activity in addition to glutathione and superoxide dismutase levels.

Key words: *sperm count, Ubiquinone-10, male fertility*

Introduction

There are many categories of causative agents that included in male infertility, drugs related infertility ,need to be investigated [1]. Impact of anticancer agents on fertility are well-known [(2, 3, 4)]. Antineoplastic drugs possess deleterious effects on germ and Sertoli cells in men, resulting severe oligozoospermia or azoospermia after chemotherapy courses. Spermatogonia are more affected by these drugs than that in other developing-stage due to differences in rate of cell division between them . Most alkylating chemotherapy agents (procarbazine) are toxic to spermatogenic cells and caused long lasting azoospermia due to their ability to cross-link DNA [2]. In addition, there are patients within their reproductive age, therefore investigation a method to protect spermatogenesis and fertility is very important. It is obvious that cytotoxic drugs damaged mainly rapid dividing cells such as spermatogonia but the mechanism that govern 'spermatogenic arrest' to decline damage of testis is still unclear [5]. About 30 % to 80 % of subfertility cases in male resultant from sperm damaging caused by oxidative stress [6]. Susceptibility of spermatozoa to oxidative stress and reactive oxygen species (ROS) was documented by many previous studies that considered (ROS) involved in sperm damage as well as male infertility [(7,8)]. Ubiquinone-10 is a molecule possess antioxidant ability, contribute in the respiratory chain. Light thrown on the ability of antioxidant to reduce male infertility and investigate whether antioxidant supplementation to infertile men with can enhances seminal indices. Ubiqunone-10 was one of various compound that tested due to its role in energy metabolism and antioxidant status via its function as a liposoluble chain-breaking for lipoproteins and cell membranes [6].Considerable amount of Ubiqunone-10 present in seminal plasma and spermatozoa to reduces oxidative stress and protect sperm viability [9],Seminal plasma Ubiqunone-10 concentration is significantly related with sperm number and motility [10]. The present study aimed to assessment ability of Ubiqunone-10 to achieve recovery of procarbazine-induced infertility in a male rat .

Materials and Method

Animals and experimental design

Twenty- eight Adult male albino rats ,aged three months and their weight range 250-300 gm, were used in the present study. Rats were reared in metal cages and food and water were ad libitum. Male rats were distributed into four groups (7 rats each group), as following: Vehicle control group, rats were firstly gavaged with propylene glycol 20% then intraperitoneally injected with 0.25 ml of PBS after on hour of gavage . Ubiqunone-10 group, rats were orally administered 10 mg/kg BW of ubiqunone-10 (dissolved in propylene glycol 20%) via gavage [11]. Procarbazine group, rats were injected intraperitoneally with 30 mg/kg BW of procarbazine (NATULAN® 50 mg, Sigma-Tau Pharmaceuticals Company, France) according to [12]. Ubiqunone-10 plus procarbazine group, rats were gavged with ubiqunone-10 then injected with procarbazine within one hour interval. Ubiqunone-10 (NOW Health group Inc. USA). All treatments were performed daily for 9 weeks. At the end of the experiment, blood samples were collected via cardiac puncture. Serum separated and frozen at –20°C till perform the biochemical and hormonal measurements. Animals were anesthetized by chloroform the sacrificed in order to harvest epididymis of each rat and right testis to perform epididymal sperm analysis and processed for enzymatic antioxidant assessment respectively .

Hormone Assay

Serum Testosterone, FSH and LH concentrations were measured by use commercial ELISA kits (American Laboratory Products Company (ALPCO) , USA) according to the instructions of manufacture.

Antioxidant Indices Assessment

The right testis of each rat was homogenized in (1-4 v/v) of PBS (pH 7.4) thereafter, the homogenate was centrifuged (10 000×g for fifteen minutes at 4°C) . The resultant supernatant used to perform enzymatic antioxidants measurement. Estimation the testicular SOD and CAT activity as well as GSH level were performed according to procedures described by Misra and Fridovich [13], Clairborne [14] and Jollow et al [15] respectably.

Epididymal sperm analysis

The cauda epididymis was excised and sperm suspension obtained by minicing it to release spermatozoa onto a Petri dish to mixed with 2 ml of physiological saline (0.9% NaCl) at 37 °C. sperm concentration and progressive motility of sperm were performed by using methods described by Belsey et al [16] while Sperm viability and morphological abnormalities of sperm were assayed according to Wells and Awa [17].

Statistical Analysis

All data were statistically analyzed by using Statistical Package for Social Sciences(SPSS, version 19). One-way analysis of variance (ANOVA) were carried out to compare between groups of study and least significant differences(LSD) used to identify significance of the differences between means and P value less than 0.05 were considered significant. Obtained results were expressed as the mean plus minus standard error.

Results and Discussion

The means of serum testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels are present in Table 1. The LH and FSH levels were significantly ($P \leq 0.05$) increased while testosterone levels decreased in rat treated procarbazine as compared with all other groups. Procarbazine produce toxic effects on rodent spermatogenic epithelium and induces sterility in male rodents [18] largely by killing stem spermatogonia.. Primary gonadal dysfunction is resultant from testicular damage lead to deleterious effect on spermatogenesis and/or Leydig cells dysfunction [19] According to above mention effect of procarbazine, the elevation of FSH in the present study may be resulted from impairment of spermatogenesis, whereas decrease of testosterone levels that associated with LH elevation may happen as a result of Leydig cell dysfunction.

The exposure to cytotoxic chemotherapy and radiotherapy causes rise in FSH levels co-incident with low sperm counts, therefore, the of suppressive effects of testosterone and analogs of gonadotrophin on spermatogonial furthermore, chemotherapy and radiotherapy are often used in combination associated with greater testicular dysfunction and germinal epithelial damage. To lead to an availability of therapy on male infertility after cancer treatment [22]

Qu et al., [22] were conclude that the therapy on male infertility after chemo- and radiotherapy is an available because sever testicular dysfunction and damage of germinal epithelia resulted post cancer treatment.

Results in table 1 also revealed that ubiquinone 10 reduces the toxic effect of procarbazine represented by elevation in testosterone level and reduce in levels of FSH and LH in ubiqunone-10 plus procarbazine group although their value still less than that recorded in control group. Ubiquinone 10 also called coenzyme Q10 supplementation was found to ameliorate the reduction in testosterone induced by chemical reproductive toxicants, mainly by neutralizing the damaging effect of the generated free radicals [23] . The mechanism by which ubiquinone 10 could counteract testicular toxicity is associated with its potent antioxidant properties and with ability to enhance transcription of steroidogenic enzymes [24]. Therefore , the enhancement of hormone levels in the present study may resulted from reduce damage of germinal epithelia by anti-oxidant effects exerted by ubiquinone.

According testicular antioxidant status, The changes in some enzymatic antioxidants levels in testis were shown in Table 2. Significant decline in the activity of SOD, CAT, GSH levels was observed in rats treated with procarbazine as compare to other groups. Co-dministration of ubiquinone 10 to procarbazine treated rats caused elevation in activity of testicular SOD, CAT, and GSH in compare to procarbazine group. However, above mentioned antioxidants exhibit non-significant differences between rats received ubicoinone 10 alone and rats of control group. Procarbazine is one of Alkylating agents that produce reactive oxygen species (ROS) causing decrease cell capability to detoxifying thiols and depletion of antioxidant enzymes [(25,26)].

Table 1: Effect of Ubiqunone-10 on serum hormone level in Procarbazine- treated male rat. (Mean ± SE)

Hormones Group	Testosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)
Vehicle control	A 2.52 ± 0.63	C 1.13 ± 0.13	C 6.52 ± 1.23
Procarbazine	C 0.68 ± 0.09	A 3.41 ± 0.05	A 16.46± 3.42
Ubiqunone-10	A 2.85 ± 0.91	C 1.21 ± 0.12	C 5.79 ± 1.08
Ubiqunone-10 Plus Procarbazine	B 1.73 ±0.46	B 2.71 ± 0.08	B 9.33 ± 2.91

Different letters represent significant difference between groups ($p \leq 0.05$).

Superoxide dismutase stimulates the radical of superoxide peroxide dismutation into plus oxygen molecule plus H2O2 ,the later converted into H_2O and molecular oxygen by CAT [27]. GSH, have an important role in cellular redox balance and participate in free radicals scavenging inside cells and considered the first guard against oxidation [28]. In the present study, decline in levels and activity of these antioxidants due to procarbazine treatment was corroborates with result of previous studies on testis [29] and liver [30] which may be resultant from testicular oxidative damage .

In the present study, ubiquinone significantly emulates the redox balance in the rat's testis which also in coordinate with previous findings in human [31] and rats [32] We suppose that the improvement of antioxidant capacity and the reduction of oxidative stress caused by procarbazine due to co-admenstration of ubiquinone could be associated with the improvement of sperm indices that observed in table 3.

Table 2: Effect of Ubiqunone-10 on testicular SOD and CAT activity as well as GSH level in Procarbazine-treated male rat. (Mean ± SE)

Antioxidant Group	SOD (U /mg protein)	CAT (µmole / min/ mg protein)	GSH (µg /g of tissue)
Vehicle control	A 10.17 ±2.14	A 0.31 ± 0.03	A 8.72 ± 1.09
Procarbazine	C 4.09 ± 1.05	C 0.12 ± 0.01	C 2.88± 0.93
Ubiqunone-10	A 10.55 ± 2.23	A 0.28 ± 0.12	A 8.61 ± 1.67
Ubiqunone-10 Plus Procarbazine	B 7.63 ±1.91	B 0.19 ± 0.08	B 4.71 ± 0.54

Different letter denote presence difference between groups ($p \leq 0.05$).

Results of epididymal sperm analysis in table (3) revealed that treatment with procarbazine for 9 weeks lead to a significant decrease in sperm count, studies [33,34] reported the ability of a single dose of procarbazine to cause toxic effect on spermatogenic epithelia of rodent. Moreover, Gould et al., [18] found that the procarbazine may cause most harmful effect on spermatogenic cells throughout other agents included in the MOPP chemotherapy protocol. The exact mechanism by which procarbazine can resulted the toxicity remains unknown. Conversion of procarbazine to an alkylating intermediate via bioactivation is necessary in both ,the anticancer activity and spermatotoxicity of the drug [35]. The antioxidant properties of ubiquinone 10 make it involved in biochemistry of sperm and male infertility. Sperm number and motility are correlated with concentration of ubiquinone 10 in seminal fluid [39] . However, ubiquinone 10 in the present study improve sperm count, motility and viability as well as reduces abnormal sperm percentage may be via elevation in the coenzyme Q10 level or due to improve the antioxidant status in the testis

Table 3: Effect of Ubiqunone-10 on sperm count, motility, viability and abnormality in Procarbazine-treated male rat. (Mean ± SE)

Antioxidant Group	Sperm count ($\times 10^6$/ml)	Motility (%)	Viability (%)	Abnormality (%)
Vehicle control	A 62.53 ±3.24	A 58.40 ± 9.10	A 74.23 ± 13.12	C 6.18 ±1.27
Procarbazine	C 2.15 ± 0.76	C 18.17 ± 2.19	C 14.16± 3.25	A 45.22±8.19
Ubiqunone-10	A 67.21 ± 4.93	A 61.73 ± 11.02	A 78.18 ± 16.62	C 4.06±0.92
Ubiqunone-10 Plus Procarbazine	B 18.18 ±2.47	B 33.17 ± 3.94	B 27.36 ± 7.13	B 19.34±3.41

Different letter denote presence difference between groups ($p \leq 0.05$).

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Veterinary Medicine, University of Kerbala and all experiments were carried out in accordance with approved guidelines.

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