AMELIORATING EFFECT OF UBIQUINONE 10 ON KISSPEPTIN-10 INDUCED-REPRODUCTIVE CHANGES IN MALE RAT DURING PREPUBERTAL AGE

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ABSTRACT:

Objectives: to investigates the effects of pulsatile Kisspeptin administration for 12 days on gonadotropin and testosterone production and maturation of immature rat male gonads, as well as to assess the protective role of ubiquinone 10 when rats were exposed to Kisspeptin in the male reproductive system during the prepubertal phase.

Materials & methods: thirty-six male rats were used in the experiment. The rats divided into four groups (eight each), the first group were given DMSO as control, the second group were given kisspeptin as experimental, the third group were given kisspeptin + ubiquinone as treatment and the fourth group was given ubiquinone only as care. At the end of the experiment, all rats in each group were sacrificed. After this, Plasma LH, FSH and testosterone concentrations were measured, while total spermatid head count, elongated spermatid and daily sperm production were calculated.

Results. At the end of the treatments, the result shows a significant decrease in follicular stimulating hormone (FSH), Luteinizing hormone (LH) and testosterone hormone while show significant increase in Inhibin B in male rats treated with 50nmol/kg B.W of kisspeptin during the prepubertal stage compared with other groups.

Coadministration of ubiquinone with kisspeptin results in an improvement in LH, FSH, testosterone and Inhibin B levels in the present study. While other results concerning a total number of spermatid heads, elongated spermatid and daily sperm production show a significant decrease in male rats treated with 50nmol/kg B.W of kisspeptin compare with other groups. Coadministration of ubiquinone with kisspeptin results in a significant elevation in all parameters treated studied.

Key words: kisspeptin, Spermatogenesis, Hypothalamic-pituitary axis.

I. INTRODUCTION

Kisspeptin is a neuropeptide family formed by the cleavage of a 145 amino acid precursor peptide encoded by the KISS1 gene [1]. Kisspeptins, which were initially discovered as metastasis suppressors, are now recognized to serve critical regulatory functions in reproduction [2]. Kisspeptin is generated by two primary neuronal populations in the hypothalamus: the rostral periventricular region of the third ventricle (RP3V) and the arcuate nucleus (ARC). These neurons project to and activate gonadotrophin-releasing hormone (GnRH) neurons in the hypothalamus (through the kisspeptin receptor, Kiss1r), stimulating GnRH production [3]. In addition, other peptide fragments of the kisspeptin precursor, such as kisspeptin-14, kisspeptin-13, and kisspeptin-10, have also been discovered [4]. All kisspeptin fragments have a C-terminal decapeptide that is required for biological activity, and all kisspeptin forms exhibit comparable agonist activity for kisspeptin receptors [5]. Vulnerability in Windows, often known as critical periods, is a common organizational effect feature [6]. Exposures that occur after critical times will not release regulatory influences [7]. There is evidence that the endocrine regulatory effects of synthetic estrogenic compounds such as inappropriate exposures to kisspeptin during early critical
periods in rodents lead to disruption of puberty onset and disturbed gonadotropin secretion [8,9]. Exposure of kisspeptin in prepubertal rats induces testicular degeneration, which can be avoided by pretreatment with the antioxidant such as ubiquinone.

CoQ10, also known as ubiquinone, is a hydrophobic molecule [10] which happens in the inner membrane of the mitochondria, where it plays an important role in the electron transport chain and produces ATP [11]. CoQ10 is an antioxidant and free radical eliminator, as well as a super vitamin (vitamin Q), that aids in the protection of DNA, cell membrane lipids, and proteins from the dangers of oxidative damage, aids in the regeneration of vitamin E, and promotes optimum energy levels [12,13]. CoQ10 is often present in seminal fluid, where it enhances testicular and sperm function [14].

In this study, we analyzed LH, FSH, and testosterone levels, as well as we calculate spermatid heads count, elongated spermatid heads, and daily sperm production, to see whether there was any testicular degeneration. The aim of this study is to determine the effect of kisspeptin on gonadotropin secretion and the role of ubiquinone in preventing the effect of kisspeptin on testicular function in male rats that are pre-pubertal.

II. MATERIALS AND METHODS

Experimental Animals: We utilized 36 immature male rats, 35 days old, with average body weight (100-135gm) in this experiment. The rats were housed in plastic cages, and they were given tap water in glass bottles on a daily basis, as well as food and drink.

In the current research, thirty-six prepubertal male rats were randomly divided into Four groups comprising (8 animals each), They are:

The first group was given DMSO intraperitoneally twice daily for 12 days, considered as a control group.

the second group was administrated kisspeptin at a dose (50nmol/kg B.W) intraperitoneally, twice daily for 12 days.

the third group was received ubiquinone 10 (10mg /kg B.W) orally and kisspeptin 10 (50nmol/kg body weight) intraperitoneally, twice daily for 12 days.

the fourth group was intubated orally ubiquinone 10 only (10mg /kg B.W) twice daily for 12 days.

At the end of the experiment, all rats in each group were sacrificed.

parameter of study

1-Hormone analyses: Standard solid phase radioimmunoassay (RIA) was used to measure concentrations of plasma luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone as documented by [15].Inhibin B Was measured according to [16].

2-Testicular spermatid head count: The total number of spermatid heads in a testis was calculated as described by[17] using the formulae:

Total number of spermatid heads = \( \frac{\text{mean count of spermatid heads}}{0.00004 \text{ ml}} \times 50.5 \text{ ml} \)

Elongated spermatids per gram of testis weight = \( \frac{\text{Total number of spermatid heads}}{\text{testis weight}} \)

Daily sperm production (DSP) was calculated as

Daily sperm production per gram testis weight per day = \( \frac{\text{Total number of spermatid heads per grm of testis weight}}{6:10\text{(time divisor)}} \)

III. RESULTS

1-effect of kisspeptin and ubiquinone 10 on serum hormones levels of pituitary – Gonad axis of pre-pubertal male rat:
The statistical analysis of variance revealed presence of significant decrease (P<0.05) in means of FSH serum level in rats treated with the kisspeptin group compare to other groups. On the other hand, Coadministration of ubiquinone with kisspeptin considerably increases its value. Regarding the LH serum level there was a significant decrease (p<0.05) in the group of rats treated with kisspeptin compared to the other groups. In contrast, Kisspeptin administration with ubiquinone concurrently resulted in a substantial increase in its value. Concerning testosterone level, the rats treated with kisspeptin only show a significant decrease (p<0.05) in their value compared with other groups. Kisspeptin coadministration with ubiquinone improved its value as well.

While Inhibin B level, show increase highly significant (p<0.05) in male rats treated with kisspeptin when compare with control group, ubiquinone +kisspeptin group and ubiquinone alone group. In compared to kisspeptin alone, coadministration of kisspeptin with ubiquinone improves its value, but it was not significantly different in the control group. In comparison to the other groups, ubiquinone alone shows a substantial reduction in Inhibin B.

Table (1) effect of kisspeptin and ubiquinone 10 on serum hormones levels of pituitary – Gonad axis of pre-pubertal male rat (mean ±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>FSH µIU/ml</th>
<th>LH µIU/ml</th>
<th>Testosterone ng/ml</th>
<th>Inhibin B pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A</td>
<td>1.91±0.11</td>
<td>1.88±0.18</td>
<td>B</td>
<td>2.13±0.23</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.03±0.21</td>
<td>2.03±0.21</td>
<td>A</td>
<td>2.83±0.26</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2.02±0.11</td>
<td>1.65±0.11</td>
<td>B</td>
<td>1.68±0.12</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>0.84</td>
<td>0.53</td>
<td>0.67</td>
<td>83.4</td>
</tr>
</tbody>
</table>

Different letters represent significant difference at (p ≤0.05).

Table (2) effect of kisspeptin and ubiquinone 10 on total number of spermatid head, elongated spermatid and daily sperm production of pre-pubertal male rat (mean ±SE)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Total number of spermatid head (× 10⁶ / ml)</th>
<th>Elongated spermatid (× 10⁶ / g of testis weight)</th>
<th>daily sperm production (× 10⁶ / g of testis weight)</th>
</tr>
</thead>
</table>

- 2-effect of kisspeptin and ubiquinone 10 on total number of spermatid head, elongated spermatid and daily sperm production of prepubertal male rat:

The statistical analysis of variance of a total number of spermatid heads, elongated spermatid, and daily sperm production in the present study reveals no significant difference between the control group and the treated group.

The result shows a total number of spermatid heads a significant decrease (p<0.05) in rats treated with the kisspeptin group compare to other groups. In contrast, The coadministration of ubiquinone with kisspeptin significantly increases its value. Regarding the elongated spermatid, there was a significant decrease (p<0.05) in the group of rats treated with kisspeptin compared to the other groups. On the other hand, Kisspeptin coadministration with ubiquinone also resulted in a substantial increase in its value. Concerning to daily sperm production, the rats treated with kisspeptin only show a significant decrease (p<0.05) in their value compared with other treated and control groups. While coadministration of kisspeptin and ubiquinone causes a significant increase (p<0.05) in its value in comparison with kisspeptin only but it was not significantly different in the control group. Ubiquinone alone reveals a significant increase in daily sperm production in comparison with all groups.
Control | B | 5.54±0.27 | B | 5.33±0.76 | B | 0.78±0.02 |
---|---|---|---|---|---|
Group 1 | (Kisspeptin 50nmol/kg B.W.) | C | 1.94±0.31 | C | 2.13±0.84 | C | 0.35±0.07 |
Group 2 | (ubiquinone 10mg/kg B.W.) | A | 7.58±0.49 | A | 6.85±0.39 | A | 1.12±0.03 |
Group 3 | (Kisspeptin+ ubiquinone.) | B | 4.93±0.24 | B | 4.52±0.46 | B | 0.74±0.06 |
LSD | | 1.235 | | 1.128 | | 0.28 |

N=6
Different letters represent significant difference at (p ≤ 0.05).

IV. DISCUSSION:

1-effect of kisspeptin and protective role of ubiquinone 10 on FSH, LH, Testosterone and Inhibin B:

In the present study, the results showed that there was a significant decrease in FSH plasma level in male rats treated with kisspeptin compared with other groups in the prepubertal stage, this study agreement with [18] which found chronic S.C. injection of 50nmol/day for 13 days of kisspeptin 54 produced a significant decrease of plasma FSH when compared to control,While this result is disagreement with [15] who found no effect on FSH level.Hormonal interference in this critical period leads to an imbalance in the work of hormones It is, therefore, possible that pulsatile kisspeptin input resulted in changes in the pattern of GnRH release in terms of frequency and pulse amplitude, the decreased level of FSH may be attributed to the inhibitory effect of inhibin, which was significantly elevated following administration of kisspeptin. Or perhaps may be due to prolonged use of kisspeptin which has a desensitizing effect on the hypothalamus and pituitary gland [18,19,15] who found LH plasma level the result shows a significant decrease (p ≤ 0.05) in male rats treated with kisspeptin group compare with treated and control groups, this finding is agreement with [20,21,18,19,15] who found LH concentration was reduced in kisspeptin group compared to control group, this decrease may result that prolonged kisspeptins administration desensitized the hypothalamic–gonadal–pituitary axis [19] or perhaps Since LH and testosterone concentrations declined significantly, it is logical to presume that the cellular degeneration was due to a lowered plasma testosterone concentration. Possibly also, greater than normal plasma kisspeptin concentration, as a result of exogenous treatment, exerted a negative feedback effect on either the GnRH secreting neurons or directly on the pituitary, owing to Kiss1r expression [5], to suppress the LH secretion and a consequent drop of gonadal steroid. The presence of a functional kisspeptin receptor on the pituitary and release of kisspeptin in ovine hypophysial portal blood suggests that kisspeptin may have a direct action on the pituitary to modulate gonadotropin secretion [22]. Regarding the testosterone plasma level, the result shows a significant decrease (p ≤ 0.05) in male rats treated with the kisspeptin group compare with other groups. This result is consistent with [20,21,15,18,19] who found a decrease in testosterone after continuous administration of kisspeptin. Also, these results are similar to the findings of [23] who revealed that testosterone levels were reduced following treatment of adult male rats with kisspeptin-54 for 13 days period. Moreover, [24] reported that continuous subcutaneous injection of kisspeptin-54 resulted in a tendency for reduction of total and free testosterone with no statistical significance. The decrease may be due to that a significant decrease in the testosterone concentration following the kisspeptin treatment was quite possibly due to significantly lowered LH concentration because secretion of testosterone is under the control of LH. This indicates that active suppression of gonadal testosterone secretion was likely the cause of the sharp decline in testosterone concentration. Testosterone release into the bloodstream acts to regulate LH secretion by the pituitary through a negative feedback loop. Elevated circulating testosterone concentrations feedback at the level of the hypothalamus and pituitary to suppress LH secretion by the pituitary, which subsequently reduces luteinizing hormone–chorionic gonadotropin receptor (LHCGR) - mediated stimulation of Leydig cells and thereby reduces testosterone production. Conversely, a fall in
circulating testosterone concentrations induces LH secretion by the pituitary and a concomitant increase in testosterone production by the Leydig cells [25]. Or perhaps the result of this research suggests that prolonged use of kisspeptin may have a desensitizing effect on the hypothalamus and pituitary gland which subsequently affect the gonadal secretion of sex hormones [18]. While the study showed a significant increase in inhibin B in male rats treated with kisspeptin compared to other groups in prepubertal stage. This result is disagreement with [26] who found continuous kisspeptin-54 administration lead to a significant decrease in circulating inhibin B after 2 day of continuous administration, also this result disagreement with [19,27] who found continuous infusions of KP lead to inhibin B levels in mice. Plasma inhibin B, produced predominantly by the Sertoli cells, usually correlates with Sertoli cell number[28,29]. Inhibin B has been proposed as a sensitive endocrine marker reflecting the state of spermatogenesis [30]. FSH which is a gonadotropin that is produced and secreted by the anterior pituita, acts on Sertoli cells in the seminiferous tubules to initiate spermatogenesis [31]. So be FSH and Inhibin B together are more sensitive than either alone in predicting the histological status of the testis and the presence of sperm in biopsy tissue[32]. This increase of level inhibin B may be due to a decrease in follicle stimulating hormone (FSH), may have a direct effect on Sertoli cells and this leading to increased coadministration of ubiquinone with kisspeptin we found that the testosterone, LH, and FSH levels were improved. Coenzyme Q10 is a natural antioxidant that plays a fundamental role in the electron transport chain [33]. CoQ10 could suppress oxidative stress in the testis by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity. This in turn can counteract oxidative damage and sustain the function of Leydig cells protecting testosterone secretion [34]. The key application of CoQ10 in the testis is to increase the level of CoQ10 and its reduced form, ubiquinol, in the semen [35]. Ubiquinol is a potent fat-soluble antioxidant that can regenerate other antioxidants including vitamins E and C [36]. It also eliminates peroxyl radicals resulting from the lipid peroxidation process [37]. It also reduces the levels of follicle-stimulating hormone and luteinizing hormone [38,39]. The investigators' highlight that a lower serum follicle-stimulation hormone implies better spermatogenesis [40]. In recent research coadministration Ubiquinone-10 with procarbazine improve hormone levels as well as testicular catalase activity, as well as glutathione and superoxide dismutase levels [41].

2-effect of kisspeptin and ubiquinone 10 on total number of spermatid head, elongated spermatid and daily sperm production of pre-pubertal male rat:

In the current study, our results showed that there was a significant decrease (p<0.05) in the total number of spermatid heads in male rats treated with kisspeptin compare with other groups in the prepubertal stage, this study agreement with [19] who found administration of kp54 (50 nmol/day) for 13 days using mini-osmotic pumps implanted subcutaneously in the interscapular region of male rats significantly decreased sperm count, this result also is in agreement with [18] who found that chronic S.C. injection of kisspeptin-54 with a daily dose of 50 nmol produced a significant decrease in the count of sperm, this result decrease may be through direct desensitization of the HPG axis [19], or perhaps that prolonged use of kisspeptin may have a desensitizing effect on the hypothalamus and pituitary gland which subsequently affect gonadal functions rather than the direct effect of kisspeptin on the gonads [18]. Or it is probable that the testicular deterioration observed following prolonged kisspeptin treatment is due to alterations in testicular blood flow. Short-term flow reductions cause apoptosis in spermatogonia and early spermatocytes[42], while concerning elongated spermatid, the results showed that there was a significant decrease (p<0.05) in the elongated spermatid in male rats treated with kisspeptin compare with other groups in the prepubertal stage, this study agreement with [15,20,21,43] who found there was a significant reduction observed in elongated spermatid after i.p. administration of 1μg kisspeptin dose, continuously for 12 days. This decrease result may be due to data at the cellular level completely supports the suppression of testosterone and LH concentration, mediated by kisspeptin at stage VII of the spermatogenic cycle, which results in exceeded deterioration of the seminiferous epithelium. Currently, a small amount of elongated and round spermatids were persistently seen, pointing towards a stoppage of germ cell maturation [20]. or perhaps that LH withdrawal leads to suppression of spermatogenesis and results in significant reductions in the numbers of pachytene spermatocytes.  

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spermatocytes, preleptotene spermatocytes, spermatogonia, and round spermatids whereas elongated spermatids remained unnoticeable[44], or because a distinct degeneration pattern of the spermatogenic cell is also seen in acute removal of the testosterone. A significant decrease was observed in elongated spermatid and spermatid heads at day 8 and afterward, as animals used were prepubertal, they did not have these cells before that age[44]. Regarding daily sperm production, the results showed that there was a significant decrease (p≤0.05) in daily sperm production in male rats treated with kisspeptin compared with other groups, this study agreement with [15,20,21] who found there was a significant reduction in daily sperm production after administration of kisspeptin, continuously for 12 days. This decrease result may be or due this degeneration was linked to a reduction in plasma testosterone. However, the tendency toward lower testosterone levels might suggest a deficiency in the Leydig cells' steroidogenic activity[26]. Or because of dramatically reduced serum hormone levels (LH, FSH, and testosterone levels), testis weight by direct desensitization of the HPG axis [19], perhaps significant decreases in germ cells, daily sperm production, total support capacity of Sertoli cells, Sertoli efficiency and meiotic index, and an increase in the coefficient of mitosis indicated germ cell loss, which may have occurred through apoptotic mechanisms [20]. Co-administration of kisspeptin and ubiquinone resulted in a significant enhancement in the total sperm count, elongated spermatid and daily sperm production, the improvements may be due CoQ10 act as an antioxidant, an energy booster, a membrane stabilizer, and a regulator of mitochondrial permeability transition pores [36]. The majority of CoQ10 in sperm cells is concentrated in the mitochondria of the mid-piece, and energy-dependent activities in the sperm cell are dependent on CoQ10 availability [45]. CoQ10 levels in seminal fluid have a direct relationship with sperm parameters [46]. Due to its participation in mitochondrial bioenergetics and antioxidant characteristics, exogenous treatment of CoQ10 raises both ubiquinone and ubiquinol levels in the sperms and can be useful in enhancing sperm kinetic aspects in patients with idiopathic asthenozoospermia [14,35]. The findings show that supplementing with Ubiquinol aids in boosting sperm count and motility [47]. Reduced oxidative damage in the presence of the lipophilic antioxidant ubiquinol may explain why testicular cells release a normal quantity of testosterone. Flagella with a lengthy exposed structure are more vulnerable to oxidative degradation. The presence of ubiquinol may have decreased oxidative stress and avoided oxidative damage to the flagella structure, which aids in sperm movement, by preserving flagella size, which aids in sperm motility [48].

V. CONCLUSION:

Based on our findings, we infer that kisspeptin has a negative impact on reproductive and testicular function when administered at the prepubertal stage.

REFERENCES: