# Zinc modulates heat-induced degenerative effects in mice testes

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#### Summary

This study was designed to investigate whether administration of zinc sulphate could improve the response of mouse testis to degenerative effects of short term exposure heating. Fifty-four adult male Balb/C mice were anaesthetized and the scrotums of 36 of them were immersed for 15 min in a waterbath at 43°C. Immediately after the heating, half of the heated animals (n = 18) were injected with saline solution (HS) and the other half (n = 18) were given 10 mg/kg of zinc sulphate intraperitoneally (HZ), and this was continued every other day for 60 days. Control group of mice (n = 18) were treated identical to the HS group with the exception that the waterbath was held at 23°C. Mice were sacrificed at 15, 30 and 60 days after heating and the testes were gently excised. Seminiferous tubules diameter and percentage of spermatogenesis were measured in each testis. There was a significant reduction in the mean seminiferous tubules diameter and the percentage of spermatogenesis up to day 30 after heating in the HS group in comparison with the control group (172.5  $\pm$  2.55 µm vs 208.2  $\pm$  1.53 µm and 18.00  $\pm$  0.77% vs 99.33  $\pm$  0.33%, respectively) but in the HZ group, only initial decline (up to day 15) was observed in the mean seminiferous tubules diameter (199.1  $\pm$  1.90 µm vs 206.0  $\pm$  2.51 µm, not significant) and the percentage of spermatogenesis (91.33  $\pm$  0.95% vs  $97.67 \pm 0.66\%$ , P<0.001); then, a progressive recovery was seen up to the end of the experiment. Histopathological observations showed progressive degeneration up to day 30 after heating and then normal structure was returned in the HS group. In the HZ group, normal structure was returned up to day 15. The results from this study indicated that administration of zinc sulphate may improve the spermatogenesis process after testicular damage caused by a short term exposure of the mouse testis to heat.

Key words: Heat, Seminiferous tubule, Spermatogenesis, Testis, Zinc sulphate

# Introduction

It is well known that exposure of the testis to heat for a short single episode results in disruption of spermatogenesis and, subsequent recovery begins about 40 or even 60 days later (Setchell, 1998). The nature of heat exposure is critical since 43°C for 15 min induces specific damage limited to spermatocytes whereas 45°C for 15 min results in generalized non-specific damage to many different germ cell types (Jegou *et al.*, 1984).

Zinc (Zn) is an indispensable element for optimum growth and reproduction in males

and females. Zn participates in the mechanisms of the major metabolic pathways involving protein synthesis and turnover, carbohydrates, energy, nucleic acids, lipids, and heme syntheses. Zn has an important role in gene expression and embryogenesis (Aggett and Comerford, 1995). Zn deficiency in rabbits reduces plasma progesterone and estrogen and increases plasma prostaglandin  $F_{2\alpha}$  levels (Sharma and Joshi, 2005). In humans, Zn plays an important role in the physiology of spermatozoa, in sperm production and/or viability in the prevention of spermatozoa degradation, and in sperm membrane

stabilization (Lewis-Jones et al., 1996). Some studies have shown that Zn therapy has beneficial effects on the testicular function in the infertile men (Wong et al., 2002). Zn also has antioxidative properties thus may also act to reduce the reactive oxygen species leading to an increase in male fertility (Powell, 2000). The aim of this to investigate whether study was administration of Zn could improve the response of the mice testes to the degenerative effects of short term exposure heating.

## **Materials and Methods**

#### Animal treatment

A total of 54 adult Balb/C mice aged about 8 weeks and weighing about 25 g purchased from Neuroscience Research Center (Kerman, Iran). Mice were cared and used in accordance with the International Guiding principle for Biomedical Research Animals at Kerman University of Medical Science. Animals were fed with standard laboratory diet (Javeneh Khorasan Co., Mashhad, Iran) and water ad libitum and housed under controlled temperature (22  $\pm$ 2°C) and light (12 hr light/12 hr dark). The mice were anaesthetized with intraperitoneal administration of xylazine (10 mg/kg, Alfasan, Holland) and ketamine (100 mg/kg. Rotexmedica, Germany), then, the scrotum and hind legs of 36 out of 54 mice were immersed for 15 min in a waterbath at 43°C. Immediately after the heating, half of the heated animals (n = 18) were given 10 mg/kg of zinc sulphate (Merck, Germany) intraperitoneally (heat and zinc treatment animals, HZ). The administration of zinc sulphate was continued for 60 days every other day. Other half of the heated mice (n =18) were injected intraperitoneally with saline solution (heat and saline treatment animals, HS), and this was also continued every other day for 60 days. Control group of mice (C, n = 18) were treated identical to the HS group with the exception that the waterbath was maintained at 23°C.

#### Sampling

Mice were killed by cervical dislocation at 15, 30 and 60 days after heat treatment,

and the testes were gently excised. The testes were stored in 10% buffer formaldehyde for at least 72 hrs, embedded in paraffin wax for section cutting, washed with xylene, and finally stained with haematoxylin and eosin for histopathological examination.

#### Histopathological evaluation

Histopathological evaluation of testes was done using two parameters by an expert pathologist in a blind fashion. The first parameter was the mean seminiferous tubules diameter in each testis. The ten smallest, roundest tubules were identified and measured with an ocular micrometer under light microscopy. Mean diameter, in microns, was then determined for each group. The second parameter was the percentage of spermatogenesis. For this purpose, two hundred seminiferous tubules were examined under light microscopy. The presence of spermatozoa within the seminiferous tubule was considered as the evidence of spermatogenesis. Lack of spermatozoa even in the presence of orderly progression of primary and secondary spermatocytes was not considered as the evidence of spermatogenesis for the purpose of this experimental study (Boran and Ozkan, 2004).

## Statistical analysis

Data were analysed by using ANOVA, followed by Tukey's test for multiple comparisons. Results were expressed as mean  $\pm$  standard error. The analysis was performed using the SPSS<sup>®</sup> software for windows (SPSS Inc, Chicago, IL, USA).

## Results

# Mean seminiferous tubules diameter

The mean seminiferous tubules diameter  $(\mu m)$  in sections of testes of the control, HS and HZ groups are shown in Table 1.

The mean seminiferous tubules diameter in the testes was reduced after heating in both the HS and HZ groups in comparison with the control group (197.0  $\pm$  2.70 vs 206.0  $\pm$  2.51, P<0.01), but it was not statistically significant in the HZ group (199.1  $\pm$  1.9 vs 206.0  $\pm$  2.51). The mean seminiferous tubules diameter showed a markedly reduction up to day 30 in the HS group  $(172.5 \pm 2.55 \text{ vs } 197.0 \pm 2.70,$ P < 0.05) and then increased up to day 60  $(207.0 \pm 1.45 \text{ vs } 172.5 \pm 2.55, P < 0.05)$ , but there was not significant difference in comparison with the control group at the same time  $(207.0 \pm 1.45 \text{ vs } 203.5 \pm 1.62)$ . Tubular diameter showed initial reduction in the HZ group (P>0.05), then a recovery was observed up to day 30 (203.5  $\pm$  1.86 vs  $199.1 \pm 1.90$ , P>0.05). However, up to day 60, the mean seminiferous tubules diameter in the HZ group remained higher than those in the control group  $(217.0 \pm 1.89 \text{ vs } 203.5)$ ±1.62, P<0.001).

Table 1: Tubular diameter  $(\mu m)$  in the testes of the control (C), HS (heated and treated with saline) and HZ (heated and treated with zinc sulphate) groups

Days after heating	С	HS	ΗZ
15	206.0	197.0	199.1
	$\pm 2.51^{a}$	$\pm 2.70^{a, **}$	$\pm 1.90^{a}$
30	208.2	172.5	203.5
	$\pm 1.53^{a}$	±2.55 <sup>b, ***</sup>	$\pm 1.86^{a}$
60	203.5	207.0	217.0
	$\pm 1.62^{a}$	$\pm 1.45^{ac}$	±1.89 <sup>b, ***</sup>

Values are means  $\pm$  SEM. <sup>a, b, c</sup> At each column different superscript shows significant difference (P<0.05). \*Significantly different from C on the same day after heating: \*\*p<0.01; \*\*\*p<0.001

#### Percentage of spermatogenesis

The percentages of spermatogenesis in the testes of the control, HS and HZ groups are shown in Table 2.

During 15 days, the percentage of spermatogenesis in the testes was reduced in both the HS and HZ groups in comparison with the control group  $(71.17 \pm 1.16 \text{ and})$  $91.33 \pm 0.95$  vs  $97.67 \pm 0.66$ , P<0.001). It was decreased significantly until day 30 after heating in the HS group  $(18.00 \pm 0.77)$ vs  $71.17 \pm 1.16$ , P<0.05) and then recovered progressively but still significant difference from the control group on day 60 after heating was observed ( $92.17 \pm 0.94$  vs 98.50 $\pm 0.43$ , P<0.001). In the HZ group, the of spermatogenesis percentage was increased progressively from day 15 to day 60 after heating such that there was no significant difference from the control group

at the same time  $(96.17 \pm 0.70 \text{ vs } 98.50 \pm 0.43, P > 0.05)$ .

Table 2: Percentages of spermatogenesis in the testes of the control (C), HS (heated and treated with saline) and HZ (heated and treated with zinc sulphate) groups

Days after heating	C	HS	HZ
15	97.67	71.17	91.33
	$\pm 0.66^{a}$	$\pm 1.16^{a, ***}$	$\pm 0.95^{a, ***}$
30	99.33	18.00	94.50
	$\pm 0.33^{a}$	±0.77 <sup>b, ***</sup>	$\pm 0.92^{ab, ***}$
60	98.50	92.17	96.17
	$\pm 0.43^{a}$	±0.94 <sup>c, ***</sup>	$\pm 0.70^{b}$

Values are means  $\pm$  SEM. <sup>a, b, c</sup>At each column different superscript shows significant difference (P<0.05). \*Significantly different from C on the same day after heating: \*\*\*\*p<0.001

#### **Histopathological observations**

The control animals exhibited normal testicular structure in the histopathological cross sections. The testes of the mice in the HS group showed progressive degeneration with some evidence of edema and thickening of the capsule up to day 30 after heating and then normal structure was returned and only few degenerated tubules were seen on day 60. Although, in the HZ group, normal structure was returned up to day 15 but some of the degenerated tubules were remained. However, the testes of the mice in the HZ group showed normal structure thereafter (Fig. 1).

On day 15, hypocellularity of interstitial tissue and reduction of Leydig cells was seen in the HS group and then they reappeared on day 30. The appearance of Leydig cells in the HZ group was similar to the HS group.

## Discussion

This study demonstrates that the spermatogenic damage caused by a short term exposure of the mouse testis to heat can be improved quickly by administration of zinc. The brief exposure of the scrotum to the heat results in damage to the most heat sensitive germ cells, the pachytene spermatocytes and early spermatids (Setchell et al., 2001). These cells are lost a gap is created in the spermatogenic process

Fig. 1: Photomicrographs of H&E stained sections of mouse testis. (a) A normal seminiferous tubule with evidence of spermatogenesis, all layers of spermatogenic epithelium can be seen: lumen (L), spermatogonium (sg), spermatocytes (sp), spermatid (sd) and (b) degenerated seminiferous tubule with spermatic arrest, only a single layer of spermatogonium can be seen: lumen (L), spermatogonium (sg),  $(\times 400; Bar = 25 \ \mu m)$ 

as observed in our study. Since the length of time from initiation of stem cell division to formation of spermatozoa is around 35 days for mice (de Rooij and Russell, 2000), the chosen period of time (8 weeks) provided sufficient time to monitor the potential recovery of spermatogenesis in surviving spermatogonia in both the HS and HZ treated animals. According to the report by Setchell (1998), the recovery was seen approximately 40 days after a single exposure to heat, but our findings revealed that more than 60 days were needed to complete recovery and treatment with zinc is able to reduce the duration of recovery significantly.

A number of studies have shown that Leydig cell function is altered following spermatogenic damage induced by cryptorchidism, fetal irradiation in utero, vitamin A deficiency and hydroxyurea treatment (Rich et al., 1979; Risbridger et al., 1981a, b). Our histological observations show that the spermatogenic damage caused by a short term exposure of the mouse testis to heat is accompanied by reduction in Leydig cells and is reversible in association with restoration of spermatogenesis. This is in agreement with a report by Jegou et al. (1984) who suggested that the restoration of normal Leydig cell function is in concert with the recovery of seminiferous tubule function. In the present study a quick recovery in the spermatogenesis occurred following zinc therapy but the reappearance of Leydig cells were similar to the heat exposure group (HS). It seems that zinc did not affect on the reappearance of Leydig cells. Therefore, the positive effects of zinc on spermatogenesis might be via other mechanisms.

Early studies have shown that heat induces cell death in the testis. Cell death has been observed in meiotic primary spermatocytes and mitotic spermatogonia (Khan and Brown, 2002). According to the previous reports, zinc is able to stabilize the structures of proteins, preserves the integrity of subcellular organelles, and prevents destruction of DNA by inhibiting degrading enzymes (Bedwal and Bahuguna, 1994). Vital enzymes in rapidly growing tissues such as gonads and tissues in regeneration process those involved in nucleic acid and protein syntheses are zinc metalloenzymes (Prasad and Oberleas, 1973).

Zinc is a cofactor for more than 200 enzymes, so has multiple important functions. One of its most important functions is its participation in the antioxidant defense system (Sahin and Kucuk, 2003). The mechanism by which zinc exerts its antioxidant action is not well defined. However, it has been suggested that it increases the synthesis of metallothionein, a cystein-rich protein, which acts as a free radical scavenger (Webb and Cain, 1982) and hence enhances fertility potential (Bray *et al.*, 1997).

Normal testicular function requires hormonal stimulation by pituitary gonadotrophins (LH and FSH), which are in turn controlled by pulsatile secretion of gonadotrophin-releasing hormone (GnRH) from the hypothalamus (Garner and Hafez, 2000). In zinc deficiency, cells are unable to form sex steroids, leading to arrest of spermatogenesis and impairment of fertility (Lei et al., 1976; Om and Chung, 1996). Zinc plays an important role in the synthesis and excretion of luteinizing hormone and follicle stimulating hormone (El-Tohamy et al., 1997). Hambidge et al. (1986) reported that zinc is essential in the production of many sex hormones including testosterone and gonadotrophin-releasing hormone.

It is concluded that zinc sulphate administration seems an effective way in improving the testicular damage induced by short term exposure of the mouse testis to heat. Therefore, the present report should be extended for the improvement of fertility in male animals which maintained at high ambient temperature. More research is needed.

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