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Testicular Torsion Following Reperfusion Injury in Rat Model: Can Vitamin E Palliate this Injury?

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ABSTRACT

This study seeks to investigate in a rat model the effect of vitamin E following reperfusion injury/testicular injury. Twenty (20) Wistar male rats weighing between 100-150g ranging in age from 4-7 weeks were used for this study and were grouped into five groups of four rats each. Group one was administered with intramuscular (IM) administration of vitamin E (200mg/kg bw) and undergone testicular torsion with reperfusion for an hour, group two rats had concurrent administration of vitamin E (200mg/kg bw) followed by testicular torsion with reperfusion for an hour, while group three undergone torsion and was treated with vitamin E (200mg/kg bw) after an hour of testicular torsion at 720^0 clockwise rotation. Group four undergone testicular torsion for an hour after which detortion was done and reperfusion was allowed for another one hour. While group five served as the control rats. At the end of the experiment testes of all the treated rats were exposed and semen was obtained for sperm characteristics (sperm motility and count). The results showed that following testicular torsion, sperm motility was reduced significantly while sperm count was unaffected. Intramuscular administration of vitamin E following torsion showed insignificant decrease ($p>0.05$) in sperm function.

Keywords: Reperfusion injury, progressive sperm motility, sperm count, vitamin E, surgery.

INTRODUCTION

Reperfusion injury is a temporary interruption of blood flow to a tissue which results in damage to the tissue. It can also be defined as a surgical emergency in humans that causes testicular injury and sub-fertility [1]. It has usually been assumed that this damage occurs during the period of hypoxia and is due to depletion of Adenosine Triphosphate (ATP). Another possibility is that the deleterious effects actually occur during reperfusion and are due to free radical generation. Thus, one can imagine an accumulation of reduced substances during hypoxia such that reperfusion and re-oxygenation results in a burst of free radical production [2]. The mechanism of reperfusion injury has been elegantly studied in feline intestine and the role of O_2^- confirmed [2,6]. There are various antioxidant agents that can serve as free radical scavengers or have free radical scavenging properties; among these antioxidants are vitamin E. Vitamin E, an essential fat soluble vitamin helps to stop the reaction of free oxygen radical [superoxide(O_2); hydrogen peroxide (H_2O_2) and hydroxyl (OH^-)] in the tissue vitamin E is a potent antioxidant and study has proved that vitamin E has a protective effect against testicular lipid peroxidation induced by both iron and ethanol [3]. Hence, this work was carried out to ascertain whether of administration of vitamin E will have beneficial effects on reperfusion injury occurring after torsion and detorsion of the testis using animal model.

MATERIALS AND METHODS

Animals

Twenty (20) male wistar rats weighing between 120-150g were assigned into five groups of four rats each under controlled light (12L: 12D cycle) and temperature (24 ± 1^0C) condition. The rats were fed with normal pellet and water was provided ad libitum. This study was conducted in accordance with the recommendation from the declaration of Helsinki on guiding/principles in care and use of animals.

Experimental group

Group one was treated with vitamin E (200mg/kg bw; intramuscular administration) and undergone testicular torsion. Group two undergone testicular torsion followed by intramuscular administration of vitamin E (200mg/kg bw) an hour after torsion. Group three undergone only testicular torsion. Group four served as the positive control, the rats were anesthetized and had testicular torsion for one hour after which detorsion was done and reperfusion was allowed for another one hour. Group five served as the normal control (had no torsion).

Testicular Reperfusion

All experimental animals were anesthetized with ketamine (4mg/kg bw) before undergoing testicular torsion. The rats undergone torsion an hour after administration of vitamin E (200mg/kg bw) and torsion was carried out by opening both scrotal sacs and twisting the testes and the spermatic cords clockwise (at an angle 720^0). Testicular torsion was maintained at this position (720^0) for an hour, after which the testis was untwisted and reperfusion was allowed for another one hour before the testis was excised and delivered. This procedure was carried out in all the experimental animals except in the positive control.

Sperm Profile

Semen was collected via the caudal epididymis for estimation of sperm count and sperm motility.

Epididymal sperm motility

Progressive motility is a forward movement of spermatozoa which is indicative of characteristic of good semen. Semen progressive motility was determined by conventional method. Semen was squeezed unto a pre-warmed slide and two drops of warm 2.9% sodium citrate was added. After which, it was then covered-slip and examined under the light microscope at X40 objective.

Epididymal sperm count

The caput from the epididymis was homogenized in 5ml of normal saline in a measuring cylinder and the change in volume was measured. A further dilution of 1/200 was made and sperm count was determined using the new improved neauber counting chamber in the hemocytometer.

Statistical analysis

Chi-square and Analysis of variance (ANOVA) were used. The P-value less than 0.05 considered as significant difference. All computations were performed using the statistical package (SPSS-PC1 for windows; SPSS; Chigaco IL).

RESULTS

Table 1: Comparison of progressive sperm motility in control and experimental groups following ischemia and reperfusion injury

Progressive Sperm Motility (%) Class	Group 1	Group 2	Group 3	Group 4	Group 5
A	32.6±12.80^{c}	21.0±1.00^{b}	3.0±1.00^{d}	0.00±0.00	61.0±7.10^{a}
B	36.7±9.70^{c}	41.0±1.00^{b}	5.5±0.50	0.00±0.00	22.5±2.50^{a}
C	19.3±10.30^{c}	25.5±0.50^{b}	67.5±7.50^{d}	0.00±0.00	12.5±0.50^{a}
D	13.3±7.50^{c}	12.5±0.50^{b}	19.0±1.00^{d}	75.0±2.50^{e}	4.0±2.80^{a}

Data are expressed as Mean ±SD. Values in the same roll with different superscript are significantly different at $p<0.05$.
Key: A-Progressive directional (fast), B-Progressive directional (slow), C-Vibrational, D-Non motile.

Table 2: Comparison of the effect of vitamin E administration on sperm count of ischemic and reperfusion injury in Wistar rats.

Group	Sperm count (10^6/ml)
1	41.65±18.70^{c}
2	19.85±2.10^{b}
3	25.05±8.50^{ab}
4	16.38±5.90^{b}
5	32.6±0.10^{a}

Data are expressed as Mean ±SD. Values in the same column with different superscript are significantly different at $p<0.05$.

DISCUSSION

Testicular torsion is the twisting of the testis on its connection and usually experienced in young boys in their teenage years. It can cause venous obstruction, edema, hemorrhage, arterial occlusion which could lead to gangrene and atrophy of the testis if unrelieved within few hours. This study was carried out to examine the role of vitamin E in male Wistar rats that undergone testicular torsion following reperfusion injury. The testis produces sperm and male sex androgen principally testosterone. The sperm are formed in the long, convoluted seminiferous tubules that are joined by straight tubules to the rat testis. Torsion and subsequent detorsion of the spermatic cord could result into cellular injury called reperfusion injury and thereby releasing free radicals. According to world health organization (WHO) classification and adaptation by Rothmann *et al.*,[4,14] the progressive sperm motility was divided into four classes as given above. The effect of vitamin E was tested on sperm motility after ischemia and reperfusion injury. In group one vitamin E(IM) treated rats for an hour before torsion, the result in table 1 shows that this group had most of their sperm motility to be progressive directional with a very slow movement (they belong to class B). In group two, the rats were given vitamin E (IM) at the same time followed by torsion and it was observed to have most of their sperm motility to be progressive directional with a very slow movement (class B), while in group three, the rats were administered vitamin E (IM) followed by an hour of testicular torsion and it was observed that the sperm had the highest percentage of its motility to be vibrational. Group four rats were artificially induced with torsion but not administered with vitamin E, most of the sperm were non motile. However, the results obtained in all the experimental groups showed that there were reductions in progressive sperm motility when compared to their control counterpart after ischemia and reperfusion injury and that vitamin E administration had palliative effect on this reduction. In group one treated rats (rats were administered vitamin E an hour before torsion) and group two rats (treated with vitamin E and at the same time had torsion) showed statistically significant difference ($p<0.05$) in sperm count when compared with the control group (group five), although a decrease in sperm count was observed in this group. Group three rats showed significant reduction ($p<0.05$) in sperm count after ischemia and reperfusion injury when compared with the control group. Group four rats were induced with torsion but not treated with vitamin E also showed significant reduction ($p<0.05$) when compared with the control rats. The results from the present study showed that sperm function was reduced after torsion as this is similar to the report by Greenstein *et al.*,[5]. However, there were several studies on different animal species which showed that unilateral testicular torsion could produce pathological changes in the contralateral testis [6,7] with reduction in fertility profile [3] and abnormal sperm production [8]. However, the exact mechanism of testicular damage following torsion/reperfusion injury has not been fully elucidated. Studies by Tanyel *et al.*, [9]; Kizilcan *et al.*[10] reported that unilateral testicular torsion produces a reduction of blood flow in the non-twisted testis and which gradually increases after the detorsion procedure which therefore involves the generation of toxic reactive oxygen species

(ROS) which can damage majority of the cellular components by the peroxidation of cell membrane lipids [11,12]. However, with vitamin E co-treatment the results showed a notable reversal in sperm motility and count which were differ in the groups that undergone testicular torsion in which there was reduction in sperm motility as obtained in the present results which was significant ($p<0.05$) when compared with the normal and the positive control rats. These results suggest that vitamin E administration can be taken for effective treatment of testicular torsion in animal model, since it was observed that vitamin E caused a reversal in the sperm profile of rats that undergone testicular torsion.

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