HISTOLOGICAL EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES SIZE 10 NM IN MICE TESTES

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Received: Dec. 2016 / Accepted: May 2017 / Published: Jun. 2017

ABSTRACT:
In the present study, the histological effect of titanium dioxide nanoparticles (TDN) on testicular tissue of mature Mus musculus mice was investigated. The animals were divided into six groups, control group treated with TDN free saline and five groups treated with TDN as follow: 5, 10, 50, 100 and 150 mg/kg B.W. The results showed that TDN has histological effects on testicular tissue like sever congestion, mild edema between seminiferous tubules (STs) and decrease the thickness of germinal epithelium at low concentrations. While, the histological changes at high concentrations involved disturbance in STs diameters, sever edema between STs, sever vaculation in the germinal epithelium and necrosis in spermatogonia, germinal epithelium and Sertoli cells.

KEYWORDS: Mus Musculus Mice, TDN, Histological, Seminiferous Tubules, Sertoli Cells.

1. INTRODUCTION
Titanium (Ti) is found in normal animals’ tissues, but only in trace amounts (Schroeder et al., 1963). But there is no proof of Ti being a vital element for human or animals (Dankovic et al., 2007). Titanium dioxide (TiO2) amid most oxidation forms used worldwide in consuming and usage ways (Hongbo et al., 2013). Many researches pointed to that TiO2 nanoparticles (NPs) were more cytotoxic to Leydig cells than diesel exhaust and carbon black NPs, and impacted gene expression, proliferation and viability of these cells (Komatsu et al., 2008). In nanomedicine, intravenous and subcutaneous injection is a unique way to deliver TiO2 NPs into human body (Zhao and Castranova, 2011). Many researches have revealed that TiO2 fine particles (FPs) and NPs prompt genotoxicity and cytotoxicity in various cultured cell lines in animal model (Coskun et al., 2004). So our research aims to reveal the cytotoxic effect of TiO2 on adult mice testes.

2. MATERIALS AND MEHODS

2.1 Titanium dioxide
Titanium Oxide Anatase nanopowder, APS: 10 nm was requested form M K Impex Corp. 6382 Lisgar Drive Mississauga, Ontario L5N 6X1 Canada, (mknano, www.mknano.com).

2.2 Experimental animals
Forty two albino male mice (four months old) with average weight (25 gm) of strain Balb/c were used in this study. These animals were housed in plastic cages under standard laboratory conditions, including temperature at 22± 2°C and 12 hours light and 12 hours dark cycle. They were given standard diet as pellet and water ad libitum.

2.3 Experimental design
This experiment was designed to study effect of 5, 10, 50 100 and 150 mg/kg body weight (BW) TiO2 on histological feature of male albino mice testes. The animals of this study were randomly divided into six groups (n=7) for each group, as follow: first group is (control group), the males of this group were treated with TDN free normal saline (0.9%). While other five groups were intraperitoneal (IP) injected with 5, 10, 50 100 and 150 mg/kg BW TiO2 respectively. TiO2 was administered daily for fourteen days. At the end of the experiment all animals were subjected to postmortem dissection to obtain the testes (Liu et al., 2009).

2.4 Histologic examination
All animals were subjected to anesthetizing with 0.2 ml ketamine/xylazine (1:1 volume/volume). Then the testes were removed and fixed in bouine solution for 8-24 hours. After fixation the fixed testes were embedded in paraffin wax, sectioned at 5 µm using rotary microtome (microTec Laborgerate GmbH Rudolf-Diesel-Straße, Walldorf, Germany) and stained with hematoxylin and eosin (H and E). Then the general feature of the testicular sections were examined under light microscope (Culling et al., 1985).

3. RESULTS
Figure 1 showed the testicular cross section of control group. This testis was surrounded by a thick capsule of dense connective tissue, the tunica albuginea and within the testis the well development of seminiferous tubules (STs) were observed. In addition to active spermatogenesis the spermatogenesis process was increased compared to the control group (Figure 1). Whereas treatment with 10 mg/kg BW TiO2: NPs caused shrinkage of STs which associated with edema and hypoplasia of germinal epithelium (Figures 4, 5). Germinal epithelium hyperplasia, sever edema and obvious decreased in spermatogenesis cells were observed in nearly all the tubules. In the testicular cross sections of 5 mg TiO2: NP/kg BW (Figures 2, 3), the spermatogenesis process was increased compared to the control group (Figure 1). But treatment with 100 and 150 mg/kg BW TiO2; NPs resulted in acute necrosis and pronounced cytoplasmic vaculation of germ cells towards the periphery of STs. In addition to the destruction of these tubules and severe loss of germ cells (8-12).
Figure 1. Histological section of control mice testes showing normal structure of seminiferous tubule (H&E x 100).

Figure 2. Histological section of mice testes treated with TiO2 5 mg/kg body weight for 14 days showing sever congestion of blood vessel (arrow) and increases of spermatogenesis (head arrow) (H&E x100).

Figure 3. Histological section of mice treated with TiO2 5 mg/kg body weight for 14 days showing sever congestion of blood vessel (arrow) and increases in spermatogenesis (head arrow) (H&E x200).

Figure 4. Histological section of mice testes treated with TiO2 10 mg/kg body weight for 14 days showing decrease in spermatogenesis (head arrow) sever congestion of blood vessel (arrow) and edema between STs (H&E x100).

Figure 5. Histological section of mice testes treated with TiO2 10 mg/kg body weight for 14 days showing decrease thickness of germinal epithelium (line) with sever congestion of blood vessel and edema between STs (arrow) (H&E x200).

Figure 6. Histological section of mice testes treated with TiO2 50 mg/kg body weight for 14 days showing hyperplasia in germinal epithelium (line), sever edema between STs (arrow) and decreases of spermatogenesis (head arrow) (H&E x100).

Figure 7. Histological section of mice testes treated with TiO2 50 mg/kg body weight for 14 days showing hyperplasia in germinal epithelium, sever edema between STs, decreases of spermatogenesis (double head arrow) and sever congestion of blood vessel (head arrow) (H&E x200).

Figure 8. Histological section of mice testes treated with TiO2 NP 100 mg/kg body weight for 14 days showing sever vacuolation and necrosis in germinal epithelium (head arrow), sever edema amid STs and no spermatogenesis with cellular debris in the lumen of STs (arrow) (H&E x100).
Titanium dioxide nanoparticles are vastly used like rubber, plastics, ceramics, paints, (Ashraf et al., 2009) and as drug constituents, food colorant vehicle, (Medina et al., 2007; Jiang et al., 2008) and in water purification (Fujishima et al., 2008; Wang et al., 2008; Trouiller et al., 2009). Although, intravenous and subcutaneous injection of TDN is unique way to deliver TDN into human body, however, the transplanted graft Nano-medicine application showing another way to deliver TDN to the human body (Zhao and Castranova, 2011). So, in the present study TDN size 10 nm was delivered intraperitoneally to Mus musculus mice. The observations of the current results showed the appearance of symptoms of acute toxicity with increasable doses, which reveals passive behavior, loss of appetite, tremor, and lethargy, that is confirmed with Ma et al., (2010) by using 50 nm TDN (Wang et al., 2007).

4. DISCUSSION

On the contrary of our results, Mahrous (Mahrousa, 2004) recorded that oral treatment of male rats with 4 mg/kg B.W of TDN for 90 days resulted a significant decrease in their body weight. Wang et al, (2007) are found that using acute oral administration of TDN 5 mg/kg B.W decrease body weight of all treated mice, this may return to the way of TDN delivered to body, where IP route affect on signaling process (testes) whereas the oral route affect metabolic processes. Ahotupa and Huhtaniemi, (1992) showed that TDN induced spermatogenic injury may be cause to formation of free radicals in the testicular tissue and spermatogenesis, this comes confirmed with the recent results.

It has been reported that TDN causes stress due to high level of nitric oxide in rat serum, (Herrero et al., 1997) which leads to decrease of cholesterol the precursor of testosterone, and finally disturbances of gonadotrophin releasing hormone (Ferrini et al., 2001) and decrease spermatogenesis and testicular inflammation, this is confirmed with recent results except 5 mg/kg B.W that show increasing of spermatogenesis. Many studies showed that TDN have the ability to penetrate the blood-testis barrier and induce toxic effects on male germ cells (Komatsu et al., 2008; Gao et al., 2013), and forming reactive oxygen species then it increases antioxidant enzyme activity (Afaq et al., 1998) like glutathione reductase, superoxide dismutase (Nabela et al., 2010) and same result reported as used ultrafine titanium dioxide that leads to testicular histologic damages, these findings come confirmed with recent results which show acute histologic damages in germ epithelium and testicular tissue in addition to significant decreasing in spermatogenesis.

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