Preventing Effect of Vitamin E on Oocytes Apoptosis in Morphine-treated Mice

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SUMMARY: Several studies have shown that Morphine Sulfate affects on fertility, embryogenesis and consequent pregnancy loss and ultrastructural alterations of oocytes in animal model. This study was done to determine the effect of morphine sulfate on oocytes apoptosis and preventive role of daily supplementation of Vitamin E on oocytes apoptosis in morphine sulfate-treated mice. Twenty-four NMARI female mice were randomly allocated into four experimental groups. For 15 days, control group received saline (0.2 ml/day by subcutaneous injection), group I Vitamin E (60 mg/kg/day orally), group II Morphine Sulfate (10 mg/kg/day by subcutaneous injection) and group III Morphine Sulfate with Vitamin E (60 mg/kg/day orally). Then, animals were superovulated with PSMG (10 Units) and 10 Units of HCG. The next day the animals were sacrificed, oocytes were flushed from each fallopian tube. The collected oocytes were subjected to determine apoptosis by Tunnel assay with using Fluorescent Microscope. According to our results, the number of retrieved oocytes were 121, 132, 86 and 114 in control, experimental group I, II and III, respectively. Morphine Sulfate treatment increased apoptosis in oocytes to 17.44% whereas oocytes apoptosis was 4.13% in Controls. Supplementation with Vitamin E in Morphine Sulfate-treated mice reduced the oocytes apoptosis to 7.01%. This study showed that Morphine can increase apoptosis in oocytes and Vitamin E treatment significantly reduces oocytes apoptosis in the Morphine Sulfate-treated mice.

KEY WORDS: Oocytes; Morphine sulfate; Vitamin E; Apoptosis; TUNEL assay; Mice.

INTRODUCTION

Opium substance consumption in young people is increased in comparison with last decade. More than 90 percent of women consuming Morphine Sulfate as a component of opium takes place in childbearing age (Vucinovic et al., 2008; Dehghan et al., 2010).

Opium addiction causes menstrual period, reduces CNS repose to excitation of visceral nerve in cervix of uterus, decrease of oxytocin secretion and myometrium contraction (Shin & Eisenach, 2003; Kayacan et al., 2007; Yoo et al., 2001; Nacitarhan et al., 2007; Kowalski et al., 1998).

Morphine Sulfate (C17 H19 O3 N) is one of the 40 alkaloids present in opium from Papaver somniferum which as one of the main opium substances causes disorder in uterus cycle, reduce of pregnancy chance and inhibition of normal of ovulation in mice (Siddiqui et al., 1997; Lakhman et al., 1989). Morphine Sulfate reduces volume of placental blood, length and weight of fetuses and cerebral cortical layers and number of neurons in frontal lobs (Sadoraie et al., 2008). Morphine Sulfate can transfer from placenta barrier and due to morphine receptor in placental vile and fetal tissue has long time effects (Kopecky et al., 1999; Ray & Wadhwa, 1999).

Opioids have oxidative properties and due to this spatiality increase apoptosis in many of cells by producing free radicals (Singhal et al., 1994; Pan et al., 2005). Several studies have shown that Morphine Sulfate increase apoptosis in neurons, glial, hepatocytes, cells of immune systems and epithelial cells in endometrium of uterus (Dehghan et al.;
Lin et al., 2009; Li et al., 2009; Wang & Han, 2009; Svensson et al., 2008; Singh et al., 1999; Payabvash et al., 2006). Morphone sulfate can increase the generation of free radicals by acting as a pro-oxidan (Singhal et al., 1994).

Lipid peroxidation increase following blocking of anti-oxidant enzyme. This process leads to the formation of free radicals or reactive oxygen species (ROS) (Niki et al., 2005). These free radicals or ROS caused damage to the cell membrane and DNA fragmentation (Yang et al., 1998). Also, Oxidative stress cause chromosomal instability and program cell death (Kumar & Cotran 2003). Tunnel assay is a well develop method for evaluation of apoptosis or program cell death (Gavrieli et al., 1992; Paszkowski et al., 2002). Program cell death is known as main mechanism in oocytes death (Morita & Tilly, 1999; Tilly, 2001). Also studies have shown that anti-oxidant component reduce the oxidative stress in liver tissue (Zhang et al., 2004).

Vitamin E as a lipid-soluble substance has anti oxidant properties (Brigelius-Flohe & Traber, 1999). Vitamin E has important role in prevention of lipid production and oxidative stress reaction by scavenging free radicals. Due to anti oxidant properties (Brigelius-Flohe & Traber, 1999; Sheppard et al., 1993; Burton & Ingold, 1986), It can reduce the senile oxidative stress reaction on number and quality of oocytes (Tafin et al., 2002).

Furthermore, several studies have shown that Vitamin E and its various components reduce the oxidative stress reaction by scavenging free radicals in cells and cell organelles (Hassa et al., 2007; Kunitomo et al., 2009; Sen et al., 2007; Mokhtar et al., 2009). With Regard to impotent the number of oocytes in fertility and increase of opium consumption in child- bearing age women, this study was done to determine the effect of daily supplementation of Vitamin E on oocytes apoptosis in morphine sulfate -treated mice.

**MATERIAL AND METHOD**

Twenty-four female mice were randomly allocated to four separate groups of six animals each control group (Group C) received saline (0.2 ml/day by subcutaneous injection). Animals in Vitamin E treated group (I) were received Vitamin E (T-3251; Sigma) (60 mg/kg/day orally). Animals in Morphine Sulfate -treated group (group II) were received Morphine Sulfate (10 mg/kg/day by subcutaneous injection). Also Female mice in Morphine with Vitamin E treated group (group III) were received Morphine Sulfate (10 mg/kg/day by subcutaneous injection) with Vitamin E (60 mg/kg/day orally). The treatments were scheduled for 15 consecutive days.

**Superovulation protocol.** The animals were superovulated following these schedules. Mice were treated with an intraperitoneal injection of 10 IU pregnant mare serum Gonadotrophin (PMSG; Intervet International B.V., Netherlands). Human chorionic gonadotropin (10 IU; Intervet International B.V., Netherlands) was injected intraperitoneally 48 hours later. The next day the animals were sacrificed by cervical dislocation, oocytes were flushed from each fallopian tube using a hypodermic needle with media (a Mem) under a dissecting microscope, and the retrieved cumulus oocytes complexes were examined by TUNEL Assay.

**TUNEL method.** The retrieved cumulus oocyte complexes were incubated for 60 minutes in 2% paraformaldehyde and 2 minutes in triton X solution. Tunnel assay (*In situ* cell death detection kit- Roche Germany) was used to determine Failure rate of DNA (DNA damage). Incubation with Propidium Iodide (P4170; Sigma) solution was used for background staining (Hassa et al.; Kunitomo et al.). Then Failure rate of DNA were determined by fiorescent microscope (BX-51 Nikon –Japan) with Excitation wave length in the range of 450-500nm and detection in rang of 515-565 nm.

**Statistical analysis.** Data analyzed by One way ANOVA and Post Hock tests. A P value less than 0.05 was taken as statistically significant.

**RESULTS**

The number and percent of retrieved and apoptotic oocytes of mice in four experimental are depicted in Figure 1. According to our results, the number of retrieved oocytes were 121, 132, 86 and 114 in control, experimental group I, II and III, respectively.

The number and percent of apoptotic oocytes was 5 (4.13%), 7 (5.30%), 15 (17.44%) and 8 (7.01%) in control,
Vitamin E treated mice, Morphine Sulfate treated and nicotine and Vitamin E treated mice, respectively.

Oocyte apoptosis significantly increased in Morphine Sulfate treated mice in comparison with controls (p<0.5). Also oocyte apoptosis in Morphine Sulfate –vitamin E group significantly reduced in comparison with Morphine Sulfate treated mice (p<0.5). There was no significant difference between apoptotic cell number in controls and Morphine Sulfate –vitamin E group.

DISCUSSION

This study showed that Morphine Sulfate exposure increased apoptosis in mice oocytes. Our findings are comparable to previous studies (Dehghan et al.; Pan et al.; Singhal et al., 1999; Zhang et al.; Nasiraei-Moghadam et al., 2010; Bhat et al., 2004).

Degahn et al. reported that interaperitoneal administration of morphine 10 mg/kg for 15 days in mice dams cause signs of apoptosis and inflammation with increase of blood vessels in uterus. Furthermore disruptions of nuclear envelop in epithelial cells of endometrium and irregular space between nucleolus and chromatin was founded.

Also, Singhal et al. (1999) have indicated that morphine with increase of free radicals such as SOD (superoxide desmotase) and katalase cause increase of gene expression of BAX and Caspase 3 and reduce of Bcl2 and increase apoptosis in T lymphocytes and Jurkat cells.

In other study, Nasiraei-Moghadam et al. reported that oral administration of morphine sulfate in mice dams during the first days of gestational age cause increase of gene expression of BAX and reduce of Bcl2 and increase apoptosis in neuroblasts of offspring. Also, Pan et al. showed that heroin administration in mice reduces endogen antioxidants in blood stream and increase of ROS in white cells and finally it causes oxidative damage in proteins and lipids of brain and liver.

Morphine sulfate by production of NO (nitric oxide) and dysfunction of NADPH oxidase produce super oxide (O3), this process cause binging of apoptosis in macrophages (Bhat et al.).

Furthermore, Zhang et al. reported that interaperitoneal administration of morphine sulfate cause DNA damage and increase of oxidative enzymes in hepatocytes. He also indicated that anti- oxidants such as ascorbic acid and Glutathione reduce negative effects of morphine in these cells. Thus, we can conclude that morphine sulfate increase Lipid peroxidation following blocking of anti-oxidant enzyme. This process leads to the formation of free radicals or ROS (Niki et al.). These free radicals or ROS caused damage to the cell membrane and DNA fragmentation (Yang et al.). Also, Oxidative stress cause chromosomal instability and program cell death (Kumar & Cotran). Program cell death is known as main mechanism in oocytes death (Morita & Tilly; Tilly).

Also, in our study Supplementation with Vitamin E reduced the oocyte apoptosis rate in morphine sulfate-treated mice. This reduces of oocyte apoptosis can be due to antioxidant properties of vitamin E.
Vitamin E and its various components reduce the oxidative stress reaction by scavenging free radicals in cells and cell organelles (Brigelius-Flohé & Traber; Sheppard et al.; Burton & Ingold).

Vitamin E, in fact, is the major peroxyl radical scavenger in biological lipid phases such as membranes. Its antioxidant action has been ascribed to its ability to chemically act as a lipid-based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation and protecting the organism against oxidative damage (Hassa et al.; Kunitomo et al.; Sen et al.; Mokhtar et al.). Several studies reported that with vitamin E an antioxidants substance has preventive effects (Zhang et al.; Hassa et al.; Train et al.).

Zhang et al. showed that anti-oxidants such as ascorbic acid and Glutathine reduce negative effects including DNA damage and increase of oxidative enzymes due to morphine in hepatocytes. Also, Hassa et al. in an experimental animal study reported that fertilization and cleavage rates were affected mainly in the smoke-exposed female mice population and treatment with vitamin E did affect the fertilization, cleavage, and embryo development rates of smoke-exposed female mice. Indeed, Train et al. in an animal model have shown that oral administration of antioxidant (vitamin E and C) can counteract the negative effect of female aging on number and quality of oocytes.

CONCLUSION

This study showed that morphine sulfate exposure increases apoptosis in oocytes and Supplementation with Vitamin E reduce the oocytes apoptosis in morphine sulfate-treated mice.

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