Environmental Toxicology and Pharmacology 54 (2017) 133–141

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Research Paper

Melatonin protect the development of preimplantation mouse embryos from sodium fluoride-induced oxidative injury

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ARTICLE INFO

Keywords:
Sodium fluoride
Melatonin
Antioxidant
Embryo

ABSTRACT

Recently study shows that melatonin can protect embryos from the culture environment oxidative stress. However, the protective effect of melatonin on the mouse development of preimplantation embryos under sodium fluoride (NaF) induced oxidative stress is still unclear. Here, we showed that exposure to NaF significantly increased the reactive oxygen species (ROS) level, decreased the blastocyst formation rates, and increased the fragmentation, apoptosis and retardation of blastocysts in the development of mouse preimplantation embryos. However, the protective of melatonin remarkable increased the of blastocyst formation rates, maintained mitochondrial function and total antioxidant capacity by clearing ROS. Importantly the data showed that melatonin improved the activity of enzymatic antioxidants, including glutathione(GSH), superoxide dismutase(SOD), and malonaldehyde (MDA), and increased the expression levels of antioxidative genes. Taken together, our results indicate that melatonin prevent NaF-induced oxidative damage to mouse preimplantation embryo through down regulation of ROS level, stabilization of mitochondrial function and modulation of the activity of antioxidases and antioxidant genes.

1. Introduction

A numerous researches have demonstrated that oxidative stress is the main source of embryonic development and proliferation defects (Guerin et al., 2001), the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to oxidative stress, and oxidants stress is a negative effect produced by free radicals, reactive oxygen species (ROS) can be accumulated in a cellular condition (Blazquez-Castro et al., 2012). Some external factors such as culture condition can enhance the ROS levels generated by embryos (Choi et al., 2008). ROS is an unavoidable factor generated in gamete maturation fertilization and embryo growth (Mohammadi Roushandeh and Habibi Roudkenar, 2009). ROS include a number of reactive molecules derived from oxygen such as superoxide anion radi (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (-OH) (Fantone and Ward, 1982). It was found that some ectogenesis factors like sodium fluoride, a kind of ionic compound of fluorine which have high reactivity, can make those oxidative stress markers significantly increased.

Although low dose of fluorine is beneficial to body health, chronic ingestion at high doses can have adverse effects on human health (Fleming and Greenfield, 1954), recently a huge amount of fluoride has been released into environments and fluoride pollution seems to be increased(Ando et al., 2001). Fluoride exposure increases the O_2^- concentration and its downstream oxide such as hydrogen peroxide, peroxynitrite, hydroxyl radicals, thereby resulting in ROS production, (Goh and Neff, 2003). Moreover, fluoride alter the activity of antioxidant enzymes such as superoxide dismutase (SOD) and Catalase (CAT), and giutathione levels in the body. These actions often resulting in excessive production of mitochondrial ROS level (Oyagbemi et al., 2016). The overproduction of ROS leads to macromolecule oxidation, which caused free radical attack of mitochondrial membrane phospholipids, DNA breakage, nuclear chromatin condensation and cell apoptosis(Fantone and Ward, 1982). While antioxidant treatment consistently protects cells from lipid peroxidation caused by fluoride exposure, suggesting that oxidative damage is the major mode of action of fluoride.

Melatonin(N-acetyl-5-methoxytryptamine) is an endogenously produced indoleamine, which is extensively existed in animals, plants and bacterial. Melatonin has been proved to be a powerful direct free radical scavenge. The antioxidant effects of melatonin including single electron transfer, hydrogen transfer, and radical adduct formation (Tan

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http://dx.doi.org/10.1016/j.etap.2017.06.014
Received 5 March 2017; Received in revised form 10 June 2017; Accepted 19 June 2017
Available online 29 June 2017

Table 1
Protective effect of melatonin on the development of preimplantation mouse embryos against NaF-induced oxidative injury.

Groups	No. of oocytes cultured	GV stage	No. of embryo(%)		
			MII stage	Two-cell stage	Blastocyst stage
Control	358	343(96.2)	305(85.3)	190(78.1)	223(62.3)
NaF	371	322(87.1)	181(48.8)*	113(35.2)**	99(26.7)**
NaF + Mel	376	345(92.5)	286(76.2)*	252(67.2)**	198(52.7)**
H_2O_2	184	162(88.1)	115(62.5)*	97(52.7)**	45(24.5)**
Ascorbate	202	198(98.1)	177(88.2)	162(80.6)	132(65.3)

Differences between the groups were calculated using the x2-test.
Mel: Melatonin.
*P < 0.05, ** P < 0.01, *** P < 0.001 versus the GV stage number of oocytes.

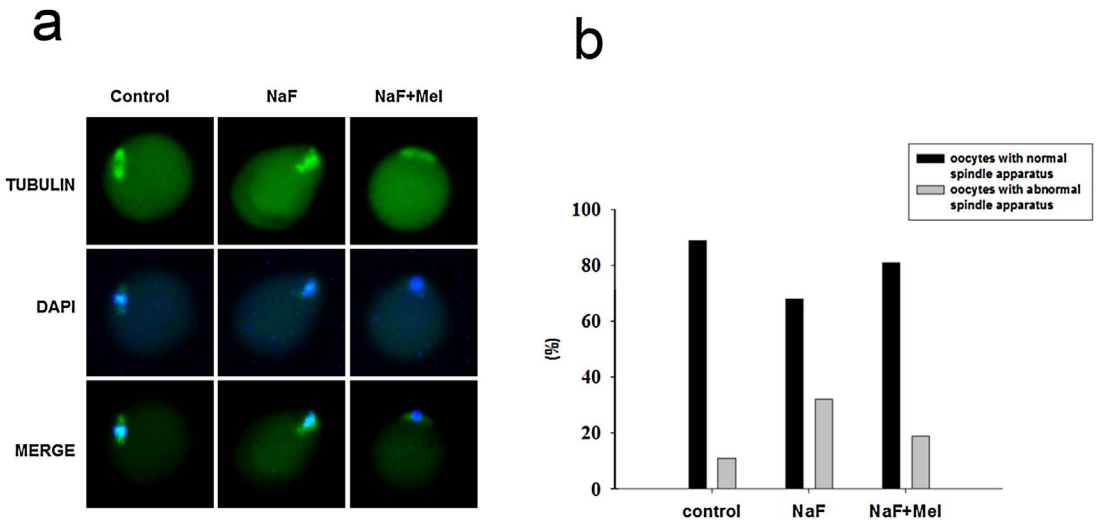

Fig. 1. Protective effect of melatonin on the development of preimplantation mouse embryos against NaF-induced oxidative injury. (a) Representative images of normal or abnormal chromosomes and spindle apparatus. A normal spindle apparatus was characterized as having chromosomes located exclusively on the equatorial plate and a barrel-shaped structure. (b) Percentage of oocytes with normal or abnormal spindle apparatus.

et al., 2012). Ramis et al. found that melatonin can directly scavenge the highly toxic ·OH in vivo. Besides directly remove ROS, melatonin also stimulates antioxidant enzymes, suppresses pro-oxidant enzymes (Ramis et al., 2015). In addition, melatonin as a lipophilic molecule, can be accumulated in high concentrations in mitochondrial, make it one of the most important factors to clear the ROS in mitochondrial (Manchester et al., 2015; Ramis et al., 2015).

Thus, elimination of NaF-induced oxidative stress in the development of mouse preimplantation embryos by melatonin seems feasible. However, little research has been designed to explore the beneficial effect of melatonin on NaF-induced oxidative damage in preimplantation embryo. In this study, we found that melatonin can protect mouse oocytes from NaF-induced morphological development injury, growth retardation, oxidative stress and apoptosis.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict accordance with the guidelines for the care and use of animals of Northwest A & F University. All animal experimental procedures were approved by the Animal Care Commission of the College of Veterinary Medicine, Northwest A & F University. Every effort was made to minimize animal pain, suffering, and distress and to reduce the number of animals used.

2.2. Reagents

All reagents were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated.

2.3. Animals and collection of oocytes

Kunming mice (7–10 weeks old) used for this experiment were purchased from Xi'an Jiaotong University. Feeding in the room temperature animal room, keep 12 h light and 12 h dark cycle and provide free access food and water. Injection female mice of 10 IU pregnant mare serum gonadotropin (PMSG; Sansheng; Ningbo, Zhejiang, PRC) to super-ovulated and inject 10 IU human chorionic gonadotropin (hCG; Sansheng; Ningbo, Zhejiang, PRC) follow 46–48 h later. Super-ovulated female was selected and humanely sacrificed by cervical dislocation. Collected GV stage oocytes from the oviduct ampullae, digested with 1 mg/ml hyakuronidase to remove the cumulus cells which aroud oocytes and culture in KSOM.

2.4. Medical treatment

Selected one group of the obtained oocytes exposed to different concentrations of NaF (0–0.8 mM) for 4 h, and hydrogen peroxid (0.35 mM) (Yu et al., 2014) for 4 h as positive control, another group exposed to 0.5 mM NaF, melatonin (50–100) and added ascorbate acid 50 μM(Kere et al., 2013) for 4 h the same in positive control group, washed extensively and cultured in KOSM for 12 h. Control group cultured in KSOM for 12 h. Select a part of three group oocytes in vitro fertilization, and cultivation to the blastocyst stage.