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Toxoplasma gondii isolate with genotype Chinese 1 triggers trophoblast apoptosis through oxidative stress and mitochondrial dysfunction in mice

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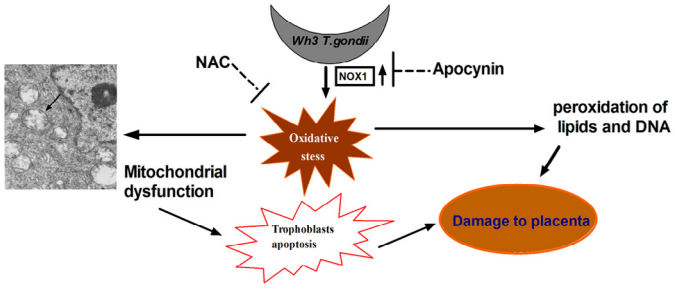
HIGHLIGHTS

- Maternal infection with Wh3 *Toxoplasma gondii* induces trophoblast apoptosis.
- Wh3 strain promotes ROS production and peroxidation damage to placental tissues.
- Generation of ROS in Wh3 strain infection is mainly through NADPH oxidase.
- Wh3 infection contributes to oxidative stress-induced mitochondrial dysfunction.

GRAPHICAL ABSTRACT



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ABSTRACT

Congenital toxoplasmosis may result in abortion, severe mental retardation and neurologic damage in the offspring. Placental damage is considered as the key event in this disease. Here we show that maternal infection with *Toxoplasma gondii* Wh3 isolate of genotype Chinese 1, which is predominantly prevalent in China, induced trophoblast apoptosis of pregnant mouse. PCR array analysis of 84 key genes in the biogenesis and functions of mouse mitochondrion revealed that ten genes were up-regulated at least 2-fold in the Wh3 infection group, compared with those in the control. The elevated levels of reactive oxygen species (ROS), malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG), as well as the decreased glutathione (GSH), were observed in the infected mice. The mRNA levels of NADPH oxidase 1 and glutathione peroxidase 6 (GPx6) were significantly increased. The production of excessive ROS was NADPH oxidase-dependent, which contributed to mitochondrial structural damage and mitochondrial dysfunction in placentas, followed by the cleavage of caspase-9 and caspase-3, and finally resulted in apoptosis of trophoblasts. All the above-mentioned phenomena were inhibited by pretreatment with the antioxidant of *N*-acetylcysteine (NAC). Taken together, we concluded that Wh3 infection during pregnancy

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may contribute to trophoblast apoptosis by oxidative stress-induced mitochondrial dysfunction and activation of the downstream signaling pathway.

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1. Introduction

Reactive oxygen species (ROS) are free radicals containing one or more unpaired electrons which can damage a wide variety of biomolecules and cell structures. ROS generation exerts a bactericidal function in specialized phagocytic cells (El-Benna et al., 2008). However, the imbalance in ROS production and antioxidant capacity could bring out oxidative stress (OS), which has been considered as one of the major factors causing cell apoptosis (Ueda et al., 2002). Improving evidences suggest the important roles of OS in microbe-induced cell apoptosis, such as gram-negative bacteria, *Toxoplasma gondii*, *Fasciola hepatica*, as well as in placental-related diseases (Nishikawa et al., 2007; Pal et al., 2010; Sebai et al., 2011; Siemieniuk et al., 2008; Wang and Hirsch, 2003). Apoptosis is a normal physiological phenomenon in trophoblast cells throughout gestation, and is vital for normal placental development and fetal growth (Huppertz et al., 2006). The apoptotic process of trophoblasts might be induced or inhibited by many stimuli, for example, trophoblast apoptosis is greatly increased in the case of spontaneous abortion (Kokawa et al., 1998). OS and apoptosis can happen simultaneously in a gestation-related disease.

T.gondii is a ubiquitous parasitic protozoan responsible for abnormal pregnant outcomes, when pregnant women are primo-infected. Because the placenta plays a crucial role in prevention and expression of fetal disease (Abbasi et al., 2003), the severity of congential toxoplasmosis correlates with the extent of placental damage. Placental OS in the patients is closely associated with unexplained intrauterine growth restriction and early onset preeclampsia (Burton et al., 2009), while OS plays an intermediary part in the generation of these syndromes (Redman and Sargent, 2005). Previous studies demonstrated that LPS, an active component of gram-negative bacterial cell wall, could induce OS in several tissues, leading to preterm labor in mice (Wang and Hirsch, 2003). Components similar to LPS have been found in *T.gondii*, such as glycosyl phosphatidylinositol (GPI) (Hunter and Sibley, 2012). More interestingly, the effector dense granule protein 15 (GRA15) carried by type II strain of *T. gondii* directly activates NF-κB and drives host macrophages to polarization of classically activated macrophage (caM), which generates ROS and nitric oxide (NO) responsible for OS (Melo et al., 2011; Rosowski et al., 2011). The action of OS, however, in congenital birth defects caused by *T.gondii* remains unknown.

Mitochondria perform important cellular functions, including calcium regulation, ATP production, the control of apoptosis, alteration of the reduction-oxidation potential, and free-radical scavenging (Reddy, 2006). Mitochondria are both the most important endogenous source of ROS production and the major target for ROS-induced cellular injury. OS-mediated damage to mitochondrial DNA (mtDNA) can lead to a vicious circle of ROS production and further mtDNA damage, which might be a key mechanism that has been implicated in the pathogenesis of bacterial infection (Galley, 2011; Kuwabara and Imajoh-Ohmi, 2004). The progression of systemic inflammatory response can result from the cellular damage and induced cell death, which partially depends on mitochondrial dysfunction characterized by elevated production of ROS, increased membrane permeability and eventual release of cell death mediators, such as Cytochrome c (Cyt.c) (Exline and Crouser, 2008). Generally, pregnancy is often accompanied by mild OS arising from increased placental mitochondrial activity and ROS generation for maternal and fetal metabolism (Jauniaux et al., 2000). Our previous study showed that RH *T.gondii* infection deteriorated OS of placenta, and contributed to the increased ROS production, cell apoptosis and placental damage. Genotyping of *T.gondii* isolates from animals and humans indicated that Chinese 1 is dominantly circulating in China, which shares the polymorphisms of ROP16I/III and GRA15II with types I, III, and II strains (data not shown here). Among the genotype Chinese 1, Wh3 exhibits high virulence similar to type I RH strain (Chen et al., 2011, 2012; Jiang et al., 2013).

To unveil the underlying mechanisms of miscarriage caused by *T. gondii* Chinese 1 strains, we set up the pregmant mice model infected with Wh3 and explored the putative role of ROS in Wh3 infection by using the ROS quencher, *N*-acetylcysteine (NAC), and specific NADPH oxidase inhibitor (apocynin). Additionally, attention was focused on damage to mitochondrial structure and function, as well as activation of the downstream pathways.

2. Materials and methods

2.1. Materials

The Mouse Mitochondria RT^2 Profiler™ PCR Array kits were obtained from SABioscience Company (Frederick, MD, USA). Annexin V-EGFP/PI kit for apoptosis assay was obtained from BestBio (Shanghai, China). TUNEL kit for apoptosis detection *in situ* was purchased from Roche Diagnostics (Mannheim, Germany). *N*-acetylcysteine (Sigma, St. Louis, MO) was reconstituted in physiologic saline solution. 4′-Hydroxy-3′-methoxyacetophenone (apocynin) and anti-caspase primary antibodies were obtained from Santa Cruz Biotechnology (Shanghai, China). Commercial assay kits for MDA and GSH were produced by the Jiancheng Institute of Biotechnology (Nanjing, China); the kit for 8-OHdG was obtained from Cell Biolabs (San Diego, CA). The kits for caspase-3, caspase-8 and caspase-9 activity, and tissue mitochondria isolation kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Anti-alpha tubulin, anti-Cyt.c, anti-Cyt.c oxidase IV (COX IV), anti-NADPH oxidase 1 (NOX1) antibodies and the horseradish peroxidase-conjugated secondary antibody to rabbit were obtained from Abcam Ltd (Hong Kong, China). The Super Signal West Pico ECL kit was purchased from Thermo Scientific (Beijing, China). Cell culture media and reagents were obtained from Invitrogen (Shanghai, China).

2.2. Mice model and treatment

Wh3 *T.gondii* was maintained in BALB/c mice by intraperitoneal passage at 72h intervals. Eighty CD1 female and forty male mice were maintained in the animal facility of the Laboratory Animals Centre of Anhui Medical University, with a 12h light/dark cycle. The Ethics Committee for Animal Research of Anhui Medical University approved the study (No. AMU26-08061). All animal care and experimental procedures were in accordance with the Chinese National Institute of Health Guide for the Care and Use of Laboratory Animals. Females were caged overnight with males (4:2) and successful mating was verified next morning. The presence of a vaginal plug was designated day 0 of gestation (GD0). Treatments of pregnant females were performed as described previously (Liu et al., 2013). Briefly, all pregnant females were grouped into Wh3 infection, NAC pretreatment and control groups randomly. On GD8, the mice were intraperitoneally injected with 200 tachyzoites in 0.1 ml 0.9% sterile saline solution (Wh3 infection and NAC pretreatment groups), or only 0.9% sterile saline solution (the control group). Additionally, on GD8 and GD9, mice in the NAC pretreatment group were intraperitoneally infused with NAC (100 mg/kg of body weight).

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