THIAMETHOXAM INHIBITS BLASTOCYSTS EXPANDING AND HATCHING VIA ACTIVATION OF ROS-INDUCED DNA DAMAGE CHECKPOINT IN PIGS

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Introduction

Thiamethoxam (TMX) is a neonicotinoid insecticide. It has specific high toxicity to insects. So far, residues of TMX have been detected in rice hull, bran, and polished rice grains. The quantified values are greater in hull and in rice bran. Early embryo quality is vital for fertility. Overmuch production of ROS can override an embryo's antioxidant defenses producing oxidative stress and triggering apoptosis, necrosis and/or permanent DNA-damage response (DDR) in the developing early embryo. Relative studies point that TMX hepatotoxicity is significant to mammals in acute test, but it is unknown on accumulated chronic toxicity to early embryo development of mammals. Therefore, it is necessary to make it clear.

Materials and Methods

The embryos were obtained by *in vitro* maturation of oocytes, parthenogenetic activation and *in vitro* culture early embryo during which embryos were exposed to TMX. Localization or relative content of TUNEL, BrdU and DNA damage checkpoint components were tested by immunofluorescent staining. Genes expression related with ROS and hatch were quantified by RT-PCR. The ROS content was quantified using the DCHFDA method. Activity of $p34^{cdc2}$ was tested with MESACUP CDC2 kinase assay kit. Data were analyzed using SPSS software.

Results and Discussion

Expanding and hatch of blastocysts treated with TMX decreased by 21.73% and 16.71% compared with control, respectively. The rate of cell proliferation decreased by 44.33% compared with expanding blastocyst of control. Increased ROS, γH2AX and mRNA of *sod1*, and decreased transcription of *Mnsod* and *Gpx1* appeared in TMX group. In addition, activity of MPF decreased by 31.41% in expanding blastocysts. These data indicate that TMX has a bad impact on blastocyst quality. In conclusion, TMX inhibits blastocysts expanding and hatching by ROS-induced DNA damage checkpoint activation, which is consistent with relevant studies in zebrafish embryos.