Original Article

Effects of Tempol on in vitro Development of Mouse Embryos under Oxidative Stress

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Abstract

Background: Etiologically, oxidative stress can be considered as one of the reasons for defective embryonic development which leads to developmental arrest due to necrosis or apoptosis. Under in vivo conditions, multiple mechanisms act to protect the embryo against reactive oxygen species (ROS), but under in vitro conditions most of these mechanisms are absent leading to higher levels of ROS in the culture medium. The objective of this study was to compare the antioxidant effects of Tempol, 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl, a permeable synthetic antioxidant, on mouse pre-implantation embryonic development in vitro conditions in the presence or absence of oxidative stress.

Methods: Mature oocytes from mouse were retrieved following ovarian stimulation by the administration of Pregnant Mare Serum Gonadotropin (PMSG) and hCG. Upon in vitro fertilization, the zygotes were cultured in different groups in HTF medium containing 4 *mg/ml* BSA. To study the effects of oxidative stress on embryo development, the zygotes were cultured for an hour in a medium containing different concentrations of H_2O_2. After washing, the zygotes were transferred to the culture plate. The zygotes were later placed in the media containing different concentrations of Tempol following their culture in 10 μM H_2O_2 for one hour to study the effects of different concentrations of the substance in the absence of other oxidative stresses. The data were later compared and statistically analyzed.

Results: The pre-implantation embryonic development decreased significantly in the case group, compared to the control group after a short exposure to H_2O_2, – the effect being more noticeable in higher concentrations. Tempol reduced the impairments resulting from the oxidative stress to some extent. Under in vitro conditions and a concentration of 0.5 μM, Tempol improved embryonic development quality, quantitatively and morphologically. Tempol increased the percentage of two-cell embryos from 91.78% in the control group to 96.99% ($p < 0.05$), blastocysts from 67.80% in the controls to 81.33% ($p < 0.05$) in the cases, and significantly decreased embryonic arrest from 32.19% in the controls to 18.67% in the cases ($p < 0.05$).

Conclusion: ROS has a major role in embryonic arrest, witnessed in embryo cultures in vitro conditions. The present study showed that supplementation of embryo cultures with Tempol improved the embryonic development. It seems that addition of permeable synthetic antioxidants, such as Tempol, to embryo cultures could protect embryos from oxidative damage and improve embryonic development.

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