

An Ultrastructural Study on the Apoptotic Features of Spermatogenic Cells following Busulfan Treatment in Adult Mice

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Abstract

Introduction: Chemotherapy has adverse effects on spermatogenesis and results in reversible or irreversible oligozoospermia or azoospermia. Little is known about the ultrastructural alterations of male germ cells following busulfan administration, as an anti-cancer drug. The present study aimed to study the ultrastructural alterations or apoptotic features of male mouse germ cells, following administration of various doses of busulfan.

Materials & Methods: This study was performed on thirty-two 8-week-old adult male mice. Animals were divided into four groups. The mice on the control group were treated with DMSO and in the second, third and forth groups were treated with 10mg/kg, 20mg/kg and 40mg/kg busulfan, respectively. Busulfan was injected as a single dose intraperitoneally. Thirty-five days after the treatment, the mice testes were dissected and prepared for apoptosis study by transmission electron microscopy (TEM) and TUNEL assays. The results were analyzed by ANOVA test using SPSS software and a $p < 0.05$ was regarded statistically significant.

Results: A single dose of busulfan induced ultrastructural features of apoptosis in all the experimental groups such as: chromatin marginalization of germ cell nuclei, especially in spermatogonia, deformation of nuclei, separation of germ cells and presence of large spaces between adjacent cells, cell shrinkage, occasional presence of vacuoles in germ cells and apoptotic bodies in sertoli cells. Ultrastructural features of apoptosis were more frequent in spermatogonia and primary spermatocytes. Apoptosis was observed less frequently in the control group ($2.77 \pm 0.57\%$), particularly in primary spermatocytes. However, the number of apoptotic cells in 10mg/kg and 20mg/kg busulfan treated groups were ($58.23 \pm 7.32\%$ and $54.09 \pm 6.23\%$) significantly higher than the number of apoptotic cells in 40mg/kg treated group ($28.16 \pm 5.67\%$), ($p < 0.001$).

Conclusion: Administration of a single dose of busulfan in various doses of 10mg/kg, 20mg/kg and 40mg/kg, induces apoptosis in male germ cells, especially in spermatogonia and primary spermatocytes. Degree of apoptosis in male germ cells in busulfan treated groups is not dose dependent. Fewer number of apoptotic cells in 40mg/kg busulfan treated group may be due to more declines in the number of germ cells in comparison to 10 and 20mg/kg busulfan treated groups.

Key Words: Busulfan, Chemotherapy, Germ cell, Apoptosis, Spermatogenesis, Azoospermia, Sertoli cell, Testis.

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