Investigating the protective effects of *dexamethasone* against *cisplatin-induced* damage in a Spermatogonial Stem Cell *(SSC)* model assessed using flow cytometry

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INTRODUCTION

- Childhood cancer survival has improved, but infertility is a major long term side effect
- Cisplatin is highly gonadotoxic, damaging SSCs, which are essential for lifelong sperm production.
- Prepubertal boys can not bank sperm and rely on their SSC pool for future fertility
- Glucocorticoids may offer chemoprotective benefits by reducing apoptosis through activation of the glucocorticoid receptor (GR)

AIMS

This study investigates dexamethasone, a GR agonist, as a potential chemoprotectant against cisplatin-induced spermatogonial damage.
Dexamethasone was used as a co-treatment for 4 hours directly alongside cisplatin for and as a pre-treatment 20 hours prior to cisplatin exposure.

METHODOLOGY

Mouse-Derived Type B Spermatagnonial Stem Cell Line

Co-Treatment → 4-hour Dexamethasone *(1 μM)* + Cisplatin co-exposure *(0.39 μM-25.00 μM)*
Pre-Treatment →20-hour single Dexamethasone *(1 μM) pre-*exposure & 4-hour single cisplatin exposure *(0.39 μM-25.00 μM)*

Day 1:
- Cells detached from culture flasks using TrypLE, resuspended in complete media,
- Viable cells counted via trypan blue and haemocytometer.
- Cell suspension prepared at 20,000 cells/mL; ~8,000 cells seeded per well in 24-well plates.
- Cells incubated 24 hours to allow adherence (incubation with chemoprotectant for pre-treatment exposure was added if required)

Day 2:
Cells at ~50% confluency treated with:
1. Cisplatin alone (to assess chemotherapy toxicity)
2. Dexamethasone alone (to examine chemoprotectant effects)
3. Combination (cisplatin + dexamethasone, to test protective effects)
4. Untreated control (baseline)

Day 3:
Cells harvested using TrypLE, resuspended in buffer with serum, and centrifuged.
Resuspended cells were stained with:
- Hoechst 33342 – DNA content and cell cycle analysis
- Annexin V – early apoptosis
- DRAQ7 – necrotic/dead cells
Samples transferred to flow cytometry tubes for acquisition and analysis.

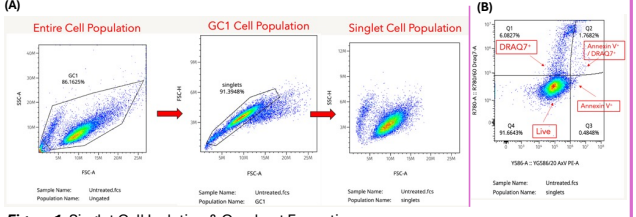

Figure 1: Singlet Cell Isolation & Quadrant Formation
(A) Flow Cytometry gating strategy for singlet cell population isolation
(B) Identification of Early Apoptotic (Annexin V$^+$) / Late Apoptotic (Annexin V$^+$/DRAQ7$^+$) & Necrotic (DRAQ7$^+$) cell populations in singlets by flow cytometry

RESULTS

During early apoptotic stages, dexamethasone presented protective effects at various cisplatin concentrations

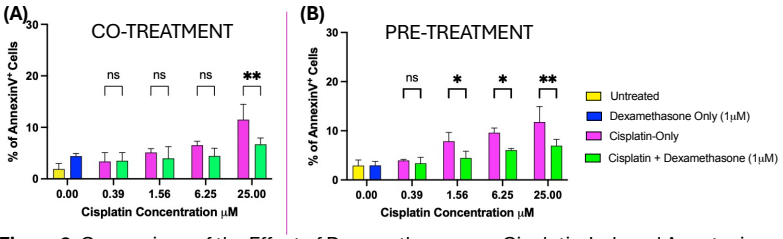

Figure 2: Comparison of the Effect of Dexamethasone on Cisplatin-Induced Apoptosis During **(A)** 4-hour Co-Treatment (n=3) **(B)** 20-hour Pre-Treatment (n=3) Window
* p < 0.032 ** p < 0.0018

Cisplatin exerts its cytotoxic effects by binding to DNA and forming cross links, which distort the DNA helix. Hoechst dye works by binding to the minor grooves of the DNA.

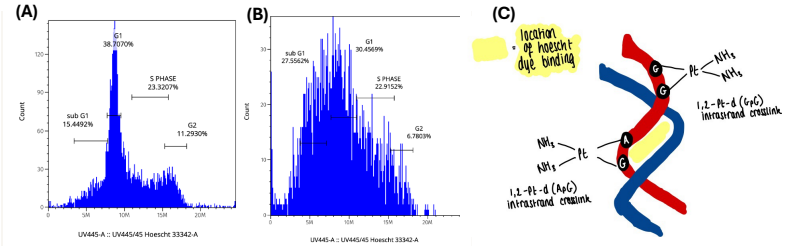

Figure 3: Cell cycle analysis using Hoechst staining was inconclusive for both co- and pre-treatment groups, as no distinct sub-G1 population could be identified in cells that had been treated with cisplatin. Cisplatin forms interstrand cross-links, affecting the shape of the DNA helix. Hoechst is then unable to correctly bind. Untreated & Dex-Only treated had expected profiles.
(A) Untreated sample with a distinct sub-G1 peak
(B) Cisplatin-Only sample @ 6.25 μM with no distinct sub-G1 peak
(C) Typical site of Hoechst binding at highlighted area

Treatment with dexamethasone alone revealed a dose-dependent increase in cells in the Sub-G1 phase of the cell cycle

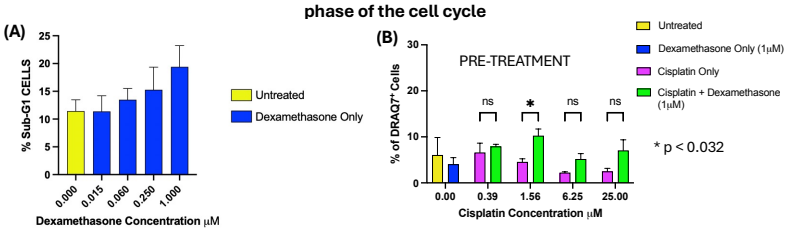

Figure 4: At 1 μM dexamethasone, the highest proportion of cells was observed in the sub-G1 population. High Sub-G1 population indicates increased cell death. Pre-treatment exposure with 1μM chemoprotectant found increased necrosis in combination treatment group.
(A) Percentage of cells in the Sub-G1 phase of the cell cycle
(B) Effect of dexamethasone on cisplatin-induced necrosis during 20-hour treatment window

CONCLUSIONS

- Dexamethasone can partially protect SSCs from cisplatin-induced damage by reducing early apoptosis.
- Cell cycle analysis with Hoechst staining was inconclusive due to cisplatin-induced DNA distortion.
- Prolonged dexamethasone exposure at high concentrations may drive stressed cells toward necrosis.

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