The glucocorticoid (GC) dexamethasone plays a key role in the treatment of Acute Lymphoblastic Leukaemia (ALL).

Despite good prognosis in childhood ALL, treatment failure due to therapy resistance is still a problem. GC response is also a key prognostic indicator of outcome. However, mechanisms underlying GC resistance are poorly understood [1].

The PreB697 cell line shares a number of features of primary patient ALL samples, making it a good model to investigate resistance.

Previous studies in PreB697 cells have examined a number of possible GC resistance mechanisms [2,3] (figure 1), but not addressed whether decreased intracellular dexamethasone could cause resistance.

Improved understanding of GC resistance mechanisms could aid the development of therapies for its pharmacological reversal.

To determine whether variation in intracellular dexamethasone levels could contribute to dexamethasone resistance in an ALL cell model

3. Methods

Initially, the relative sensitivity to dexamethasone of the parental 697 cells and GC resistant sub clones (created under GC selection pressure [3]) was confirmed with alamar blue drug sensitivity assays.

An LCMS assay was developed and validated for analysis of dexamethasone concentrations in human plasma and cell lysates.

For measurement of intracellular dexamethasone concentrations, Cells were seeded 36 hours prior to incubation with 100-1000nM dexamethasone over 4 hours or 500nM dexamethasone over 1-2 hours. Cell pellets were lysed by acetonitrile and centrifugation at 16,000g. Dexamethasone concentrations were determined using LCMS.

The LCMS was performed on an API Q Trap 3200 LC/MS/MS with a Gemini 3μ C18 110A column (50x3mm). Mobile phases were (A) 0.1% formic acid and (B) acetonitrile.

4. Results I: Sensitivity to dexamethasone differed significantly between parental 697 cells and GC resistant sub clones

Figure 3: Intracellular dexamethasone concentration (pmol/10^6 cells) after incubation with clinically relevant concentrations of dexamethasone over 4 hours. Results presented are ±SEM from 3 experiments and are compared to parental 697 cells (blue) under the same experimental conditions. There was no difference between any cell line and its 697 comparison (p=0.056 two way ANOVA with Sidak correction for multiple comparisons).

Figure 4: Intracellular dexamethasone (AUC pmol/10^6 cells.h) after incubation with 500nM dexamethasone over 8 hours. Results presented are ±SEM from 3 experiments and are compared to parental 697 cells (black) under the same experimental conditions. There was no statistically significant difference between any of the cell lines (p=0.45 students t test).

6. Conclusions

- Intracellular dexamethasone concentrations did not differ between GC sensitive and resistant cells and therefore cannot explain the GC resistance seen in the ALL PreB697 cell line model.

- Further investigation is needed to determine whether this finding is relevant to resistance in patients, where extracellular factors could also impact on intracellular dexamethasone levels.

References