

Evaluation *in vitro* of glucocorticoid sensitivity in transplant patients

Yan Chen, M.D., Ph.D.^{1,2}, Vera Pravica, M.D., Ph.D.^{1,2} and Ian V Hutchinson, Ph.D.^{1,2}

¹National Institute of Transplantation, Los Angeles, CA and ²University of Southern California, Los Angeles, CA.

Introduction and aims

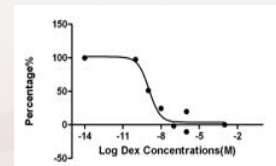
There is currently a lack of predictive methods to define glucocorticoid (GC) sensitivity, which is extremely variable among individuals. In particular, identifying patients in whom steroids will have immunosuppressive and toxic activities is important in transplantation. Therefore we have sought to establish an *in vitro* pharmacokinetic assay to investigate leukocyte proliferation and cytokine production, with the intention of applying such a test in transplantation.

Methods

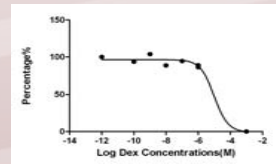
Peripheral blood mononuclear cells (PBMCs) were obtained from 21 healthy volunteers (n=21). These cells were stimulated to proliferate *in vitro* using PMA/ionomycin. Dexamethasone (DREX) was titrated into such cultures. Proliferation was measured by BrdU incorporation. Synthesis of cytokines (IL-2, IFN- γ , IL-4 and TNF- α) was measured using specific ELISA assays.

Individuals showed big differences in suppression of proliferation by DEX, and could be classified into GC sensitive, intermediate and resistant groups by equally dividing the study cohort. Therefore the defining IC₅₀ corresponds to 2x10⁻⁷M and 5x10⁻⁵M Dex, respectively. The dosage vs. Dex response curves of a GC sensitive and resistant individual are exemplified on Figure 1. A strong correlation was found between the parameters I_{max} and LogIC₅₀ (Pearson's R = -0.84, P=0.000). Validation experiments demonstrated excellent inter- and intra-assay reproducibility. The GC-sensitive individuals expressed significantly lower levels of IL-2, IL-4 and TNF- α than the insensitive ones (P<0.05), while there was no difference in the expression of IFN- γ between GC sensitive and resistant groups (Table 1).

Results



a



b

Figure 1: Dose-response curves from (a) a steroid-sensitive individual (LogIC₅₀=-8.9/IC₅₀=1.2x10⁻⁹ R²=0.95) and (b) steroid-insensitive individual (LogIC₅₀=-5.0/IC₅₀=9.9x10⁻⁶ R²=0.98). DEX concentrations are shown on the X axes by the logarithmic values. PBMC proliferation percentages are presented on the Y axes.

Table 1: Comparison of mean cytokine production between the steroid sensitive (n=7) and insensitive groups (n=7)

TNF α	Sensitive	Insensitive	P value (2-tailed)
10 ⁻⁹ Dex	27.7%	32.2%	0.40
10 ⁻⁷ Dex	31.7%	52.1%	0.02*
10 ⁻⁶ Dex	78.3%	85.7%	0.47
IL-2	Sensitive	Insensitive	P value (2-tailed)
10 ⁻⁹ Dex	38.9%	60.4%	0.03*
10 ⁻⁷ Dex	57.5%	72.3%	0.11
10 ⁻⁶ Dex	79.9%	97.1%	0.06
IFN- γ	Sensitive	Insensitive	P value (2-tailed)
10 ⁻⁹ Dex	55.2%	56.0%	0.95
10 ⁻⁷ Dex	63.7%	60.0%	0.77
10 ⁻⁶ Dex	97.4%	94.2%	0.83
IL-4	Sensitive	Insensitive	P value (2-tailed)
10 ⁻⁹ Dex	12.0%	17.7%	0.44
10 ⁻⁷ Dex	23.8%	34.6%	0.36
10 ⁻⁶ Dex	60.8%	82.3%	0.01*

*P<0.05

Discussion

Tests on PBMC proliferation and cytokine production *in vitro* have been used to investigate individual GC sensitivities. Here, the *in vitro* pharmacokinetics of Dex suppression in the proliferation assay divided the individuals according to Dex sensitivity and this was reflected by suppression of cytokine synthesis. Of note, IFN- γ expression was not related neither to DEX dose nor to the production of other cytokines, perhaps because of the gene promoter lacks an NF-B motif. The steroid level achieved in patients is about 10⁻⁶ to 10⁻⁸M. Analysis of GC sensitivity using an *in vitro* assay prior to initiation of steroid therapy may help clinicians make a rational choice of immunosuppressant agent and appropriate dose, thereby limiting the unnecessary exposure of patients to an agent with numerous side effects.