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MULTIPARAMETRIC EFFECT: CONCENTRATION ANALYSES.

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1. ABSTRACT

Immunosuppressant drug toxicity currently competes with acute rejection, as the major cause of efficacy failure of potent new agents in clinical transplantation. The development of mechanistic drug targets as surrogate endpoints for use in the clinic has been facilitated by fluorescent imaging techniques which measure multiple cytokines and cell surface receptors on stimulated (peripheral blood) lymphocyte responses. However, the promise of delivering customized drug therapy to the transplant recipient remains unfulfilled. In this brief review, computational algorithms that can relate multiparametric effects to clinical drug concentrations of immunosuppressants are discussed. Based on Hill equations, these pharmacodynamic modeling techniques have been used to simulate single-agent effects, combination regimen effects, as well as the individual response to combination regimens. The potential implications of these models crystallize the clinical challenges confronting practitioners of clinical, post-transplant immunosuppression.

2. INTRODUCTION

In recent years, considerable effort has been directed in the field of organ transplantation toward generating qualitative information that can classify individuals as either "immune responders" or "non-responders". For the most part, this effort comprises extended genotyping, and characterizes inherited traits of transplanted subjects (1). With the availability of potent new drugs, an increasing appreciation of life-threatening side effects has stimulated interest in measuring drug effect in hopes of delivering customized immunosuppression. This attitude represents a convergence brought about by diagnostic platforms, which can measure cell distribution and function using multiple phenotypic markers, and by the recent availability of a diverse array of computational modeling algorithms that can relate multiparametric phenotypic data to clinical endpoints.

The following sections review analytic approaches previously reported by us (2-4). They have been used to relate the output from multiparametric flow cytometry, to immunosuppressant concentrations, and to the stable post-transplant course. The input consists of the measured expression of cytokines and cell surface markers on immunologically competent peripheral blood cells (PBL, peripheral blood lymphocytes) subjected to mitogenic stimulation. These have been conducted in the presence of various concentrations of immunosuppressants, alone and in combination.

The analytic approaches have been grouped to address clinical need of monitoring single agents, two agents used in combination, and determining individual variation in response to two immunosuppressants in combination.

3. MODELING THE EFFECT OF A SINGLE IMMUNOSUPPRESSANT

Calcineurin inhibitors, represented by Cyclosporine (CSA) and Tacrolimus (TAC) are potent immunosuppressants that inhibit gene transcription of cytokines such as interleukin-2 (IL-2). This gene transcription results from activation of the enzyme calcineurin phosphatase in response to transplanted antigen (5). Because intracellular IL-2 content can be measured easily in T-cells subjected to stimulation with mitogens such as phorbol-myristic acid (PMA)-Ionomycin, this assay system was used to evaluate the frequency of T-helper cells, that expressed intracellular IL-2 in the absence or presence of varying amounts of CSA in 13 renal transplant recipients (6). To this end, whole blood samples were obtained before transplantation, and at the end of the first post-transplant week, immediately before and 2 hours after the morning dose of CSA. The results plotted in Figure 1 suggested a non-linear relationship. This non-linear relationship was modeled by modified Hill equations relating drug concentration to effect as follows:

$$E=(E_0+((E_{max}-E_0)*C)/(EC_{50}+C)$$

Where E=effect, E_0=effect at drug concentration=infinity, E_{max}=effect at drug concentration (C) zero (7). The effect: concentration threshold was the EC_{50}, or the CSA whole

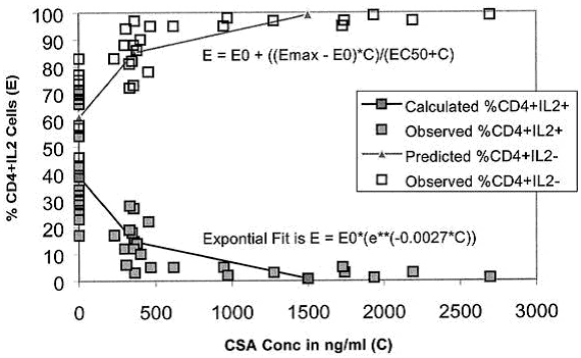

Figure 1. Pharmacodynamic relationship between effect (E) and concentration (C) predicted by the exponential fit as well as by the Emax model. Although E can be expressed as either $\%CD4^{+}IL\text{-}2^{+}$cells or $\%CD4^{+}IL\text{-}2^{-}$ cells, EC_{50} prediction in both models was based on E represented by $\%CD4^{+}IL\text{-}2^{-}$ cells. The predicted EC_{50} was 250 ng/ml by exponential fit, and 249 ng/ml by the E_{max} model.

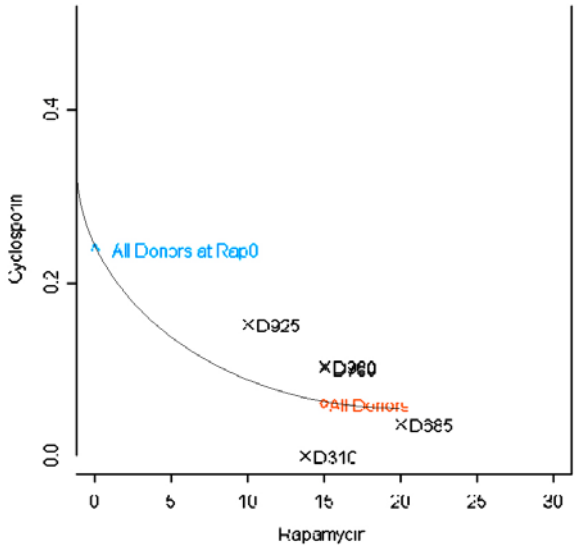

Figure 2. Mean concentrations of CsA alone (triangles) and with SRL (circle) associated with half-maximal inhibition of CD54 in the population of 5 normal human subjects are shown below. Concentration mixtures of the two drugs, which seek to minimize exposure to CsA are also shown for each of the 5 subjects.

blood concentration in ng/ml, which resulted in half-maximal reduction in the frequency of CD4+IL-2+ cells.

The relationship could also be defined by an exponential fit:

$$E=E_0*(e^{**}(-0.00027*C))$$

to yield an EC_{50} which was virtually identical to that obtained by the modified Hill equation (250 versus 249 ng/ml, respectively, Figure 1). In both cases, the predicted EC_{50} of ≅ 250 ng/ml CSA represented a trough concentration targeted by transplant practitioners, as a therapeutic amount of CSA in the first post-transplant month. These models have also been referred to as E_{max} pharmacodynamic (PD) models (Winnonlin, Pharsight, Palo Alto, CA., reference 7).

4. MODELING THE EFFECT OF A COMBINATION REGIMEN

In clinical practice, immunosuppressants are usually given in combinations of two or more agents to achieve optimal anti-rejection effect. The CI class of agents can be titrated to desired concentration targets with commercially available immunoassays (Abbott Diagnostics, Chicago, Ill.). However, when they are combined with the antiproliferative class of immunosuppressants, there is an enhanced likelihood of drug toxicity. This has been seen in clinical trials, in which Sirolimus (SRL) or Mycophenolate Mofetil (MMF), two members of the antiproliferative class, were added to CSA to prevent rejection in primary renal transplantation (8-10). Although the incidence and severity of rejection was lowered, patients receiving these two newer agents experienced more toxicity during phase III evaluations. To this date, neither agent is monitored routinely in clinical practice, largely due the lack of an easily accessible immunoassay, and to a practice pattern in which antiproliferative drugs have been administered on the basis of dose thresholds. This represented to us an opportunity to develop a composite measure of immunosuppression for combinations incorporating these two classes of immunosuppressants, as well as to evaluate individual variation in response to combination immunosuppression (4).

The combination was simulated by adding multiple concentrations of CSA and SRL, alone and in combination, to whole blood from five healthy human subjects. As described previously, several costimulatory receptors on the surface of pokeweed mitogen-stimulated B-cells were inhibited in a concentration-dependent manner by the CI and the antiproliferative drugs, alone and in combination. The basic approach to analyzing the data by the modified general empiric E_{max} model for multiple drugs is:

$$\%inhibition=100-[E_{max1}*([CSA]/EC_{50SRL})+E_{max2}*([SRL]/EC_{50SRL})]/[1+([CSA]/EC_{50CSA})+([SRL]/EC_{50SRL})]$$

Non-linear regression is used to fit the general empiric model. The results are shown graphically in a thin-plate spline (TPS) contour plot (Figure 2). This contour plot provides potential explanations for several clinical observations.

Any position on the contour represents a threshold effect, e.g EC_{50}, in the example shown in Figure 2. This effect is plotted as a function of increasing whole blood concentrations of the two immunosuppressants, CSA and SRL. The effect contour demonstrates that a threshold effect can be achieved, either by dominant exposure to one agent, e.g. CSA, or the other, e.g. SRL, as would exist at the extremes of the contour. This supports the clinical practice of using certain drugs interchangeably, e.g. CSA in

exchange for TAC, when there is efficacy failure associated with the use of CSA. However, it also supports the intuitive notion that the same interchangeability can exist for agents belonging to different mechanistic classes, e.g. between CSA and SRL. This can only be assumed if the marker CD54, whose response to different concentration mixtures of the two drugs CSA and SRL, is known to be a good surrogate for the clinical endpoint of rejection-free survival. Another assumption is that the marker CD54 is a good surrogate for the anti-rejection effect of either agent. In the absence of a prospective study, which could relate the expression of such markers to clinical endpoints and to drug levels in transplanted subjects, we have sought clinical validation in a logistic regression analysis of retrospective data from a double-blinded, randomized, dose-controlled, prospective phase III trial of these two agents in adult renal transplantation (9, 10).

For the analysis of clinical trial data, an acceptable post-transplant course on the CSA+SRL regimen was defined as a probability of acute rejection of 15% or less within the first 75 days after renal transplantation. Rejection-free survival was associated with a mean SRL $C_0 \cong 10$ ng/ml of SRL, while rejectors demonstrated mean SRL $C_0 \cong 4$ ng/ml. No difference was seen in CSA C_0 between these two outcome groups. However, when subjects were grouped by mean CSA $C_0 \cong 150$, 300, and 450 ng/ml, the acceptable post-transplant rejection probability of 15% could be achieved by mean CSA C_0 no greater than 150 ng/ml, in the presence of SRL$\cong$10 ng/ml, whole blood. This combination mixture was represented within the contour plot relating half-maximal inhibition of CD54, to CSA and SRL whole blood concentrations. At 10 ng/ml SRL, half-maximal inhibition of CD54 could be achieved by 120 ng/ml of CSA, a number not far removed from 150 ng/ml CSA, predicted by clinical trial data.

5. ESTIMATING INDIVIDUAL VARIATION IN RESPONSE TO A COMBINATION REGIMEN

The TPS contour plot modeled by the general empiric E_{max} model also yields individual concentration mixture thresholds for CSA+SRL, which cause half-maximal inhibition of the B-cell marker CD54 (Figure 2.) (4). If the clinical goal was to minimize dependence on CSA, and maximize the exposure to SRL, and the five healthy human PBL represented transplanted subjects, then the mean concentration predicted to be effective in this population of n=5 would be SRL $C_0 \cong 15$ ng/ml + CSA $C_0 \cong 72$ ng/ml. However, the individual members would require concentration mixtures varying widely from each other, and from the predicted population mean. For example, subject D310, who needed SRL only, could experience CSA toxicity, if administered the drug combination predicted by the population mean. On the contrary, subject D925, who needed more CSA than predicted by the population mean, could experience rejection, if she was prescribed the population mean drug concentration mixture. These observations provide one explanation of why individual variation leads to individual outcomes not fully represented by data regarding population means. Pre-transplant determination of this individual variation could achieve expected outcomes in a larger majority, than is possible with population-based data.

6. SUMMARY

The abovementioned approaches have been useful to us in understanding the effect of immunosuppressants on mechanistic endpoints, in practical terms. As mentioned in previous sections, their clinical relevance is hinted at in the similarities of predicted EC_{50}'s of CSA and the CSA+SRL combination, to the trough levels of these regimens, which have been associated with a rejection-free, stable course, early after renal transplantation in clinical practice. Absolute proof must await prospective clinical validation to link expression thresholds for the various drug targets, to drug concentrations, and their clinical consequences. The clinical consequences of inadequate, adequate and excessive immunosuppression are respectively, rejection, stable post-transplant course, and toxicity. This may also allow surrogate endpoints of immunosuppression, to function simultaneously as endpoints of post-transplant clinical states (11). These hypotheses are under investigation in our laboratory (12).

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7. REFERENCES

1. Hutchinson IV. DM Turner, D Sankaran, MR Awad, PJ Sinnott: Influence of cytokine genotypes on allograft rejection. *Transpl Proc,* 30(3):862-863, (1998)

2. Sindhi R, MF LaVia, E Pauling, J McMichael, G Burckart, S Shaw, LA Sindhi, R Livingston, S Sehgal, and J Jaffe: Stimulated response of peripheral lymphocytes may distinguish cyclosporine effect in renal transplant recipients on a cyclosporine+rapamycin regimen. *Transplantation,* 69(3), 432-436 (2000)

3. Sindhi R, J Allaert, D Gladding, B Koppelman and JF Dunne: Cytokines and cell-surface receptors as target endpoints of immunosuppression with cyclosporine A. *J Interferon Cytokine Res,* 21:7: 507-514 (2001)

4. Sindhi R, J Allaert, D Gladding, P Haaland, JF Dunne, and S Sehgal: Modeling individual variation in biomarker response to combination therapy-Potential clinical Implications. *J Immunol Methods,* 272(1-2):257-72 (2003)

5. Liu J, JDJ Farmer, WS Lane, J Friedman, I Weissman, S Schreiber: Calcineurin is a common target of immunophilin-cyclosporine A and FKBP-FK506 complexes. *Cell,* 66:807-815 (1991)

6. Jung T, U Schauer, C Heusser, C Neumann, CHL Reigler: Detection of intracellular cytokines by flow cytometry. *J Immunol Methods,* 159:197-201 (1993)

7. Gabrielsson J, D Weiner D: Pharmacokinetic and Pharmacodynamic Data Analysis. Concepts and Applications. Swedish Pharmaceutical Press. Stockholm, Sweden. 423-579 (1994)

8. Sollinger HW for the U.S. Renal Transplant Mycophenolate, Mofetil Study Group: Mycophenolate Mofetil for the Prevention of Acute Rejection in Primary Cadaveric Renal Allograft Recipients. *Transplantation,* 60:225-232 (1995)
9. MacDonald, RAPAMUNE Global study group: A worldwide, phase III, randomized, controlled safety and efficacy study of sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation*, 71 (2):271-280 (2001)
10. Kahan BD, The Rapamune US Study Group: Efficacy of SRL compared with Azathioprine for reduction of acute renal allograft rejection: a randomized multicenter study. *Lancet*, 356 (9225): 194-202 (2000)
11. Lesko, LJ, AJ Atkinson, Jr:: Use of biomarkers and surrogate endpoints in drug development and regulatory decision-making: criteria, validation, strategies. *Annu Rev Pharmacol Toxicol*, 41:347-366 (2001)
12. Sindhi R, Principal Investigator: Pharmacodynamic thresholds of Immunosuppression. National Institutes of Health (National Institute of Allergy and Immunology) RO1A14956-01. September (2001)

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