A Novel Three-Dose Regimen of Daclizumab in Liver Transplant Recipients With Hepatitis C: A Pharmacokinetic and Pharmacodynamic Study

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This study evaluated the pharmacokinetics and pharmacodynamics of a novel 3-dose regimen of daclizumab in de novo hepatitis C liver transplant recipients. In 30 of 156 recipients receiving daclizumab, mycophenolate mofetil, tacrolimus, and no steroids (Arm 3 of Hep C 3 Liver Study), daclizumab (2, 2, and 1 mg/kg, respectively) was given on days 1, 3, and 8 posttransplant, respectively, with trough, peak (Cmax), and CD25 saturation (CDsat) measured sequentially. Mean daclizumab Cmax was 50.3 µg/mL on day 1, and mean trough levels were 21.8, 25.7, and 9.9 µg/mL on days 3, 8, and 30, respectively. A significant decline in CDsat (mean, 15.7% to 4.7%) was observed on day 1 and was sustained throughout the study (2.8% on day 30). Daclizumab concentration ≥5 µg/mL was the level where most of the effect on CDsat was noticed. Elevated baseline CDsat was observed in African Americans, patients weighing ≥75 kg, and patients >60 years of age. After 365 days, 2 patients had experienced 3 rejections, 10 patients had recurrent hepatitis C, 4 patients died, and 2 grafts were lost. In conclusion, this novel 3-dose regimen is effective in rapidly achieving high therapeutic concentration of daclizumab and a significant decline in CDsat lasting over 30 days. Liver Transpl 12:585-591, 2006. © 2006 AASLD.

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Daclizumab (Zenapax, Roche Laboratories, Basel, Switzerland) is a highly specific humanized anti-interleukin 2 receptor (IL-2R) monoclonal antibody approved for induction therapy in renal transplant patients. Daclizumab specifically binds to the CD25 subunit of activated lymphocytes and specifically interferes with IL-2 signaling and receptors by inhibiting binding and phosphorylation of the IL-2R beta and gamma chains. Due to its high affinity for the CD25 subunit, daclizumab is an effective IL-2 inhibitor suppressing T-cell activity in the cellular immune response to allograft rejection.1,2

Most studies conducted to describe the pharmacokinetics and pharmacodynamics (PK/PD) of daclizumab examined a 5-dose regimen in kidney transplant patients.3-5 In one study, peak serum concentrations (Cmax) rose between the first dose (21 ± 14 µg/mL) and the fifth dose (32 ± 22 µg/mL). The mean trough serum concentration before the fifth dose was 7.6 ± 4.0 µg/mL, and the systemic clearance was 15 mL/hr, with a terminal half-life of 20 days (480 hours).5

Only a few clinical trials have evaluated daclizumab as an induction agent in liver transplantation, leaving limited knowledge of its PK/PD. One important study conducted in 28 patients post–liver transplant, evaluated the PK/PD of a 2-dose regimen of daclizumab (1 mg/kg on day 0 and 0.5 mg/kg on day 4).6 Daclizumab therapeutic levels were recorded up to 14 days posttransplant, and CD25+ cells were significantly reduced in all patients 28 days posttreatment, with only 1 episode of mild rejection. Daclizumab half-life was 99
hours, total body clearance was 57 mL/hr, and no significant decrease in CD3+, CD4+, or CD8+ lymphocytes was found. Most clinical trials of daclizumab in liver transplantation have used either a single-dose regimen of 1-2 mg/kg within 24 hours posttransplant, or a 2-dose regimen of 1-2 mg/kg administered within 24 hours posttransplant and on day 7-14 posttransplant. These 2-dose regimens proved to be effective treatment, providing effective prevention of acute rejection, allowing for minimization of calcineurin inhibitors, and enabling early steroid withdrawal, which may positively affect the rate of recurrence of hepatitis C.

METHODS

Study Design

The Hep C 3 Liver Study is an open-label, randomized, prospective, multicenter study designed to compare the safety and efficacy of 3 immunosuppressant treatment regimens in patients receiving a liver transplant for end-stage liver disease caused by chronic hepatitis C. A total of 312 patients were enrolled at 18 centers in the United States and randomized to 1 of 3 treatment arms: tacrolimus/stereoids (Arm 1), tacrolimus/mycophenolate mofetil/steroids (Arm 2), or daclizumab/tacrolimus/mycophenolate mofetil without steroids (Arm 3). The daclizumab PK/PD substudy of the Hep C 3 Liver Study, which required additional Institutional Review Board (IRB) approval, included 30 patients from treatment Arm 3. This substudy enrolled over a period of 11 months from 5 participating sites. All patients in treatment Arm 3 were administered daclizumab, 2 mg/kg on day 1 and day 3, and 1 mg/kg on day 8. The first dose of tacrolimus was administered within 72 hours after transplantation. Tacrolimus dosing was aimed to achieve whole blood trough levels of 10-15 ng/mL within 72 hours of the initial dose and for the first 6 weeks, and 5-12 ng/mL thereafter. Mycophenolate mofetil was given at a dose of 2-3 g/day for the duration of the study with initial dose given within 12 hours after transplant. The dose of mycophenolate mofetil could be reduced due to adverse events including leucopenia. Mycophenolic Acid (MPA) levels were not measured.

Collection Procedures

Blood samples for CD25 levels and daclizumab concentrations (peak and trough) were drawn on postoperative days 1, 3, 8, and 30 (trough only). The daclizumab blood samples were obtained by direct venipuncture or via an indwelling cannula from the arm opposite the daclizumab infusion. The trough and peak samples were collected 30 minutes prior to and 60 minutes after the daclizumab infusion.

For each daclizumab concentration sample, 5 mL of whole blood was drawn into a red-top collection tube and labeled with the patient number and time. The collection tube was allowed to clot at room temperature for approximately 20-30 minutes. The clotted blood was then centrifuged, divided into 2 cryovials, and frozen.

For each CD saturation sample, 10 mL of whole blood was drawn into a yellow-topped collection tube and labeled with the patient number and time. This tube was maintained at room temperature and shipped immediately to CTI Clinical Trials and Consulting Services’ laboratory (Cincinnati, OH).

Pharmacokinetic Analytical Methods

(Daclizumab Serum Concentration)

The novel BeadChip assay (BioArray Solutions, Ltd., Warren, NJ) and enzyme linked immunosorbent assay (ELISA) were used to determine daclizumab serum concentration. While in each of the 2 assay designs, IL-2Rα served to capture daclizumab. IL-2Rα produced in different expression systems were procured from different sources. Aliquots were prepared for BeadChip and ELISA and were assayed in triplicate by both methods.

Daclizumab BeadChip

The design of the daclizumab BeadChip conforms to a “sandwich” format in which an anti-human CD25 (Tacrolimus), clone 7G7B6 – murine immunoglobulin G 2ακ, covalently attached to a color-colored microparticle (“bead”), serves to display IL-2Rα, which is detected by a fluorescently (Alexa 647, Molecular Probes, Carlsbad, CA) labeled, Fυγ fragment-specific goat anti-human immunoglobulin G. IL-2Rα from R&D Systems (Minneapolis, MN) was chosen for the BeadChip. The clone 7G7 displays IL-2Rα, leaving the epitope recognized by daclizumab accessible. The daclizumab BeadChip comprises several positive, negative, and internal controls, each displayed on a designated type of bead, to permit the quantitative determination of daclizumab serum levels by analysis of images recorded from the BeadChip.

To relate normalized assay signal intensities to daclizumab serum concentration, a “master” calibration curve was constructed by “spiking” normal human (male) serum with daclizumab at the following concentrations: 3.75, 7.5, 15.0, 30, 45, and 60 μg/mL. Each set of patient sera was analyzed, in triplicate, on 8-chip carriers placed on the stage of the Array Imaging System 400 (BioArray Solutions, Warren, NJ) for automated assay image recording.

ELISA

In the ELISA design, microplates were coated with IL-2Rα to capture daclizumab. A goat anti-human Fυγ fragment specific immunoglobulin G – HRP (horseradish peroxidase) conjugate was used for detection via optical absorption of 1,3,5-trimethoxybenzene (TMB) in solution. For the ELISA, IL-2Rα from Biological Mimetics (Frederick, MD) was chosen.

A standard curve was established on each plate by analyzing in triplicate serum samples of known daclizumab concentration ranging from 6.25-400 ng/mL, published on behalf of the American Association for the Study of Liver Diseases.
and a “blank” sample of 0 concentration. Optical absorbance at 450 nm was determined by placing ELISA plates into a Labsystems Multiskan MCC/340 plate reader.

Recovery Concentrations by BeadChip vs. ELISA.

Data from the multiple triplicate data sets obtained for 3 reference (Quality Assurance, “QA”) samples, of respective daclizumab concentrations 7.5, 15, and 30 µg/L, included in the 8-chip carriers (“slides”) and in the ELISA plates, were compared to establish the correlation between these 2 different methods.

Pharmacodynamic Methods (CD25 Saturation – Flow Cytometry)

Flow cytometry was used for the quantitative analysis of CD25 T lymphocytes and was validated by performing correlation analysis with a second reference laboratory (strong correlation was found, R = 0.985).

All samples were analyzed at the central laboratory and only samples with viability test results ≥80% were processed. The analysis was performed with the Beckman Coulter Epics XL flow cytometer (Fullerton, CA), which is calibrated and tested daily with standard Flow Check beads (Polysciences Inc., Warrington, PA), Flow Set beads (Beckman Coulter, Inc., Fullerton, CA), and Cyto-Comp reagents (Beckman Coulter).

The flow cytometer provides a histogram quantifying the cells with specific antibody binding to their surface membrane antigen (CD25). Using the total complete blood count (CBC) numbers recorded by the sites, the differential white blood cell count was determined and used with the CD25% from the histogram to establish the number of CD25 T cells. For the quantitative determination of CD25 lymphocytes subset, the CD25 2A3-PE antibody was used.

Statistical Methods

Statistical analysis of the data was performed using SAS (version 8.2; SAS Institute, Inc., Cary, NC) for windows statistical software. All statistical tests were conducted at the 5% level of significance except where noted.

Demographic data, consisting of gender, race, age, and weight (taken from the study database), is summarized using descriptive statistics. Continuous data are presented as the mean ± SD, median and range, and categorical data were presented as counts and percents.

Daclizumab serum concentration and CD25 saturation levels are summarized over all patients at each time point. Additional summaries are provided for patients grouped by gender, race, weight (≤75 kg vs. >75 kg), age (≤60 years vs. >60 years), and model for end-stage liver disease (MELD) score (≤20 vs. >20). Any samples below quantifiable limits are reported as 0.

An analysis was performed to determine the effects of demographic variables on daclizumab serum concentration levels. Likewise, an analysis of variance was performed to determine the effects of demographic variables on daclizumab CD saturation levels (absolute count). Each analysis of variance model will include sample (e.g., day 1 trough, day 1 peak), gender, race, weight category (defined above), age category (defined above), and MELD score (defined above) all taken from the study database.

An analysis was performed to determine the correlation between daclizumab serum concentration and CD saturation levels.

Clinical Outcomes and Efficacy

Efficacy analysis is not included in this report because the clinical outcomes presented here are from a small number of patients (30 of 156 patients in Arm 3) who volunteered to participate in this substudy. The clinical outcomes will therefore be presented only for descriptive purposes. Thorough efficacy analysis will be an important part of the comprehensive study report that will be published in the near future.

RESULTS

Demographics

The demographic characteristics of the study participants are summarized in Table 1. There were more males than females (70% vs. 30%), and the majority were Caucasian (67%), younger than 60 years of age (87%), and heavier than 75 kg (77%). The mean age was 51.5 ± 7.1 years of age, and the mean weight was 87.2 ± 19.2 kg. The distribution of the MELD score showed 21 (70%) patients with MELD >20 and 9 (30%) with MELD ≤20.

Blood Samples and Data Sets

Thirty patients were enrolled in the study from 5 sites: New York University (n = 8), University of Texas-San Antonio (n = 8), Emory University (n = 7), Mayo Clinic
at Scottsdale, Arizona (n = 4), and Baylor University (Dallas, TX) (n = 3).

Samples from all 30 patients were available for the daclizumab concentration analysis, with 13 samples missing out of a total of 210, giving a 94% recovery rate. For the daclizumab CD25 saturation analysis, samples from 28 patients were available for the analysis, with 22 of the missing samples out of a total of 210, due to shipping errors, sample collection errors, the absence of CBC, graft loss, and patient death, giving an 84% recovery rate.

Pharmacokinetics and Pharmacodynamics

Daclizumab Concentration

High correlation of the 2 methods was found and provided the basis for the selection of the BeadChip data for the statistical analysis. The mean C\text{max} and C\text{max} (±SD) for all patients for each of the time points are presented in Figure 1. Following the first dose of daclizumab on day 1, the serum concentration peaked at 50.32 ± 14.58 \(\mu\text{g/mL}\). The highest C\text{max}, 59.57 ± 16.87 \(\mu\text{g/mL}\), was achieved on day 3. Trough levels were 21.83 ± 8.72, 25.66 ± 8.28, and 9.87 ± 4.64 \(\mu\text{g/mL}\) on days 3, 8, and 30, respectively. Analysis of variance was performed to assess the effect of race, gender, age, weight, and MELD score on the CD25%. The baseline (pretreatment) level of CD25% was significantly different between various race, age, and weight groups (P < 0.05). Patients ≤60 years of age had lower baseline CD25% saturation compared to patients >60 years old (11.1 ± 3.2 vs. 16.6 ± 8.2). Patients with weight ≤75 kg had a higher baseline level of CD25% (21.3 ± 11.4 vs. 13.7 ± 4.6). The main differences between the race groups were the higher baseline CD25% level in African Americans (19.75 ± 9.26 vs. 15.63 ± ± 8.36, 14.18 ± 6.26, and 16.25 ± 5.86) and the lower CD25% level in Hispanics on day 8 (2.48 ± 2.4 vs. 5.0 ± 0.7, 5.53 ± 3.75, and 5.95 ± 1.76).

Daclizumab Concentration/Saturation Correlation

Figure 3 presents the concentration-effect plot between daclizumab serum concentration and daclizumab saturation. Daclizumab concentration of 5 \(\mu\text{g/mL}\) seems to be the cutoff point of significant drop in CD25% level. Pearson Correlation Coefficient test was performed to assess the correlation between daclizumab serum concentration and CD25% level. A statistically significant negative correlation was found with coefficient factor of −0.41 and P < 0.0001.

Clinical Outcomes

The major clinical outcomes for this group of 30 patients who were followed for at least 1-year posttransplant are outlined below based on data recorded in the study database. Recurrence of hepatitis C occurred in 10 of the 30 patients. Five of these patients received anti–hepatitis C therapy. One patient developed mild acute rejection and another patient had 2 episodes of moderate rejection. Four patients died. Of the 4 who died, 1 developed recurrence of hepatitis C and died...
from liver failure. Of the 3 additional deaths, 2 were related to sepsis and 1 to hepatic arterial thrombosis. Two grafts were lost to primary nonfunction.

**DISCUSSION**

Most of the data related to the efficacy and safety of daclizumab have been obtained in numerous studies conducted in kidney transplantation patients.\(^3,4,14,16\)

However, the use of daclizumab in liver transplant patients has increased over the last few years.\(^6,9\) In both kidney and liver recipients, daclizumab used as an induction agent has been shown to be effective in reducing the rate of acute rejection.\(^3,7,14\) has recently been successfully utilized as part of immunosuppression protocols aimed at rapid withdrawal of corticosteroids,\(^10,11,17,18\) and has been used with reduced and delayed doses of tacrolimus in kidney-sparing protocols.\(^8,18\)

Daclizumab dosing has been most commonly 1 mg/kg intravenously, with the first dose given in the early postoperative period and a subsequent dose to be followed every 2 weeks for a total of 5 doses.\(^3,4,14,15\) Modified daclizumab dosing regimens of fewer than 5 doses (0.5-2 mg/kg/dose) have recently been reported in kidney\(^19-21\) and liver transplantation.\(^6,7\)

Several studies in liver transplantation have used 2 doses of daclizumab in the early postoperative period, day 0 and day 14,\(^11\) day 1 and day 4,\(^6\) day 1 and day 5,\(^9\) and preop and day 5.\(^7\) In the study by Koch et al.,\(^6\) a 2-dose regimen (1 mg/kg on day 0, and 0.5 mg/kg on day 4), achieved an effective concentration up to 14 days posttransplant and a reduction of CD25 lymphocyte subsets for at least 21 days. Recovery of the CD25 lymphocyte subset to the pre-treatment level was reported in that study to occur by day 56 posttransplant.

This report presents the PK/PD of a novel 3-dose regimen of daclizumab in liver transplantation recipients with hepatitis C. The subgroup of 30 patients in this report was not stratified for age, weight, race, gender, or MELD score. These variables were not evenly distributed, and their impact on the daclizumab concentration and CD25 saturation should be assessed cautiously.

The results of this substudy demonstrated that this novel 3-dose regimen of daclizumab proved to be very efficient in establishing a rapid therapeutic serum concentration of daclizumab, and a significant reduction of CD25% lymphocyte subsets for at least 30 days. The initial dose of 2 mg/kg resulted immediately in a mean concentration of 50 \(\mu\)g/mL, much higher then 10-15 \(\mu\)g/mL achieved in the Koch et al. report\(^6\) where the first dose was 1 mg/kg. With the additional doses on day 3 (2 mg/kg) and day 8 (1 mg/kg), daclizumab serum concentrations remain at therapeutic levels at least up to day 30 (9.8 \(\mu\)g/mL). A sharp, statistically significant drop in CD25% lymphocyte subsets was noticed following the first dose of daclizumab, and CD25% lymphocyte subset levels remained low from that point through at least day 30, with a slight temporary rise before the day 8 dosing.

The concentration effect plot (Fig. 3) demonstrated that the maximum suppression effect of daclizumab on the CD25% lymphocyte subset was achieved with daclizumab concentration at or above 5 \(\mu\)g/mL. It is clear from the results of this substudy that the daclizumab dosing regimen used in these patients provided an effective therapeutic suppression of CD25% lymphocytes.
subset for at least 30 days. This is at least double the time reported in a Koch et al. study\(^6\) where 2 doses of daclizumab on day 0 and day 4 provided effective suppression of CD25% lymphocyte subsets for 14 days with a trend toward recovery observed by day 28 and full recovery reached on day 56. The design of our study did not include a comparison group of 2 doses of daclizumab. The present study also did not follow the daclizumab concentration and CD25 saturation beyond day 30. Therefore, although a very low CD25% lymphocyte subset (2.78%) and a therapeutic daclizumab serum concentration (9.87 \(\mu\)g/mL) were observed on day 30, further studies would be necessary to assess the potential advantage of 3- vs. 2-dose regimens of daclizumab. Although daclizumab serum concentration on days 1, 3, and 8 were relatively higher than what has been reported,\(^6\) the serum concentrations of 9.87 \(\pm\) 4.64 \(\mu\)g/mL on day 30 reflects this specific dosing regimen. Of note is that with an SD \(\pm\) 4.64, the use of lower dosage on days 1, 3, and 8 might result in 40-50% of the patients to be under the therapeutic level of 5 \(\mu\)g/mL at day 30.

The analysis of variance multivariate analysis performed for both daclizumab concentration and CD25% lymphocyte subset failed to show any impact of age, gender, race, weight, or MELD score on daclizumab concentration levels. However, this analysis showed that the baseline (pretreatment) CD25% lymphocyte subsets were elevated in African Americans, in patients weighing less than 75 kg, and in patients younger than 60 years old. Although this higher baseline level was significant, the number of patients in each group is small. Further studies should be done before any clear conclusion can be drawn regarding differences in lymphocyte subset baseline distribution in these subgroups of patients receiving liver transplants for hepatitis C induced end-stage liver disease (ESLD). The pharmacokinetics of daclizumab in liver transplantation recipients have been reported to be different than in kidney transplantation recipients, with a shorter half-time, 99 hours vs. 273 hours.\(^6\) The reason for this difference is not clear, but it may be related to the massive shift of fluids into the extra vascular space and the loss of a large amount of ascitic fluid through the surgical drains and the wound occurring in these liver recipients during the perioperative and early posttransplant period. These fluid changes combined with low albumin and other proteins may alter the pharmacokinetics of daclizumab in these patients.\(^6\)

Therefore, data obtained on the use of daclizumab in renal transplant patients may not be applicable in liver transplant recipients and should be assessed with caution. The unique approach taken in this study of administering a third dose of daclizumab on day 8 may be an important factor to counter the negative effects of the fluid changes occurring in the first few days posttransplant.

These excellent serum concentrations and effective reduction in CD25% lymphocyte subset levels resulting from the 3-dose regimen of daclizumab used in this study were achieved in a group of sick liver transplant recipients. Seventy percent of the patients had a MELD score of \(>20\), indicating the advanced nature of their liver disease and reflecting their overall poor medical condition. This factor, combined with the significant perioperative events of a liver transplantation procedure, did not seem to have a negative impact on the effectiveness of this daclizumab dosing regimen. The use of daclizumab in the Hep C 3 Liver Study aimed at providing antirejection protection in a steroid-free immunosuppression regimen for hepatitis C liver recipients. The favorable impact of reducing or eliminating steroids and lowering the rate of acute rejection on the posttransplant recurrence rate of hepatitis C has been suggested.\(^12\)

In conclusion, this novel dosing regimen of daclizumab in hepatitis C liver recipients receiving daclizumab on days 1, 3, and 8 posttransplant provided a high therapeutic serum concentration of daclizumab and an effective reduction of CD25% lymphocyte subset lasting for at least 30 days. The BeadChip technique is effective and accurate in analyzing the serum concentration of daclizumab and is an acceptable alternative to ELISA. Excellent daclizumab serum concentration and effective reduction of CD25% lymphocyte subsets can be achieved with this dosing regimen in chronically ill hepatitis C liver recipients undergoing this major surgical procedure, with its associated fluid shift and loss. The specific design of this substudy and the lack of comparison to other daclizumab treatment regimens precludes any conclusions regarding efficacy. Future trials comparing this novel 3-dose regimen to conventional regimens are warranted.

REFERENCES


