

MONOTHERAPY WITH LF 15-0195, AN ANALOGUE OF 15-DEOXYSPERGUALIN, SIGNIFICANTLY PROLONGS RENAL ALLOGRAFT SURVIVAL IN MONKEYS¹

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Background. LF 15-0195 is a novel, more potent, and less toxic analogue of 15-deoxyspergualin, an antibiotic used as an immunosuppressive agent to prevent rejection of organ transplants. This study was undertaken to determine whether LF 15-0195 monotherapy would prevent renal allograft rejection in a nonhuman primate model.

Methods. In the study groups, recipients received LF 15-0195 monotherapy at doses of 0.065 mg/kg per day (group 2, n=4), 0.13 mg/kg per day (group 3, n=4), or 0.2 mg/kg per day (group 4, n=4), administered subcutaneously, on postoperative days 0 to 14.

Results. Group 1 consisted of untreated control recipients, all of which developed advanced graft rejection after surviving for an average of 6.5±0.6 days. LF 15-0195 treatment significantly prolonged graft survival in groups 2, 3, and 4, to 20±20 days, 49±5 days, and 39±4 days, respectively. Animals in groups 3 and 4 demonstrated no evidence of rejection during LF 15-0195 treatments. The animals maintained stable renal function for 2 weeks after LF 15-0195 withdrawal but gradually developed rejection at 5 to 6 weeks. Pathologic studies demonstrated that vascular graft rejection was attenuated in LF 15-0195-treated allografts, compared with control specimens. These groups also demonstrated transient reductions in lymphocyte counts during treatment, which returned to normal levels 2 weeks after LF 15-0195 withdrawal. Total serum concentrations of IgM and IgG decreased by a mean of 20.4% and a mean of 31.4%, respectively, at the end of LF 15-0195 treatment (postoperative day 14). LF 15-0195 did not significantly alter thrombocyte counts or hemoglobin levels. Necropsy studies showed no evidence of drug toxicity in the heart, liver, spleen, intestines, stomach, or colon.

Conclusions. LF 15-0195 monotherapy significantly prolonged renal allograft survival in monkeys. These

encouraging data suggest that this novel agent may be of future value in clinical transplantation.

Solid-organ transplantation is the most effective form of therapy for the treatment of patients with end-stage kidney, heart, or liver failure. The development of new immunosuppressive agents has significantly improved the outcomes of organ transplantation; however, rejection continues to be the single greatest impediment to success. Although currently available immunosuppressive agents such as cyclosporin A and FK506 are able to attenuate rejection, they both possess undesirable side effects and are less effective for certain patients who have received ABO-mismatched organs or who have become resistant to steroid therapy (1, 2). Consequently, there is a need for more tolerable and more potent immunosuppressive agents. The current goal of transplantation immunologic treatment is to induce a prolonged state of nonreactivity to the donor graft in the recipient while preserving an otherwise intact immune system (tolerance).

15-Deoxyspergualin (DSG) is a synthetic analogue of spergualin, an antibiotic purified from *Bacillus laterosporus* (3). DSG was initially developed as an antitumor agent because of its inhibitory effects on mitosis. Cancer trials demonstrated limited effects. Instead, DSG demonstrated immunosuppressive properties in both allograft and xenograft transplantation models involving skin, heart, kidney, pancreatic islet cells, bone marrow, and liver (4). Despite its potent immunosuppressive activity, the clinical use of DSG was limited because of its toxicity and low chemical stability in aqueous solutions. Subsequent modifications to DSG resulted in LF 15-0195. This novel analogue possesses increased aqueous stability and stronger resistance to in vivo oxidation and may be less toxic (5).

Our group and others demonstrated that LF 15-0195 is a potent immunosuppressive agent that prevents allograft rejection and induces donor-specific tolerance in rats and mice (6, 7). However, this agent has yet to be tested in a nonhuman primate transplantation model. This study was conducted to evaluate the efficacy of LF 15-0195 monotherapy in the prevention of renal allograft rejection in a life-supporting monkey kidney transplantation model.

MATERIALS AND METHODS

Animals

ABO blood group-matched, outbred, juvenile, cynomolgus monkeys were used as donors and recipients. The experiments described in this study were performed according to the principles set forth in the Canadian Council on Animal Care *Guide for the Care and Use of Laboratory Animals*.

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Major Histocompatibility Complex Typing and Donor-Recipient Selection

Donor-recipient combinations were selected on the basis of genetic nonidentity at major histocompatibility complex class II. Nonidentity was established with denaturing gradient gel electrophoresis and direct sequencing of the second exon of DRB (8).

Kidney Transplant Procedures

Donor operation. With general anesthesia, a long, midline, abdominal incision was made for dissection and mobilization of the kidneys, ureters, aorta, inferior vena cava, and renal vessels. The suprarenal aorta was cross-clamped, and both kidneys were then flushed with 2 L of ice-cold University of Wisconsin solution. The right and left kidneys were removed and stored in ice-cold University of Wisconsin solution until implantation into the recipient.

Recipient operation. With general anesthesia, the abdomen was entered through a long midline incision. Standard bilateral nephrectomies were performed. The abdominal aorta and inferior vena cava were dissected and mobilized below the renal vessels. A single donor kidney was selected, prepared for implantation, and oriented into the left lower quadrant. End-to-side anastomoses of the graft renal artery (Carrel patch) and vein to the infrarenal aorta and inferior vena cava, respectively, were performed sequentially. The graft ureter was anastomosed to the native bladder. After establishment of adequate graft reperfusion and hemostasis in the surgical field, the abdomen was closed in two layers.

Experimental Groups

Recipient monkeys were randomly divided into four equal groups for administration of varying doses of LF 15-0195. Control group 1 animals were left untreated (n=4). Low-dose LF 15-0195 therapy (0.065 mg/kg per day) was administered to group 2 subjects (n=4). Intermediate-dose (0.13 mg/kg per day) and high-dose (0.2 mg/kg per day) therapies were administered to subjects in group 3 (n=4) and group 4 (n=4), respectively. LF 15-0195 was administered subcutaneously to all groups from the day of transplantation (designated day 0) to postoperative day 14.

Total Serum IgG and IgM Level Assays

Total serum levels of IgG and IgM were measured with the Beckman IMMAGE immunochemistry system (Beckman, Fullerton, CA), as previously described (9).

Pathologic Analyses

Tissue specimens were obtained for histopathologic analyses at the time of necropsy. For light microscopy, 10% buffered formalin-fixed, paraffin-embedded material was routinely processed and stained with hematoxylin-eosin and trichrome stain. Additional 3- μ m-thick sections were obtained from the kidney and stained with the periodic acid-Schiff reaction. Slides were read by one pathologist (B.G.), without knowledge of clinical data, and were graded from 0 to 4 according to the severity of pathologic changes.

RESULTS

LF 15-0195 Significantly Prolonged Renal Allograft Survival

All kidney grafts were adequately perfused after revascularization, exhibiting normal color and producing urine immediately. Table 1 presents recipient survival data according to the different treatment regimens. Untreated animals rapidly developed graft rejection and died as a result of acute renal failure, with a median survival time of 6.5 ± 0.6 days. LF 15-0195 monotherapy with doses of 0.065 mg/kg, 0.13 mg/kg, and 0.2 mg/kg significantly prolonged renal allograft survival, compared with control animals ($P < 0.001$), with me-

TABLE 1. Renal allograft survival times, according to treatment

Group	Individual Survival Times (days)	Median Survival Time \pm SE (days)
Control	5, 6, 7, 8	6.5 ± 0.6
LF 15-0195, 0.065 mg/kg	9, 13, 27, 98	$20 \pm 20^*$
LF 15-0195, 0.13 mg/kg	28, 48, 49, 38	$48.5 \pm 7^*$
LF 15-0195, 0.2 mg/kg	30, 33, 44, 47	$38.5 \pm 4^*$

* $P < 0.001$, compared with control values (log-rank test).

dian survival times of 20 ± 20 days, 48.5 ± 5 days, and 38.5 ± 4 days, respectively.

Renal Allografts Exhibited Normal Renal Function during LF 15-0195 Treatment

Figure 1 illustrates renal function after transplantation. Untreated animals became anuric on day 5 and developed severe uremia by day 8. In contrast, all LF 15-0195-treated recipients except two in the low-dose group exhibited normal renal function during therapy. These recipients maintained renal function for 2 to 3 weeks after the withdrawal of LF 15-0195. However, the function of grafts gradually deteriorated at 5 to 6 weeks.

Recipients Treated with LF 15-0195 Demonstrated Transient Moderate Reductions in Peripheral Blood Lymphocyte Counts

Figure 2 illustrates peripheral blood lymphocyte counts after LF 15-0195 treatment. Recipients treated with LF 15-0195 demonstrated transient moderate reductions in peripheral blood lymphocyte counts, which returned to normal levels 2 to 3 weeks after the withdrawal of therapy. LF 15-0195 did not significantly affect hemoglobin levels, neutrophil counts, or thrombocyte counts (data not shown).

LF 15-0195 Significantly Reduced Total Serum IgM and IgG Concentrations

Figure 3 illustrates total serum IgG and IgM levels, as measured with a nephelometry assay. LF 15-0195-treated recipients demonstrated a mean reduction in total serum IgM levels of 20.4% and a mean reduction in total serum IgG levels of 31.4% at the end of LF 15-0195 treatment (postoperative day 14), compared with baseline levels. These levels returned to a normal range 2 to 3 weeks after therapy was withdrawn. In the control animals, total serum IgG and IgM levels were significantly increased when the graft was rejected (IgG levels of 10 ± 1 g/L on day 0 vs. 15 ± 2 g/L on day 7; IgM levels of 1.3 ± 1 g/L on day 0 vs. 1.6 ± 1 g/L on day 7; $P < 0.05$ for both IgG and IgM levels).

LF 15-0195 Attenuated Vascular Rejection

Table 2 illustrates median scores for vascular rejection in renal allografts at necropsy. Untreated allografts developed advanced cellular and vascular rejection. Renal allografts treated with LF 15-0195 demonstrated minimal evidence of vascular rejection; however, advanced cellular rejection was observed. Figure 4 shows massive infiltration by lymphocytes and evidence of tubulitis. Signs of vascular rejection, such as thrombosis and hemorrhage, were minimal in LF 15-0195-

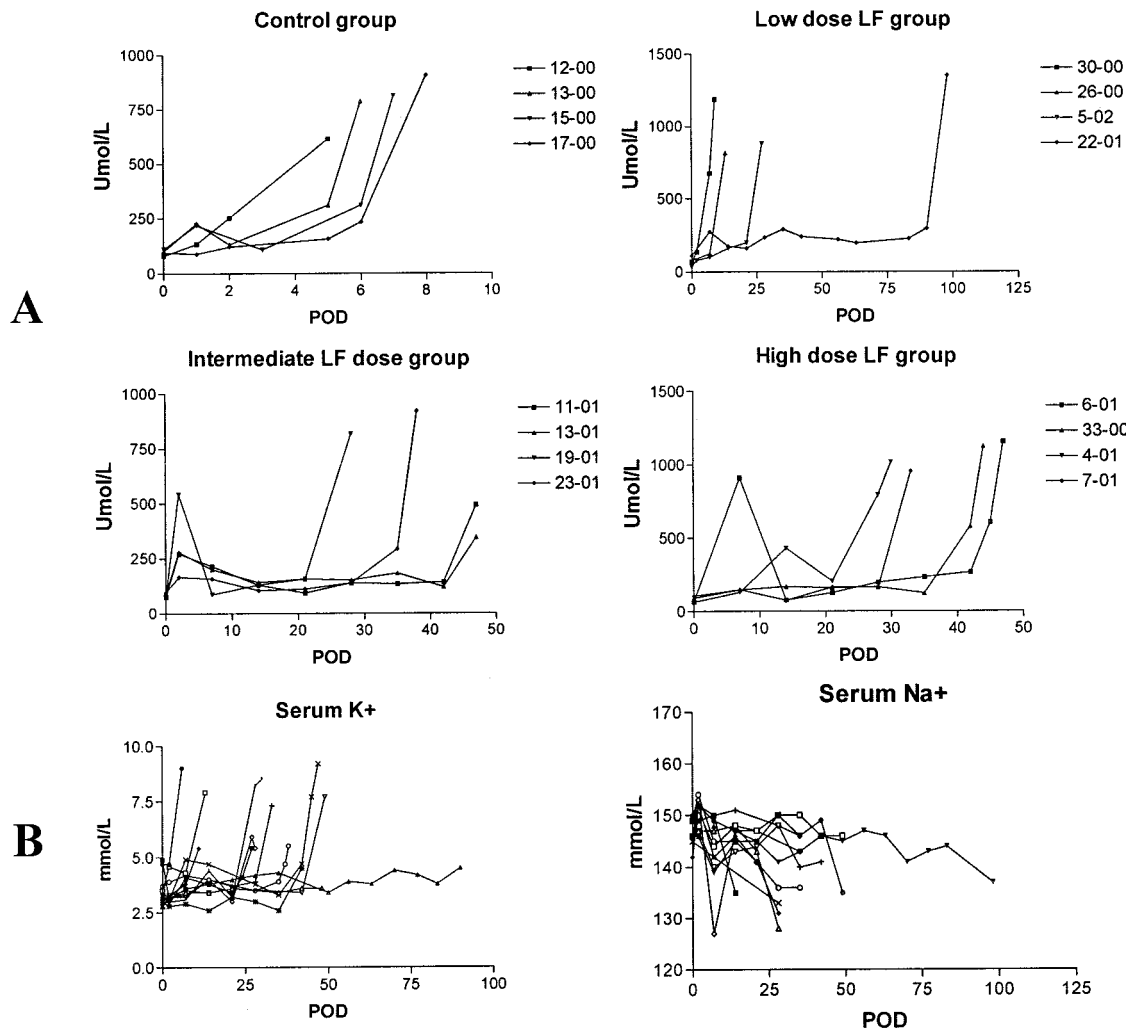


FIGURE 1. (A) Serum creatinine levels of renal allograft recipients in different groups. (B) Serum potassium and sodium levels among LF 15-0195-treated recipients. LF, LF 15-0195; POD, postoperative day.

treated allografts at necropsy. These results indicate that LF 15-0195 attenuated vascular rejection.

LF 15-0195-Treated Groups Tolerated Therapy

All low-dose LF 15-0195-treated animals tolerated therapy well, without any apparent adverse effects. Intermediate-dose and high-dose LF 15-0195-treated recipients were in good general condition, except for one animal in the intermediate-dose group and four animals in the high-dose group that developed diarrhea between day 11 and day 20. The symptoms spontaneously disappeared 1 to 3 weeks after withdrawal of therapy. Liver functions were normal in these animals. Pathologic assessments performed at necropsy demonstrated that there were no remarkable changes in the lungs, heart, liver, stomach, pancreas, colon, or lymph nodes in these animals.

DISCUSSION

This study demonstrated that short-term LF 15-0195 monotherapy significantly prolonged allograft survival, to an average of 39 ± 7 days, in cynomolgus monkeys after life-supporting kidney transplants. Data from this study also

demonstrated that vascular rejection was attenuated in LF 15-0195-treated allografts at therapeutic doses one tenth of those of DSG. There were transient reductions in lymphocyte counts during LF 15-0195 treatment, which normalized 2 weeks after drug withdrawal. Total serum concentrations of IgM and IgG decreased an average of 20.4% and 31.4%, respectively, during LF 15-0195 treatment. LF 15-0195 did not significantly alter thrombocyte counts or hemoglobin levels. There was no evidence of drug toxicity in the heart, liver, spleen, intestines, stomach, or colon. These data suggest that LF 15-0195 may be of future value in clinical transplantation.

LF 15-0195, an analogue of DSG, is a new immunosuppressive molecule that is currently under evaluation. Although the exact mechanisms of its action remain unknown, it is reasonable to think that the mechanisms are similar to those of DSG. DSG is a derivative of spergualin, an antibiotic isolated from *B. laterosporus* (10). Although it was initially developed as an antitumor agent in 1985, DSG was reported to have profound immunosuppressive properties. The modes of action of DSG and its derivatives are distinct from those of cyclosporin A and FK506, which inhibit calmodulin activation after Ca^{2+} influx. Previous studies suggested that the

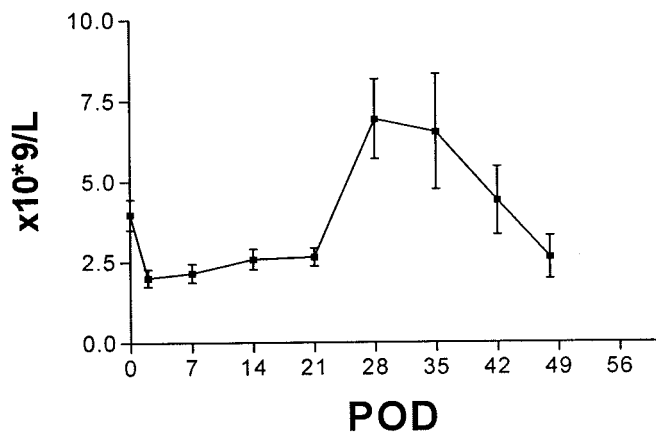


FIGURE 2. Mean lymphocyte counts for all LF 15-0195-treated recipients. These recipients exhibited transient moderate reductions in peripheral blood lymphocyte counts, which returned to normal levels 2 to 3 weeks after the discontinuation of LF 15-0195 treatment. POD, postoperative day.

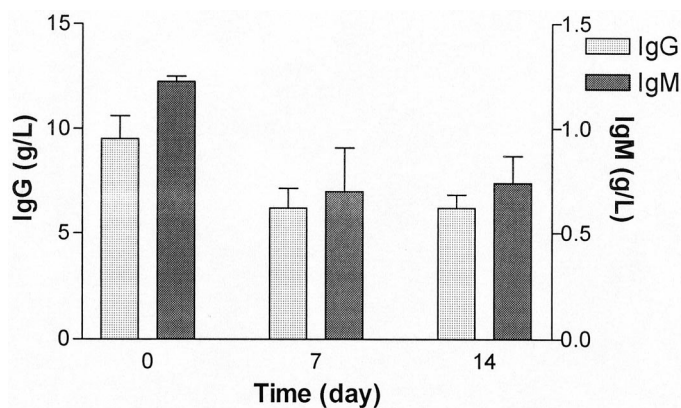


FIGURE 3. Total serum IgG and IgM levels (measured with the Beckman IMMAGE immunochemistry system). LF 15-0195-treated recipients demonstrated a mean reduction in total serum IgM levels of 20.4% and a mean reduction in total serum IgG levels of 31.4% at the end of LF 15-0195 treatment (postoperative day 14), compared with baseline levels. These levels returned to normal ranges 2 to 3 weeks after the discontinuation of therapy.

actions of DSG result from its ability to selectively suppress mixed lymphocyte responses, cytotoxic T lymphocyte induction, T cell-independent antibody formation, and interleukin-2-dependent lymphocyte proliferation. In addition, Nadler et al. (11) discovered a DSG-binding protein of the heat shock protein 70 family and deduced three hypothetical modes of

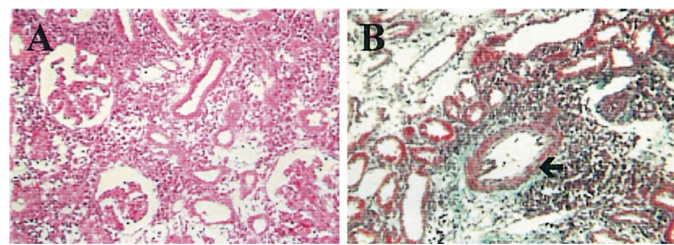


FIGURE 4. Representative histopathologic assessment of a LF 15-0195-treated renal allograft on postoperative day 49 (at necropsy), showing massive infiltration by lymphocytes and tubulitis. Evidence of vascular rejection, such as thrombosis, hemorrhage, or other vascular changes, was minimal (arrow) (A: hematoxylin-eosin; magnification × 200; B: trichrome; magnification × 200).

action, as follows. (1) DSG in conjunction with heat shock proteins may suppress lysosomal processes that are required for major histocompatibility complex class II antigen presentation. (2) Nucleic acid synthesis may be inhibited by intracellular transport of DSG by heat shock proteins. (3) DSG may inhibit normal functions of heat shock proteins (12). Our group recently demonstrated that LF 15-0195 inhibits nuclear factor-κB translocation and prevents dendritic cell maturation and regulatory T cell generation (W.M., unpublished data, 2002).

Our experiments with LF 15-0195 demonstrated that humoral rejection was significantly attenuated, with evidence of significant reductions in total serum levels of IgG and IgM after short-term treatment. These results agreed with the findings of a study conducted by Morikawa, Oseko, and Morikawa (13), which determined that DSG selectively depressed B lymphocyte differentiation and inhibition of antibody production. These properties define the role of DSG and its new analogue LF 15-0195 as down-regulators of humoral immunity, for use in future clinical transplantation trials.

DSG has been used and studied extensively in animal models; however, its potential is limited by its toxicity and instability. With the development of LF 15-0195, it may be possible to improve on the early successes with DSG. Leventhal et al. (14) studied natural antibody depletion with DSG in an immediate vascularized guinea pig-to-rat cardiac xenograft model. The study showed that DSG reduced and maintained low levels of rat anti-guinea pig natural antibody for more than 1 week after therapy. Reichenspurner et al. (15) described the prevention of hyperacute rejection in a primate xenogeneic cardiac transplantation model with DSG. That group discovered that treated cardiac grafts did not undergo hyperacute reactions but developed later cellular rejection.

TABLE 2. Median scores for pathologic changes in renal allografts at necropsy^a

Group	Scores					
	Mono.	Hemorrhage	Edema	Tubulitis	Glom.	Vascular
Untreated	4	4	4	4	4	4
Low-dose	3	1	1	1	0	0
Intermediate	4	2	0	2	0	0
High-dose	4	2	0	3	1	0

^a Scoring: 0, negative; 1, mild changes; 2, moderate changes; 3, severe changes; 4, very severe changes. Mono., mononuclear cell infiltration; Glom., glomerular changes; vascular, vascular changes.

Potential clinical applications of LF 15-0195 seem to be promising. LF 15-0195 was proved to be a potent immunosuppressant, especially in depressing the humoral response. It is likely that donor antigen-sensitized and ABO-mismatched patients could benefit the most, on the basis of the properties of LF 15-0195 and the results of previous studies with DSG. Two case reports published by Gannedahl et al. (16) described the use of DSG for patients who developed donor-reactive antibodies soon after kidney transplantation, which resulted in the deterioration of graft function. The patients were treated with plasmapheresis and DSG, which restored graft function and eliminated the cytotoxic crossmatch. Furthermore, DSG administration can result in prolonged graft survival even in the presence of preformed antibodies, as demonstrated by results obtained in discordant xenograft studies. These findings suggest that DSG may be useful in facilitating acceptance of allografts in the setting of ABO-incompatible transplantation. Takahashi et al. (17) reported on 44 patients who had received ABO-incompatible kidney transplants. DSG was administered for 5 days, starting on the transplant day, in addition to methylprednisolone, cyclosporine, azathioprine, and antilymphocyte globulin. The survival rates were 83% at 1 year and 80% at 3 years. Furthermore, DSG was reported to be very effective in reversing ongoing rejection in clinical kidney transplantation (18). Amemiya et al. (18) reported that DSG alone reversed rejection in 81% of cases, compared with 73% of cases treated with methylprednisolone. The combination of DSG and methylprednisolone effectively reversed rejection 94% of the time. On the basis of these successes with DSG, LF 15-0195 may have a role in treating donor-sensitized and ABO-incompatible patients receiving kidney transplants. There is increasing evidence that acute humoral rejection occurs more often in renal grafts than in any other solid-organ transplants (19, 20). Studies evaluating the usefulness of LF 15-0195 as an alternative to DSG would be of interest, because preliminary experiments have already proved LF 15-0195 to be a more potent and more tolerable agent than DSG.

Despite the strong immunosuppressive effects of DSG, preclinical data previously obtained with nonhuman primates demonstrated the toxicity of this agent, including weight loss, leukopenia, thrombocytopenia, anemia, and adverse gastrointestinal symptoms. Furthermore, DSG must be administered through intravenous infusion, because of its low chemical stability in solution and its tendency to elicit a strong local reaction (21). LF 15-0195 is an analogue of DSG with modifications that increase stability in aqueous solution, decrease toxicity, and improve in vivo resistance to oxidative metabolism. This study did not reveal any evidence of thrombocytopenia or fluctuating hemoglobin levels outside normal ranges. Other side effects were negligible, and histologic findings for the organs at necropsy were unremarkable.

The effectiveness of LF 15-0195 in prolonging allograft and xenograft survival in immediately vascularized, whole-organ and cellular transplantation models has been consistent and reproducible (6,22). This study provides further evidence that LF 15-0195 is more potent and more tolerable than its DSG counterpart.

CONCLUSION

LF 15-0195 monotherapy significantly prolonged allograft survival in this life-supporting kidney transplantation model in monkeys, through inhibition of antibody production and through attenuation of the humoral response. LF 15-0195 therapy seems promising because of its lack of significant side effects, its ability to maintain normal renal function for 2 weeks after drug withdrawal, and its effective dose (one tenth that of DSG). These encouraging results strongly suggest that LF 15-0195 could be of potential value in clinical transplantation.

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TRANSPLANTATION

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USE OF OLDER CONTROLLED NON-HEART-BEATING DONORS FOR LIVER TRANSPLANTATION

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Background. Use of liver grafts from non-heart-beating donors (NHBDs) warrants consideration so to expand the donor pool. Because the results of controlled NHBDs (CNHBDs) were acceptable, we have recently tried to expand the criteria to older CNHBDs. Here, we report our experience using liver grafts from older CNHBDs.

Methods. We retrospectively studied our donor records from June 1994 through December 2001. CNHBDs were divided into two groups by age: older donors (O) were more than or equal to 55 years old, and younger donors (Y) were less than 55 years old. We compared donor and recipient demographics and peak laboratory values during the first postoperative week.

Results. Twenty-five grafts from CNHBDs were transplanted in our center. Five livers were harvested from O (63±6 years) and 20 were from Y (32±15 years). No differences other than age in donor characteristics were noted between O and Y. Mean age of recipients was 50 years in both groups. Mean cold ischemic time (CIT) was 5.4 hours in O and 7.3 hours in Y ($P<.05$). Peak glutamic oxaloacetic transaminase (U/L), glutamic pyruvic transaminase (U/L), bilirubin (mg/dL), and prothrombin time (sec) during the first postoperative week were 611, 500, 3.9, and 16 in O and 846, 593, 5.9, and 17 in Y. There were no significant differences between the two groups. The graft survival at 1 year

was 80% in O and 70% in Y.

Conclusions. In our preliminary experience, recipients of liver grafts from older CNHBDs had an outcome equivalent to that of younger CNHBDs. With the strict evaluation of the donors and brief CIT, liver grafts from older CNHBDs may be used to expand the donor pool.

The excellent survival rates reported for patients after liver transplantation (LTX) have increased demand for LTX and have enhanced the disparity between the number of available donor organs and the need for such organs. This supply and demand disparity has led us to face the option of using organs from unconventional donors. Use of organs from controlled non-heart-beating donors (CNHBDs) is one option that can expand the donor pool. A report from the University of Wisconsin (UW) described encouraging results of LTX from CNHBDs (1) and Reich et al. (2) reported excellent results in eight patients who received livers from CNHBDs. We also reported acceptable outcomes of LTX from CNHBDs (3).

Our criteria for the non-heart-beating donors (NHBDs), including both controlled and uncontrolled donors, are as follows: (1) donors have no known medical condition that would exclude donation, (2) donors are not in a high-risk group, (3) cause of donor death is known, (d) donors suffered no penetrating injuries to the abdomen, (e) donors were not victims of homicides with blunt or penetrating trauma to the chest or abdomen. Moreover, we had previously applied a criteria that donors had to be less than 55 years of age. However, although it is well recognized that older donor age negatively influences the outcome of LTX (4, 5), we have no available data on the impact of NHBD age. Because the results of CNHBDs were acceptable, we have recently tried to expand the criteria to include older CNHBDs. Although use of livers from CNHBDs is still controversial and remains

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