Whole Organ Pancreas Recovery Using Ultrasonically Activated Shears

Despite the success of whole organ pancreas transplant for patients with type I diabetes mellitus, the actual number of pancreata recovered and used for transplantation falls significantly short of the potential. According to Organ Procurement and Transplantation Network data, in 2002, less than 30% of deceased donors were pancreas donors, and nearly 20% of pancreata recovered were not transplanted (*1*). Reasons cited for the low rate of pancreas procurement include lack of payment, inconsistency in organ acceptance and sharing, and the technical difficulty of the donor operation (*2*). Standard techniques for pancreas procurement have been previously described, which is usually accomplished by in situ dissection (*3*) or bench dissection after en bloc removal with other organs (*4*). Generally, this procedure is a time-consuming endeavor.

Ultrasonically activated shears (UAS) are new hemostatic devices that use ultrasonic energy to produce tissue coagulation. We hypothesized that UAS can facilitate rapid and safe pancreas recovery. Twelve consecutive cases of pancreas recoveries using UAS were studied, comparing outcomes with 12 consecutive historic cases using conventional instruments. These cases were performed by the same surgical team over a 3-year period (2000–2003). Donor age, weight, and cause of death were similar in the two groups (data not shown). UAS were used to divide the gastrocolic omentum and the short gastric vessels and to isolate the pancreas completely from the retroperitoneum. UAS were not used during dissection around the portal triad. Cannulae were placed in the aorta and the inferior mesenteric vein. At this point, the pancreas is entirely mobilized, and only the superior mesenteric artery, splenic artery, and portal vein need to be divided after aortic cross-clamping and in situ flushing. All cases were multi-organ procurements involving thoracic and abdominal recovery teams. The pancreata were transplanted into 12 recipients simultaneously with a kidney graft using standard techniques. One pancreas thrombosed in the UAS group because of cardiogenic shock and was excluded from the recipient analysis. No duodenal complications were noted. Donor operative time from initial abdominal incision to aortic cross-clamping were significantly shorter in cases using UAS. Recipient serum amylase on postoperative day 1 were also significantly lowered, although serum lipase were similar, which might indicate reduced graft injury. Perioperative transfusion requirements were also similar, suggesting adequate coagulation using the UAS as compared with conventional electrocautery and "clamp and tie" methods (Table 1).

UAS allow rapid coagulation of tissue and small vessels and have become indispensable tools in laparoscopic as well as many open procedures. However, UAS may cause injury around major pancreatic and biliary structures (*5*). Therefore, in our pancreas recoveries, we have limited the use of UAS to away from the portal structures and hepatic artery dissection. In our small series, operative time was significantly reduced without compromise to hemostasis or graft preservation. From a practical standpoint, we believe this finding will encourage more pancreas procurement for whole organ transplantation. We recommend using the UAS routinely as a valuable tool to expedite pancreas recovery and help increase the number recovered.

TABLE 1. Operative time for pancreas procurement and recipient outcomes after transplantation

	UAS	Conventional	*P* value[a]
Donor			
Operative time (min)	112±31	172±27	<0.001
Recipient			
Amylase (U/L)	308±217	519±216	<0.05
Lipase (U/L)	57±20	62±17	N.S.
PRBC transfusion (U)	3.3±1.6	3.9±1.6	N.S.

[a] Unpaired *t* test.
UAS, ultrasonically activated shears; PRBC, packed red blood cells.

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Received 1 September 2004. Revision requested 22 September 2004. Accepted 23 September 2004.
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ISSN 0041-1337/05/7906-735
DOI: 10.1097/01.TP.0000149502.18960.C8

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Mycophenolate Mofetil Is Compatible with CD28/CD154 Costimulatory Blockade in Preventing Transplant Rejection

Costimulatory signals are critically important in T-cell activation and T-cell-dependent allograft rejection. However, blocking CD28 and CD154 costimulation is not always effective in preventing transplant rejection, especially in stringent models (*1*, *2*). The apparent limitation of CD28/CD154 blockade in tolerance induction has stimulated vigorous investigation of other therapeutic reagents that can potentially synergize with CD28/CD154 blockade in tolerance induction. A practical need to study this issue is highlighted by the recent findings that not all reagents (e.g., calcineurin inhibitors) are compatible with CD28/CD154 blockade in blocking allograft rejection (*3*).

In this study, we examined the effect of mycophenolate mofetil (MMF), a potent inhibitor of the de novo pathway of purine biosynthesis (*4*), on the allograft response in vivo and its potential compatibility with CD28/CD154 costimulatory blockade in preventing T-cell activation and acute skin allograft rejection. An in vivo CFSE model, blocking CD28/CD154 costimulation with CTLA-4Ig and MR1 (0.25 mg each, i.p., days 0, 1, 2) markedly reduced the proliferation of both CD4 and CD8 T cells in vivo (Fig. 1A). However, the proliferation of CFSE labeled T cells was not completely inhibited and about 10% of the CD4+ and CD8+ T cells recovered from the allogeneic hosts still entered the cell cycle and divided multiple times. Treatment of host mice with MMF (100 mg/kg/day, i.v., days 0, 1, 2) alone exhibited some inhibitory effect on the proliferation of CFSE-labeled T cells in vivo, as compared to untreated controls. However, treatment with MMF in combination with CTLA-4Ig/MR1 showed a remarkable synergy in blocking in vivo T cell pr0-liferation, and this combined treatment nearly abolished the proliferation of both CD4 and CD8 T cells in vivo.

A skin transplant model was then used to determine the compatibility of MMF and CD28/CD154 blockade in allograft survival. BALB/c skin graft was transplanted onto C57BL/6 recipients and treated with MMF (100 mg/kg/day, i.v., daily for 14 days) with or without CTLA-4Ig (0.25 mg/day, i.p., days 0, 2, 4) and MR1 (0.25 mg/day, i.p., days 0, 2, 4, 6), and graft survival was determined and compared with untreated controls. Treatment with either CTLA-4Ig/MR1 or MMF alone failed to prevent the skin allograft rejection, despite a slight prolongation of skin allograft survival as compared to the controls (Figure 1B). In stark contrast, treatment of recipients with MMF in combination with CTLA-4Ig/MR1 markedly prolonged the skin allograft survival (median survival time, 75 days; n = 7), and 40% of the hosts accepted the skin allograft for over 100 days.

Costimulatory blockade—when successfully used with some conventional immunosuppressive drugs—may have great therapeutic potential in blocking transplant rejection. Here, we demonstrated that MMF, unlike calcineurin inhibitors (*3*), is compatible with CD28/CD154 costimulatory blockade in inhibiting T-cell proliferation in vivo and in prolonging the skin allograft survival. It has been reported that MMF, similar to rapamycin (*3*), does not interfere with priming for activation-induced cell death (*5*). This may be one of the reasons why MMF and CD28/CD154 blockade are compatible in transplant models. Our data suggests that reagents that potentially interfere with the cell cycle apparatus or events required for cell cycle progression may be compatible with costimulatory blockade in preventing transplant rejection. A better understanding of this notion is clearly important in further development of tolerance induction therapies in the clinic.

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This work was supported in part by grants from the National Institutes of Health, the Juvenile Diabetes Research Foundation, and Roche Laboratories.

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Received 31 December 2003. Revision requested 5 February 2004. Accepted 17 August 2004.

ISSN 0041-1337/05/7906-736
DOI: 10.1097/01.TP.0000146966.14808.44

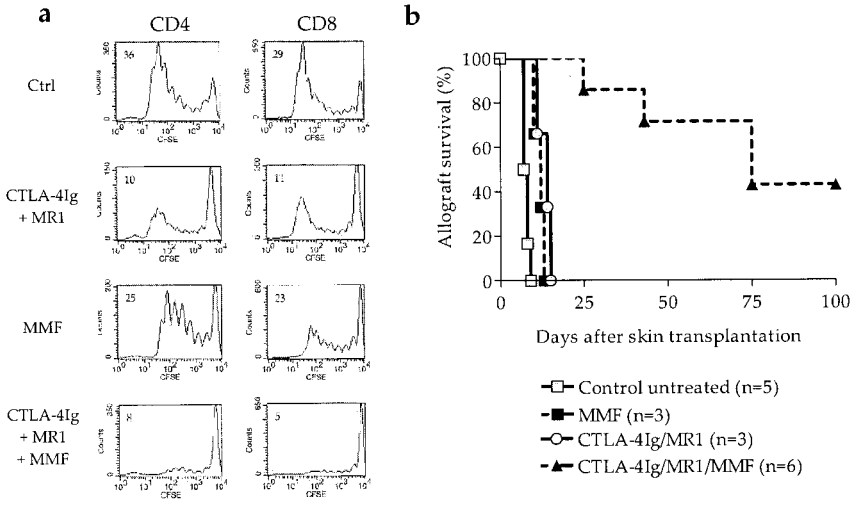

FIGURE 1. Effect of CD28/CD154 costimulatory blockade and MMF on (A) CD4 and CD8 T cell proliferation in vivo and (B) skin allograft survival in C57BL/6 hosts.

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Drug Interaction Between Itraconazole and Sirolimus in a Primary Renal Allograft Recipient

We report a serious drug interaction between sirolimus and itraconazole in a primary renal recipient. A 50-year old female patient received her first cadaveric renal allograft, six years after starting dialysis therapy. Ten years prior, the diagnosis of cutaneous scleroderma was made and the patient developed acute end-stage renal failure four years later, because of a therapy-resistant scleroderma renal crisis with thrombotic microangiopathy. Immunosuppressive therapy at transplantation consisted of sirolimus, mycophenolate mofetil (2 g/day), and corticosteroids, together with induction therapy with basiliximab. Taking into account the history of scleroderma renal crisis, it was decided to not use a calcineurin-inhibitor because of the risk for recurrence of thrombotic microangiopathy. Loading of sirolimus consisted of two days of 15 mg, followed by 10 mg for one day, and a maintenance dose of 5 mg/day. Target trough concentrations (measured by high-performance liquid chromatography (HPLC)) were between 8 and 12 ng/ml and were achieved by day 3 postoperative (Figure 1).

After a period of adequate initial graft function, serum creatinine increased on day 9 posttransplantation and a renal biopsy was performed. Histological examination revealed a Banff grade IA acute rejection (*1*) and, surprisingly, an epithelioid granuloma in the core biopsy with a PAS-positive and silver stain positive fungal structure in its center. Microscopic examination did not allow further identification of the fungus. As this unexpected detection of a full-grown fungus in the renal graft was considered a donor-related infection, the recipient was treated with itraconazole at an initial dose of 600 mg/day, followed by 400 mg/day. Blood cultures for fungal growth and *Aspergillus antigenemia* were negative. As the impact of a fungal septicemia is considered serious, it was decided to treat this patient for at least 3–6 months. Blood sirolimus trough concentration was 9.6 ng/ml on the day itraconazole was started (day 10, daily dose of 8 mg). Because the subsequent trough concentration (6.8 ng/ml on day 11) was below the preset target range, the dose of sirolimus was increased to 10 mg/day. As illustrated in Figure 1, from day 12 onward, sirolimus trough concentrations began to rise rapidly, to as much as 82.5 ng/ml on days 15 and 16 when sirolimus therapy was discontinued. Sirolimus administration was stopped by day 17, and the sirolimus blood concentrations dropped promptly thereafter. Itraconazole therapy was intended to continue for at least six months.

This report clearly demonstrates that azole antimycotic agents, known as inhibitors of CYP3A and P-glycoprotein, can cause clinically relevant drug interactions with sirolimus leading to toxic blood concentrations. Blood itraconazole concentrations up to 5 mg/L did not interfere with the sirolimus HPLC-MS-MS assay. Itraconazole was already reported to be a potentially strong competitive inhibitor of sirolimus elimination in another patient by Kovarik et al. (*2*). Fluconazole, the least potent inhibitor amongst the azoles, did not cause a significant increase in sirolimus concentration in the latter study, while others have clearly demonstrated an interaction when both drugs are combined (*3*).

In conclusion, we report a clinically relevant drug interaction between itraconazole and sirolimus in a renal recipient, resulting in toxic blood trough concentrations of sirolimus. It is therefore advisable to use, whenever possible, alternative azole antimycotics in combination with sirolimus or adhere to stringent and frequent drug concentration monitoring with rapid dose adjustments of sirolimus.

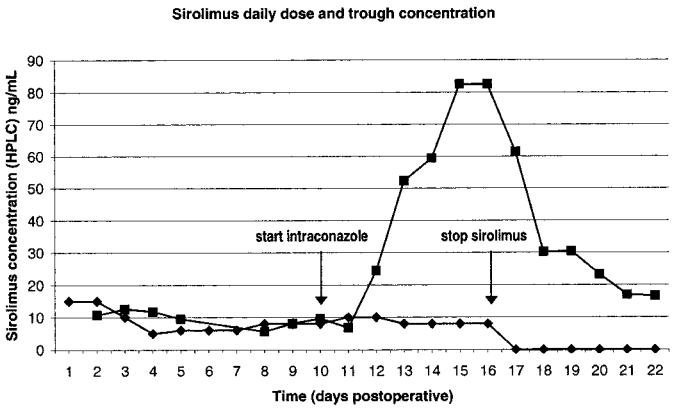

FIGURE 1. Evolution of sirolimus trough blood concentrations (■) and daily dose (♦) before and after itraconazole administration.

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Received 28 June 2004. Revision requested 3 August 2004. Accepted 6 September 2004.

ISSN 0041-1337/05/7906-737
DOI: 10.1097/01.TP.0000147462.86886.F3

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Hepatitis C Virus Recurrence and Idiopathic Thrombocytopenic Purpura after Liver Transplantation in Adult Patients: Role of Splenectomy

Idiopathic thrombocytopenic purpura (ITP) after liver transplantation can be immunological-, drug-, or virus-mediated. Hepatitis C virus (HCV) infection has been reported in cirrhotic patients as cause for ITP, but its importance after transplantation needs to be clarified.

Medical therapy is represented by IV steroids and gamma globulins; the use of polyclonal anti-D (Rh) or monoclonal anti-CD20 antibodies (Rituximab) has been reported (*1*) in cases refractory to immunotherapy. The role of surgery—like splenectomy—has rarely been explored in adult patients. Some authors (*2*) have described partial splenic embolization with gelfoam. We report a case of a 74-year-old liver transplant recipient with HCV recurrence, affected by ITP refractory to medical therapy, successfully treated in our institution (ISMETT-Palermo, Italy) by splenectomy, with a disease-free 44 months follow-up.

In 1996, the patient underwent cadaveric liver transplantation for HCV-related cirrhosis with hepatocellular carcinoma. Six months later, he developed HCV recurrence and was started on interferon (INF)-alfa (Roche, 3 M units, three times weekly) and ribavirin (Schering Plough, 400 mg, twice daily). In July 2000, thrombocytopenia was diagnosed, with a platelet count as low as 8000/mm^3. On admission, the patient presented with cutaneous hemorrhages and a moderately enlarged spleen. Coombs tests and the presence of atypical and antiplatelet antibodies were negative, while bone marrow aspirate showed an increased number of megakariocytes consistent with peripheral platelet destruction. Human immunodeficiency virus, cytomegalovirus, and parvovirus B19 infections were negative; only HCV-RNA polymerase chain reaction was positive, with a liver biopsy showing recurrent chronic hepatitis, moderately active.

All drugs possibly related to thrombocytopenia (INF, ribavirin, and trimethoprim/sulphametoxazole) were discontinued without any improvement. Treatment with intravenous gamma globulins and steroids was attempted but, due to the persistence of severe thrombocytopenia, a splenectomy was performed on August 2000. A liver biopsy showed chronic hepatitis with minimally active HCV recurrence. The spleen did not show any pathological feature. The patient was vaccinated by polyvalent *Pneumococcus* vaccine and discharged on the fourth postoperative day with a platelet count of 160.000/mm^3. Later, anti-HCV medications were started again. At a 44-month follow-up, no decrease in platelet count was found and good clinical conditions without any infection were reported, with HCV recurrence under clinical control.

To our knowledge, HCV infection in cirrhotic patients has been often reported as cause of ITP. In our case, due to lack of other possible factors, HCV recurrence has to be considered—by exclusion criteria—in the differential diagnosis of a posttransplantation ITP. In an overview of international literature on liver transplantation (Table 1), Kita et al. (*3*) failed to treat ITP using IV immunoglobulin and steroids; in one case, it was solved by splenectomy. Troisi et al. (*4*) reported three cases of hypersplenism with thrombocytopenia treated by IV immunoglobulins, steroids, and platelet transfusions. Because of persistent thrombocytopenia, a splenectomy was performed successfully in all patients, but a *Pseudomonas aeruginosa* sepsis occurred in one patient with fatal outcome. Two other cases were reported by Altaca et al. (*5*): IV immunoglobulin and methylprednisolone failed and both patients underwent successfully splenectomy. We conclude that splenectomy in adult population remains the most effective treatment after liver transplantation for thrombocytopenia refractory to medical therapy.

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Received 24 July 2004. Revision requested 10 August 2004. Accepted 31 August 2004.

ISSN 0041-1337/05/7906-738
DOI: 10.1097/01.TP.0000147787.33423.95

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TABLE 1. ITP after liver transplantation in adults requiring splenectomy

Overview	Altaca (5)	Troisi (4)	Kita (3)
Patients (n)	2 (1 pediatric)	3	1
Age (yrs)	15, 52	46, 49, 55	58
Platelet count (mm^3)			
Before splenectomy	3000, 17000	mean 17000	3000
After splenectomy	155000, 210000	mean 180000	300000
Follow-up (mos)	32, 36	Alive: 50, 72	12

ITP, idiopathic thrombocytopenic purpura.

Pure Red Cell Aplasia Associated with Parvovirus B19 Infection in Renal Transplantation: The First Case Report in Mexico

Pure red cell aplasia (PRCA) is characterized by absolute reticulocytopenia with normal leukocytes and platelets. In renal transplants (RT), PRCA is associated with erythropoietin, tacrolimus, and *parvovirus* B19 (PV B19) infections. After 1 month of living-donor RT treated with mycophenolate mofetil (MMF), cyclosporine A, plus prednisone, a 22-year-old woman had a flu-like syndrome and subsequently developed anemia without evidence of external bleeding or renal dysfunction. MMF was changed to azathioprine; however, hemoglobin continued decreasing. Bone-marrow examination showed PRCA. Positive immunoglobulin M against PV B19 were determined and confirmed with polymerase chain reaction for viral DNA. She was discharged without specific treatment, and full recovery was observed. This is the first PRCA case associated with PV B19 in RT patients reported in Mexico. It is recommended to specifically look for this agent in cases of unexplained anemia, mainly because the use of tacrolimus and MMF has been increasing in our setting.

A 22-year-old female with end-stage renal disease of unknown etiology initiated dialysis in July 2001. She received subcutaneous erythropoietin (EPO) alpha because of a hemoglobin (Hb) 6.3 g/dL in February 2003 (last Hb on dialysis 8.7 g/dL). She received living-donor related renal transplant in July 2003; immunosuppression was cyclosporine A, mycophenolate mofetil (MMF), and prednisone. Two weeks later, Hb was 10 g/dL and serum creatinine (SCr) 0.7 mg/dL. One month after transplantation, the patient complained of flu-like symptoms, and 2 weeks later, normochromic normocytic anemia was documented (Hb 7.9 mg/dL, leukocytes 11,400/μL, platelets 441,000/μL), without gross bleeding. MMF was withdrawn because Hb continued to decrease (6.7 g/dL); azathioprine was then initiated (2 mg/kg per day). In the next 2 weeks, Hb was 4.7 g/dL with reticulocytes 0%. Iron kinetics were normal, and there was no evidence of hemolysis. The patient was admitted to hospital in September 2003, and bone-marrow examination showed reticulocytes 0%, normoblasts 5%, young myeloblasts 36%, adult myeloblasts 40%, lymphocytes 11%, eosinophils 3%, monocytes 4%, and plasma cells 1%, establishing a diagnosis of pure red-cell aplasia (PRCA). One week later, positive immunoglobulin (Ig)M antibodies (and negative IgG antibodies) against PV B19 were determined and confirmed with polymerase chain reaction assay for viral DNA. She was discharged without specific treatment because Hb improvement and no acute symptoms were observed; 45 days later, Hb was 12.8 g/dL. One year after transplantation, the patient is asymptomatic; last Hb was 12.4 g/dL and SCr 0.8 mg/dL.

This is a PRCA case associated with PV B19 infection in renal transplantation with evolution similar to others previously reported (*1*). Although presence of anti-EPO antibodies (as previously described (*2*)) was not documented, the clinical course did not support their participation (patient initiated EPO 5 months before transplantation, had acceptable response, and the drug was stopped at transplantation). This patient received cyclosporine A and prednisone, which are used to treat EPO-associated PRCA (*3*). She also received MMF, which has been related to PRCA in renal transplant (*4*). However, in such patients, anemia presented 1 to 18 days after MMF treatment, whereas our patient developed anemia 6 weeks after its use; anemia subsides 4 to 9 days after MMF withdrawal (*4*), but it did not improve after 2 weeks of this drug withdrawal in the present case. Tacrolimus (not used in this case), contrary to cyclosporine, is associated with PRCA (*5*).

Evolution and spontaneous recovery of Hb in this case agrees with that reported with acute *parvovirus* infections (*1*). In contrast with the suggestion of other authors (*1*), it was decided not to provide treatment with intravenous Ig in the present case because recovery and no other tissue/organ repercussions were documented.

To our knowledge, this is the first PRCA case associated with PV B19 in a renal-transplant patient reported in Mexico or Latin America. Because PV B19 is not part of the current anemia screening in transplant patients in our setting, this entity may have been under diagnosed. It is recommended to specifically search for this agent in cases of unexplained anemia, mostly because of the increasing use of tacrolimus and MMF observed in our setting.

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Received 1 September 2004. Accepted 2 September 2004.

ISSN 0041-1337/05/7906-739
DOI: 10.1097/01.TP.0000149339.25047.F4

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Endoscopic Stenting as First-Line Treatment in Duct of Lushka Leaks after Choledochocystic Anastomosis in Two Cases of Liver Transplantation

A duct of Luschka is a persistent congenital connection between a portion of the biliary tree of the right lobe of the liver and the gallbladder. Unlike other biliary radicals, which drain liver segments, they are not accompanied by arteries and veins and measure at most 1 to 2 mm in diameter (*1*). Bile leaks attributed to a Luschka duct after laparoscopic cholecystectomy are rare but have been reported with an incidence of less than 1 in 500 patients (*2, 3*). To our knowledge, demonstration of a clinically significant duct of Lushka bile leak after liver transplantation has only been reported in one case report and was managed by reoperation with direct suturing of the duct (*4*). In this report, we relate a nonoperative treatment based on endobiliary stenting at endoscopic retrograde cholangiopancreatogram (ERCP).

Liver transplantation in our institution is performed using retrohepatic caval transection. Duct-to-duct biliary reconstruction is favored. No T-tubes are used. Two Jackson-Pratt drains are routinely placed at surgery and pulled out at 14 days postoperatively.

The first patient is a 41-year-old male who underwent liver transplantation for congenital hepatic fibrosis. Because of a significant duct-size discrepancy, an anastomosis was created between the recipient's cystic duct and the donor common bile duct (2 mm in size). On postoperative day 12, bile leakage from the wound was observed. In nuclear medicine, a hepatobiliary iminodiacetic acid (HIDA) scan confirmed a bile leak. An ERCP was performed, and the bile leak was demonstrated to be coming from a duct of Lushka in the liver bed (Fig. 1A). Stenting across the choledochocystic anastomosis was performed with a 7 F Cotton Leung endoprosthesis. The drainage from the wound resolved quickly. The patient was discharged home on postoperative day 20, and the stent was removed endoscopically 2 months later. The patient has remained asymptomatic after 30 months.

The second patient is a 70-year-old male who underwent liver transplantation for hepatitis C cirrhosis. The operation was uneventful and, again because of a size discrepancy, the recipient's cystic duct was connected to the small donor common bile duct. On postoperative day 9, the patient became jaundiced. An ERCP revealed a leak from a duct of Lushka (Fig. 1B). Treatment again consisted of stenting across the choledochocystic anastomosis with a 7 F endoprosthesis. The serum bilirubin quickly normalized, and the patient was sent home on postoperative day 25. He then developed an anastomotic stricture that required two serial dilatations. He remains asymptomatic after 20 months.

We postulate that the leaks we observed were a direct consequence of the choledochocystic anastomoses and their small size (indeed, the small caliber of the recipient's common bile duct could only accept a 7 F stent in both cases at ERCP, a most unusual situation). This type of anastomosis is rarely used at our institution, these two patients being the only ones to have received it in the past 10 years. Alternately, it is possible that the Heister "valves" found in the cystic duct may have contributed to a greater pressure in the biliary system, inducing a duct of Luschka leak and preventing spontaneous resolution with external drainage only. We concluded that the reduction in ductal pressure after stent placement encouraged rapid resolution (Lushka duct fibrosis and atrophy) (*1*, *5*). In the future, if such an anastomosis needs to be performed, we may consider immediate stent placement or, alternatively, a more conventional hepaticojejunostomy.

Although a duct of Lushka is an unusual source of biliary leak after liver transplantation, patients with a choledochocystic anastomosis may be at increased risk. We have not found an operative repair to be necessary, and ERCP stenting was an effective therapy. In our opinion, whenever possible, choledochocystic anastomosis should be avoided, but if performed, intraoperative stenting should be considered.

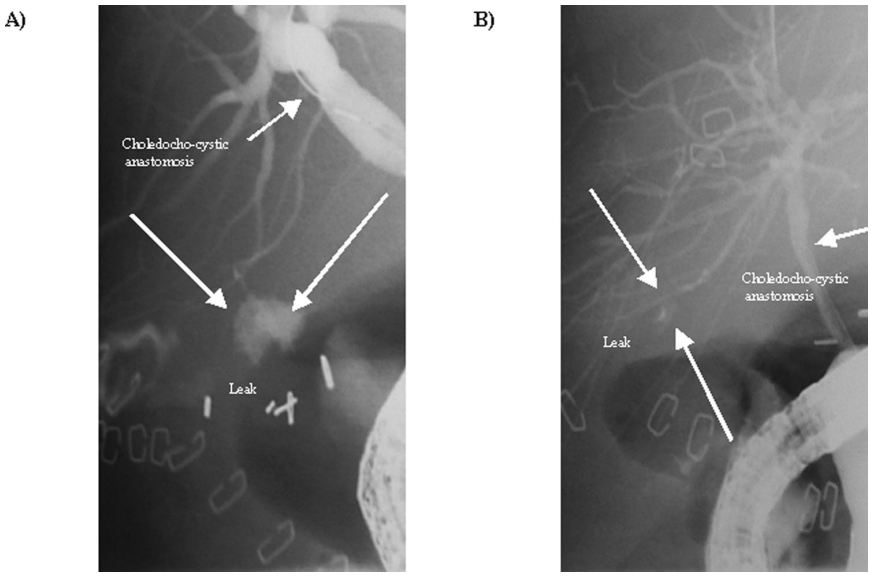

FIGURE 1. (A) Endoscopic retrograde cholangiopancreatogram (ERCP) of a 41-year-old man with duct of Lushka leak after choledochocystic anastomosis at liver transplantation. (B) ERCP of a 70-year-old patient with small duct of Lushka leak after liver transplantation.

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Received 3 August 2004. Accepted 17 September 2004.

ISSN 0041-1337/05/7906-741
DOI: 10.1097/01.TP.0000148804.85008.A2

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Recombinant Factor VIIa for Refractory Hemorrhage after Lung Transplantation

An acute complication of lung transplantation is bleeding. Postoperative hemorrhage can be divided into surgical bleeding and diffuse hemorrhage. Accordingly, therapeutic options are surgical procedures or correction of clotting. In case of postoperative bleeding, the first option is rethoracotomy to repair or exclude surgical bleeding. However, rethoracotomy for diffuse hemorrhage can lead to increased bleeding, due to new tissue damage and consumption coagulopathy. Especially when removal of the native lungs is difficult (e.g., in cystic fibrosis), diffuse bleeding can be intractable. Recombinant activated factor VII (rhFVIIa) has been advocated in patients with severe uncontrolled bleeding associated with surgery or trauma (*1*). We describe the use of rhFVIIa in a patient with diffuse bleeding after lung transplantation.

An 18-year old female with cystic fibrosis was accepted for lung transplantation because of progressive respiratory failure. She developed a tension pneumothorax with electromechanical dissociation requiring cardiopulmonary resuscitation while on the waiting list. This complication resulted in acute tubular necrosis and ventilator dependency. After recovery of renal function, the patient was still ventilator-dependent and was placed on the high-urgency transplantation list. The subsequent bilateral lung transplantation procedure lasted 8 hours. The explantation of the native lungs was laborious. At ICU admission, hemoglobin was 3.6 mmol/L. The drains produced $>$ 500 ml/hour of blood. Despite continuous transfusion of red cells, thrombocytes, and fresh frozen plasma, the patient could not be stabilized.

Six hours after the lung transplantation, the first rethoracotomy took a place. The bleeding occurred at the anastomoses of both pulmonal arteries, which were surgically repaired. There was also oozing from the dorsal thoracic wall. After return to the ICU, the patient was still hemodynamically unstable and 8 hours later a second rethoracotomy was performed. Diffuse hemorrhage was found and surgical correction appeared impossible. After return from the operating room, the patient continued losing blood, became circulatory unstable again, and ventilation was hampered because of insufficient drainage of intrathoracic blood. By then she had received 34 U of red blood cells, 25 U of thrombocytes and 35 U of fresh frozen plasma. Rescue therapy was started with rhFVIIa (Novoseven, 120 μg/kg). After starting this therapy, the patient became transfusion-independent. Three hours later, she received the same dose of rhFVIIa. Hemoglobin values remained stable, thrombocytes remained normal, prothrombin time and activated partial thromboplastin time values normalized, and problems with respiration stabilized and ultimately improved. Six days later, the patient was extubated and 3 days thereafter was transferred to the ward. She is well and at home now.

Case reports describe successes with FVIIa in the control of refractory hemorrhage in a variety of clinical situations: major trauma, thrombocytopenia, postoperative bleeding, intracerebral hemorrhage, orthotopic liver transplantation, and excessive bleeding (*1*, *2*). rhFVIIa induces rapid clot formation at vascular wall sites of damaged endothelium and has little systemic effects (*3*). However, there is some concern about thrombosis at the vascular anastomoses in transplantation surgery (*4*), but in the last six years only 24 thrombotic events have been reported (*2*). Therefore, the thrombosis risk is acceptable in case of refractory bleeding. In severely ill patients like our patient, multiorgan failure might worsen by therapy with rhFVIIa (*5*). Despite this, in patients with uncontrollable blood loss after lung transplantation, rhfVIIa is a justifiable salvage therapy. Due to the small numbers per transplantation center, a formal randomized clinical trial will probably not be feasible. Therefore, both beneficial and detrimental effects should be reported.

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Received 7 July 2004. Accepted 5 August 2004.

ISSN 0041-1337/05/7906-741
DOI: 10.1097/01.TP.0000147319.66838.71

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Intravenous Immunoglobulin and Thymoglobulin Induction Treatment in Immunologically High-Risk Kidney Transplant Recipients

Kidney transplant recipients with complement-dependent cytotoxicity (CDC) B-cell or flow-cytometry (FC) T/B crossmatch positivity are at higher risk for the development of acute and chronic rejection, and decreased graft survival due to anti-HLA donor-specific antibodies (DSA) (*1*, *2*). Intravenous immunoglobulin (IVIG) has immunomodulatory effects and has been shown to facilitate kidney transplantation in crossmatch positive patients by downregulating anti-HLA DSAs (*3*).

We have recently reported our experience with IVIG and thymoglobulin induction treatment in eight CDC and FC B-cell crossmatch positive kidney transplant recipients (four also had FC T-cell crossmatch positivity) (*4*). Only one patient with pretransplant class I anti-HLA DSAs had early acute antibody mediated humoral rejection, and none developed acute cellular rejection. After those eight patients, we treated an additional seven immunologically high-risk living-donor kidney transplant recipients (five female, two male; four white, two Hispanic, one black; three second-transplant recipients) with low dose IVIG (100 mg/kg for 3 days) and thymoglobulin (1.5 mg/kg for 5 days) induction. Five patients received maintenance immunosuppression with cyclosporin microemulsion and two patients received tacrolimus along with mycophenolate mofetil and prednisone. Five patients had FC T- and B-cell (one also had CDC B-cell) and two patients had CDC and FC B-cell crossmatch positivity. Three patients had both anti-HLA class I and II, one patient had class I, and one patients had class II DSAs, as determined by Flow Beads (Luminex). Two patients did not have demonstrated pretransplant DSAs. Two patients developed early acute humoral rejection with positive C4d staining; both had positive FC T- and B-cell crossmatch with anti-class I and II DSAs. One patient with borderline acute rejection responded to IVIG treatment and has stable renal function with a creatinine level of 1.5 mg/dl. However, the other patient with severe acute humoral rejection did not respond to IVIG treatment along with plasmapheresis and rituximab, and eventually underwent transplant nephrectomy. The remaining five patients have stable renal function with a creatinine levels between 0.8–1.0 mg/dl at median 16 months follow-up, range 6–34 months.

The patient's DSAs were studied at 6–12 months after transplantation by Flow Beads. When we evaluated all 15 patients receiving IVIG and thymoglobulin induction treatment, four patients lost pretransplant class II and two patients lost class I DSAs after transplantation. However, four patients developed de novo DSAs. These results indicate that low-dose IVIG and thymoglobulin induction treatment has immunomodulatory effects by downregulating preformed DSAs, but does not prevent the development of de novo DSAs. The immunomodulatory effects of IVIG treatment persist beyond its half-life, indicating ongoing active inhibitory mechanisms and/or induction of neutralizing anti-idiotypic antibodies. Three out of five patients with pretransplant class I DSAs developed early acute antibody-mediated humoral rejection, indicating the importance of preformed class I DSAs in humoral rejection, and lack of adequacy in preventing this event with our current protocol. In view of this, we now use higher doses of IVIG (300–500 mg/kg) treatment in patients with pretransplant class I DSAs. The effects of increasing the doses of IVIG on the development of acute rejection, DSA titers, and graft survival requires further follow-up.

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Received 3 August 2004. Accepted 9 August 2004.

ISSN 0041-1337/05/7906-742
DOI: 10.1097/01.TP.0000146845.93929.EC

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Stump Transureteroureterostomy in En Bloc Kidney Transplantation

Chronic rejection is the leading cause of graft loss (*1*) in kidney transplant recipients and therefore retransplantation has increasingly become a common procedure. Sometimes the procedure is complicated by other factors, which induces creativity.

While scheduled to have a planned bilateral native nephrectomy for recurrent symptomatic hemorrhagic cysts, a 46-year-old man was called to receive his second cadaveric kidney transplant. The etiology of his renal failure was hypertension and his first cadaveric kidney transplant still in place functioned for 6 years and was lost to chronic rejection. The organs were en bloc kidneys from a 3-year-old boy trauma victim.

Through a midline laparotomy bilateral native nephrectomies were performed. The right ileac fossa was occupied by the previous allograft, which we elected to leave in place since it was not symptomatic. Distal aorta and vena cava seemed suitable location for vascular anastomoses. There was immediate function from both kidneys. The transplant ureters were short and small compatible with donor's age and the recipient's bladder and ureters were small due to prolong nonfunctioning. Individual end-to-end anasthomosis seemed to offer the best result. The right native ureter was elected for upper moiety. The stump of the left native ureter was mobilized accompanying the gonadal vessels passed through the sigmoid colon mesentery and provided adequate length for the lower moiety. At the end of dissections all ureters were viable and bleeding from the ends. They were spatulated and anastomoses were stented (Fig. 1).

Foley catheter was left indwelling for 10 days and ureteral stents were removed 1 month following transplant. Nuclear renal scan at 3 months shows normal function and drainage. The recipient has normal serum creatinine.

The blood supply of upper, mid, and distal ureter is from renal, gonadal, and vesical arteries, respectively. The ureter also receives feeding branches from abdominal aorta and common ileac artery (*2*).

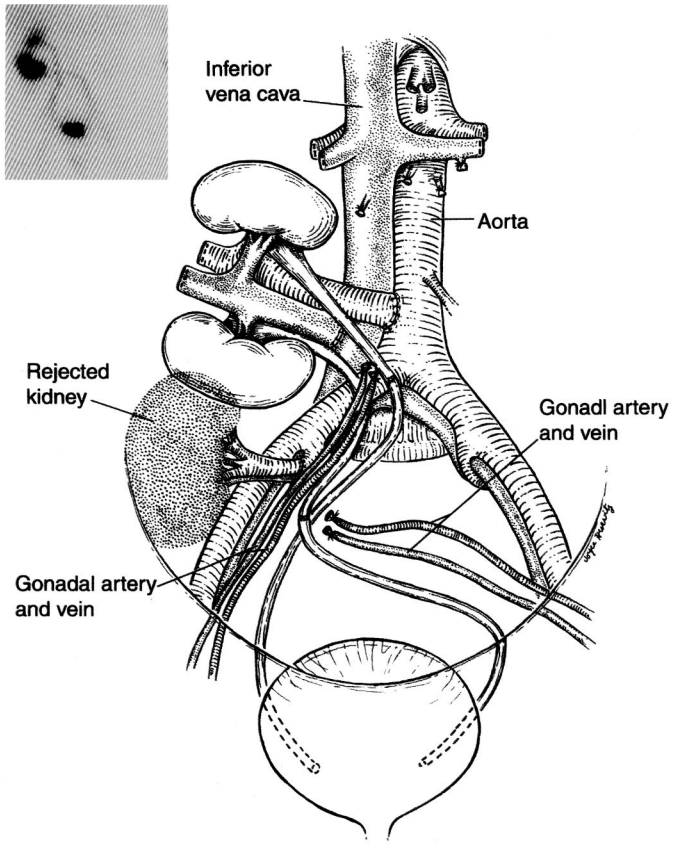

FIGURE 1. Transposition of left ureter to right accompanying gonadal vessels. Inset renal scan demonstrates good function of both renal units.

In our case on the left side while renal and gonadal arteries were disconnected from aorta it seemed that vesical arteries, branches from common ileac artery, and other sources of gonadal blood supply (cremastric and vasal arteries) in retrograde fashion provided additional supply for the ureter.

Our experience shows that stump transureteroureterostomy is a valid option in unusual circumstances however ureter always should accompany the gonadal vessels.

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Received 28 October 2004. Accepted 28 October 2004.

ISSN 0041-1337/05/7906-743
DOI: 10.1097/01.TP.0000155426.80638.A2

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Consideration of Preanalytical Conditions to Use Circulating Matrix Metalloproteinases as Diagnostic Markers

I have read with interest the report by Kuyvenhoven et al. (*1*) regarding the changed concentration of matrix metalloproteinases (MMP) 2 and 9 in patients after liver transplantation. Serum MMP-9 was significantly increased in patients with rejection compared with those without rejection and culminated at one week after transplantation. Since MMP-9 immunoreactive cells were found in great quantities in liver biopsies of the respective patients, the elevated serum MMP-9 was explained by the release from infiltrating neutrophils and lymphocytes. An impaired clearance of MMP-9 during the rejection crisis was ruled out. The authors concluded that the increased serum MMP-9 might serve as marker in patients with acute rejection (*1*). However, I would direct the interested reader's attention to the significance of blood collection as preanalytical determinant for MMP measurements to avoid diagnostic misinterpretations. Since the collection method of blood samples constitutes an essential interference factor for the measurement of circulating MMPs, I want to refer in the following to data previously published in analytical journals (*2*, *3*).

Results of the author's own experiments are summarized in Figure 1. Briefly, blood samples from eight healthy subjects were prepared in commercially available devices (Sarstedt, Nümbrecht, Germany) by centrifugation at 1600*g* for 15 minutes within 30 minutes of venipuncture. Plastic tubes were used either without additives (S-Monovette tubes 01.1728) or with kaolin-coated plastic granulate (S-Monovette tubes 01.1601) to obtain pure serum (serum-1) or serum after enhanced clotting (serum-2). Plasma samples were collected as citrate plasma (plasma-C) or heparin plasma (plasma-H) (S-Monovettes, coated with sodium citrate or lithium heparin). MMP-9 measurements were performed with an MMP-9 ELISA (MP2211, Medac Diagnostika, Wedel, Germany). Figure 1 shows that MMP-9 concentrations are about 10 times higher in serum than in plasma. In addition, different concentrations were also found in serum samples depending on the kind of serum preparation. Serum samples collected in tubes with clot activator (serum-2) had about threefold higher MMP-9 concentrations than pure serum samples (serum-1). As platelets and leukocytes contain high concentrations of MMP-9, the variable release of MMP-9 from blood cells during the platelet activation or sampling process could cause these differences (*3*). On the other hand, changes of white blood cell count depend on the time elapsed since liver transplantation, as well as complications such as acute rejection (*4*, *5*). Therefore, it can be assumed that the changed MMP-9 concentration as described (*1*) did not exclusively derive from the inflammatory cells in the transplanted liver. Kuyvenhoven et al. (*1*) used serum samples for their measurement but did not describe the kind of preparation.

In summary, in order to establish an accurate relationship between MMPs in the peripheral blood and pathological processes in tissue/organs, and to use them as diagnostic and prognostic indicators, the effect of blood sample preparation has to be notably considered. Recently, the use of blood samples collected with sodium citrate was suggested to avoid the detrimental effect of other anticoagulants or serum, and to optimize the diagnostic validity of MMPs in peripheral blood (*3*).

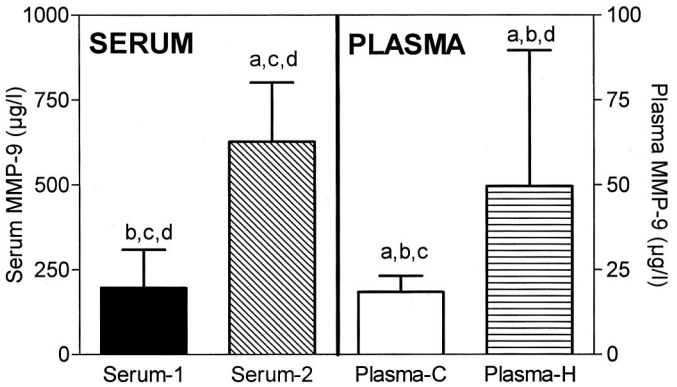

FIGURE 1. Effect of blood collection on the MMP-9 concentration in serum and plasma. MMP-9 measurements in samples prepared from blood of eight healthy adults; mean values with 95% confidence intervals are shown. *Left*: Serum-1, pure serum prepared in Monovette tubes without additive; serum-2, serum prepared in tubes containing kaolin-coated granulate as clot activator. *Right*: Plasma-C and plasma-H, prepared in tubes coated with sodium citrate or lithium heparin. Significant differences of at least $P<0.05$ (ANOVA, repeated measures; posterior test according to Tukey) between the samples are indicated by the following symbols: (*a*) significantly different from serum-1, (*b*) from serum-2, (*c*) from plasma-H, and (*d*) from plasma-C.

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Received 19 July 2004. Accepted 3 August 2004.

ISSN 0041-1337/05/7906-744
DOI: 10.1097/01.TP.0000147342.04121.D5

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Preanalytical Conditions and Circulating Matrix Metalloproteinases

We appreciate the interest of Klaus Jung in our study and his response to the manuscript on serum matrix metalloproteinases (MMP)-2 and MMP-9 after human liver transplantation. We share his concern regarding the effects of blood sample collection on the accuracy and diagnostic value of serological MMP determinations in pathological processes. We were aware of the fact that the preanalyte used might have a considerable impact on the results obtained. Exactly this concern made us perform a preevaluation of different blood samples from seven liver transplant patients, collected at six time points from pretransplantation to one year of follow-up, before we executed the complete study. We routinely collected simultaneously serum (without clotting activator), sodium-heparin, and K_2-EDTA blood samples through unforced blood sampling of the patients in the morning, using the Becton Dickinson Vacutainer system. The samples are also processed simultaneously by centrifugation and stored at -70°C until analysis.

Similar to Jung et al. (*1*, *2*) we also found considerable differences in the MMP levels, as determined by ELISA, depending on the type of analyte used (see Table 1).

Overall, EDTA-plasma samples were found to have a two- to threefold lower MMP level compared to the serum and heparin-plasma levels. However, the latter two samples had higher MMP-9 levels in serum but comparable MMP-2 levels. In addition, the correlation in the MMP levels between the different analytes were highly significant ($P < 0.0005$; i.e., MMP-9: $0.53 < r < 0.57$; MMP-2: $0.73 < r < 0.89$). More importantly, however, were the levels over time with the different analytes. As shown in Figure 1, the patterns of the MMP levels over time are very similar for the different analytes, peaking for MMP-9 (Fig. 1A) and decreasing for MMP-2 (Fig. 1B); this was less impressive for the EDTA-levels, which were consistently low for both MMPs.

These observations do indicate, in our opinion, that the type of analyte does affect the level of the MMPs; with appropriate processing, the dynamics of the levels over time within the different analytes do show a similar pattern. In addition, the consistency in both the sample preparations and MMP results of the different analytes seems to exclude a variable release of MMP-9 from blood leukocytes due to sampling as the cause of raised levels in serum, obtained without clotting activator, of our liver transplant patients with allograft rejection. Jung also suggested that the raised MMP-9 level in the patients with rejection might be related to increased numbers of white blood cells and/or platelets in the circulation, which release MMP-9. Although we did find a significantly higher serum MMP-9 level at one week after transplantation in the patients with rejection compared to the patients without rejection [233 ± 29 ($n = 13$) versus 158 ± 16 ($n = 20$) ng MMP-9/ml, $P < 0.05$], at the same time point the white blood cell and platelet counts were almost the same for both groups (respectively, 11.9 ± 1.3 versus 11.0 ± 1.3 and 156 ± 24 versus 136 ± 15; all 10^9/L). These findings support our conviction that the raised serum MMP-9 levels with liver rejection are most likely, though probably not exclusively, derived from infiltrating inflammatory cells within the liver (*3*).

Interestingly, in another study we observed a relation between citrate-plasma MMP-9 levels and severity of ischemia/reperfusion injury of the transplanted liver (*4*). Thus, the suggestion that sodium-citrate plasma might be the best analyte to be used for MMP assessments, as suggested by Jung and by Mannello et al. (*5*), might be valid, although we found relatively low preoperative levels for MMP-9 (55 ± 6 ng/ml) and for MMP-2 (782 ± 76 ng/ml) in the 24 liver transplantation patients included in this international study (*4*), a phenomenon also described in the other studies (*2, 5*). Since most of our studies on MMPs also include evaluation of their activity, either by zymography or immunosorbent activity assays, we prefer to use serum obtained without clotting activator or citrate-plasma because of the known interference of EDTA and heparin in these kinds of analyses.

In conclusion, we concur with Jung that consistency in blood sample preparation is essential in the assessment of the clinical and diagnostic value of circulating MMP levels in relation to pathological disease processes. We do think, however, that when this is executed accurately the type of preanalyte used might be more relevant to the obtained absolute MMP level and to

TABLE 1. MMP levels in different analytes of liver transplant patients

Determinant*	Serum	Heparin-plasma	EDTA-plasma
MMP-9	$246 \pm 27^{a,b}$	168 ± 23^{a}	81 ± 17
MMP-2	2846 ± 250^{b}	2755 ± 296^{a}	1098 ± 47

* n=42, in ng/mL sample; data as mean ± SEM.
[a] P<0.0005 versus EDTA-plasma; [b] P<0.005 versus Heparin-plasma.

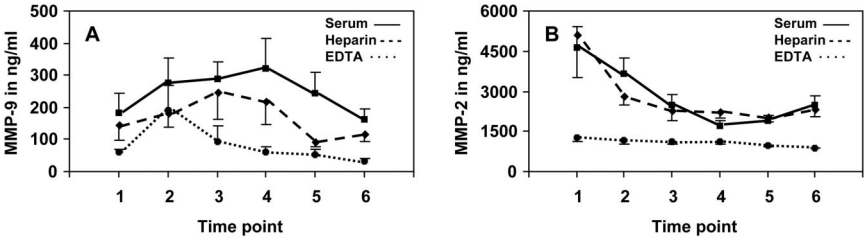

FIGURE 1. (A) MMP-9 and (B) MMP-2 levels in the different analytes of liver transplant patients. Each point represents the mean ± SEM of seven patients/samples at different time points during follow-up (*1*, pretransplantation; *2*, within 5 days posttransplantation; *3*, at 1 month posttransplantation; *4*, at 3 months posttransplantation; *5*, at 6 months posttransplantation; and *6*, at 1 year of follow-up).

the type of analysis to be subsequently performed (e.g., activity assay versus ELISA) but less to the dynamics of the changes in the MMP parameter under study.

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Received 17 August 2004. Accepted 31 August 2004.

ISSN 0041-1337/05/7906-746
DOI: 10.1097/01.TP.0000147343.33801.35

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Probable Interaction of Bergamottin and Cyclosporine in a Lung Transplant Recipient

Cyclosporine is a drug that warrants continuous monitoring due to patient variance, interactions with other medications, and interactions with concurrent ingestion of some fruit juices. Recently, it was confirmed that grapefruit juice and lime juice augment the bioavailability of medications (such as felodipine) by inhibiting presystemic metabolism mediated by cytochrome P450 (CYP) 3A4 (*1*,*2*). Other mechanisms are probable. This action appears to be directly related to the concentration of bergamottin—a substance thought to be able to irreversibly inhibit CYP 3A4 activity—in the juice. Other furanocoumarin-like substances in these juices may contribute to increased absorption of medications utilizing the CYP3A4 pathway. One patient experience has caused the authors to suspect other drinks potentially have a pharmacokinetic effect on cyclosporine.

The patient was a 32-year-old male who, for six years prior to a lung transplant, had been treated with epoprostenol infusion via ambulatory pump for primary pulmonary hypertension. The patient underwent double lung transplantation and did well, being discharged after 11 days. Since that time the patient has been maintained on several medications, including cyclosporine. Two weeks after discharge from the hospital, the cyclosporine trough serum concentration was adequate (358 ng/ml) and no signs of toxicity existed. On the next four visits, spanning 24 days, cyclosporine levels varied, and were 676, 319, 374 and 761 ng/ml respectively. These serum concentrations were not the result of altered cyclosporine doses, dosage form change, changes in the time of day of dosing or sampling, patient illness, or changes in medications given concomitantly.

A significant discovery was that the patient drank Sundrop citrus soda with breakfast on the days when higher serum concentrations were found. (Sundrop citrus soda is a registered trademark used under license by Dr. Pepper/Seven Up, Inc., 2002.) The history revealed that, over time, the rise in cyclosporine directly matched the days when cyclosporine levels were high (676 and 761 ng/ml). Dr. Pepper/Seven Up has confirmed that the citrus drink contains furocoumarins. The patient has now avoided the ingestion of this drink at the time of dosing, and cyclosporine serum concentrations have normalized and are consistently predictable.

It is the authors' opinion that practitioners should be aware of the eating and drinking history of their patients prescribed medications known to interact. It is also suggested that citrus flavorings, flavoring oils, and the presence of fresh juices in grocery products be labeled to include these items. In particular, we suggest that labeling include a caution that ingestion may interact with medications the person is taking. Patients should be instructed to take their medications without the concurrent use of these products.

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Received 10 March 2004. Accepted June 2, 2004.

ISSN 0041-1337/05/7906-746
DOI: 10.1097/01.TP.0000148913.75002.33

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Sun Drop Citrus Soda and Cyclosporine Interaction: Comments on Causality and Recommendations

I read with interest the letter by Drs. Johnston and Milstone (*1*). They reported the case of a lung transplant patient stabilized on cyclosporine at a dose that produced a therapeutic serum drug concentration soon after discharge. However, concentrations ranged between therapeutic and potentially toxic on ensuing assessments. After excluding obvious possible causes, they considered diet, which revealed that the patient had consumed a particular drink (Sun Drop citrus soda) at breakfast on the two days when serum cyclosporine concentrations were essentially doubled. Subsequent avoidance of this citrus product resulted in consistent therapeutic cyclosporine concentrations. The authors encouraged better awareness of diet as a potential cause of adverse drug interactions. Additionally, they urged labeling of citrus products in grocery stores to indicate ingredients and caution against ingestion with interacting medications. Moreover, they promoted instruction of patients by healthcare professionals about avoidance of these fruit products during pharmacotherapy.

A key discriminator of causality of an interaction is response on rechallenge. Although retrospective, the data supported consistency of effect on serum cyclosporine concentration with repeat consumption of Sun Drop citrus soda. Moreover, prospective information showed that the problem did not reoccur by exclusion of this drink from the diet. Thus, it seems logical to conclude that the potentially toxic serum cyclosporine concentrations were likely based on ingestion of Sun Drop citrus soda.

Creating a greater awareness of diet-induced drug interactions appears much needed. The public generally considers foods as safe and advantageous for human health. This concept is reinforced by lack of formal guidelines about consumption. Consequently, patients don't often feel the need to discuss their diet with healthcare professionals and practitioners don't routinely obtain accurate histories or dispense advice on diet, the exception being certain foods and use of warfarin. However, this case report provided further support that certain dietary constituents might provoke toxicity from excessive drug concentration. Consequently, taking a careful dietary history could help explain constant variability or an abrupt detrimental change in the medical status of a patient. Moreover, controlling diet may more effectively prevent unwanted drug effect than changing therapy.

Better labeling of grocery products may be also beneficial. Citrus drinks frequently do not specify what fruits or amounts are present. Yet, it has been known for more than 13 years that grapefruit can increase plasma concentrations of drugs, including cyclosporine, by enhancing oral bioavailability (*2*, *3*). More recently, Seville (bitter) orange and lime were shown to augment concentrations of the calcium antagonist, felodipine (*4*, *5*). Current labeling of Sun Drop citrus soda does indicate that it contains components from citrus fruits. Conversely, the mechanisms or specific constituents in this drink that apparently caused the markedly augmented serum concentrations of cyclosporine are not clear. Nevertheless, it seems appropriate to indicate on the label when citrus drinks contain juice or other portions from grapefruit, Seville orange, or lime and then state that there may be an unwanted interaction with certain medications. Such information would likely assist healthcare professionals and patients to optimize drug use.

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Received 8 April 2004. Accepted 2 June 2004.

ISSN 0041-1337/05/7906-747
DOI: 10.1097/01.TP.0000148914.13844.D9

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