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AIM AND SCOPE

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AIM AND SCOPE

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EDITORS FOR THIS ISSUE

REVIEW

Bioengineered stem cells as an alternative for islet cell transplantation

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Abstract
Type 1 diabetes is an autoimmune and increasingly prevalent condition caused by immunological destruction of beta cells. Insulin remains the mainstay of therapy. Endeavours in islet transplantation have clearly demonstrated that type 1 diabetes is treatable by cellular replacement. Many challenges remain with this approach. The opportunity to use bioengineered embryonic or adult pluripotential stem cells, or islets derived from porcine xenograft sources could address future demands, but are still associated with considerable challenges. This detailed review outlines current progress in clinical islet transplantation, and places this in perspective for the remarkable scientific advances now occurring in stem cell and regenerative medicine approaches in the treatment of future curative treatment of diabetes.

Key words: Islet transplantation; Hypoxia; Stem cell; Diabetes

Core tip: This paper gives a historical overview of the use of islet transplantation for the treatment of type 1 diabetes mellitus. Islet cell transplantation has seen enormous development over the years; however, this has not been without its limitations. The aim of this paper is to provide an overview of the feasibility of an alternative cell source for clinical islet transplantation.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterised by impairment of pancreatic beta cells resulting in complete insulin deficiency. Current treatment requires multiple insulin injections and dietary restriction. However, even with strict management and blood glucose level monitoring, episodes of hypoglycaemia and chronic diabetic complications (such as nephropathy, retinopathy, and neuropathy) still occur[1]. Islet transplantation offers an alternative treatment option through restoration of the physiological response to changes in blood glucose levels. However due to ongoing clinical challenges, this modality is only offered to a select group of patients.

The Edmonton group was the first to demonstrate sustained long-term insulin-independence in 2000[2] through islet transplantation and from this success the "Edmonton Protocol" was established. Islet transplantation is a relatively non-invasive procedure that involves infusion of islets containing the insulin-secreting beta cells derived from cadaveric donors, into the recipient's portal vein. Despite high rates of insulin independence one-year post-transplant, patient follow-up has demonstrated islet graft attrition with time such that insulin independence rates significantly decline 5-year post-transplant with patients being restarted on small to modest amounts of insulin[3,4].

A major caveat to the current protocol is that a subset of patients will require repeat islet transplantation. One reason for this is due to poor initial engraftment[5] resulting in a reduced initial beta cell mass. The current limitations to engraftment are multiple and include variance in the islet isolation process[6], site of transplantation[7,8], and instant blood-mediated inflammatory reactions[9,10]. The outcomes of transplanting islets into alternative transplant sites have been well studied over the past two decades, but no site has received as much attention as the subcutaneous site[11-16]. This is in large part related to its potential for less invasive retrievability, which may translate into increased safety. It should be pointed out however, that despite the obvious limitation of the intraportal hepatic site, no patient has yet been rendered insulin-free by cellular transplantation in a site other than the liver. The other reason for requiring a subsequent transplant is that islet cells undergo progressive graft failure[17,18] largely related to auto- and alloimmunity. Lifelong immunosuppression has played a central role in the success of the current islet transplantation protocol. Despite ongoing development of immunosuppression agents and optimised regimens, progressive graft loss is still an enduring issue. This is further exacerbated by the diabetogenicity of many of the immunosuppression drugs implemented in clinical practice[19]. Furthermore lifelong immunosuppression regimens are also related with significant morbidity to the patient[20,21]. As an alternative to immunosuppression, the utility of immune isolating devices is currently being explored[22].

A review of the current islet transplantation protocol indicates well-recognised limitations. Herein, we discuss the potential of using bioengineered stem cells as an alternative cell source to address the acute organ donor shortage and meet potential future need in the ever-expanding diabetes population. A historical summary will discuss the roadblocks that were overcome in developing the "Edmonton Protocol", with a highlight on the research that has evolved since describing the pathophysiology behind its current limitations. The use of immunosuppression-free regimens and the use of the subcutaneous site will be reviewed. Predicted outcomes of synergising these research areas with bioengineered stem cells will be discussed. Focus will be on the feasibility and limitations of translating this idea into clinical practice.

RESEARCH STRATEGY

Studies were identified through Medical Subject Headings in PubMed. The following text words were used: (1) ["Islets of Langerhans Transplantation" (Mesh)] AND ["Neovascularization, Pathologic" (Mesh)]; (2) ["Islets of Langerhans Transplantation/methods" (Mesh)] AND ["Subcutaneous Tissue" (Mesh)]; (3) ["Islets of Langerhans Transplantation" (Mesh)] AND ["Vasculature" (Mesh)]; (4) ["Islets of Langerhans" (Mesh)] AND ["Stem Cells" (Mesh)]; and (5) ["Islets of Langerhans" (Mesh)]) AND ["Immunosuppression" (Mesh)]. In addition, reference lists of all relevant articles were examined for further pertinent studies. Inclusion criteria included articles published in peer-reviewed journals and animal studies. Exclusion criteria included gray literature, novel lab techniques, and articles that lacked an abstract. The search was limited by the ability to access articles. Primary authors and experts in the field were not contacted to identify additional published, unpublished, or "in-progress" studies. Information was last accessed in June 2014.

DISCUSSION

Historical vignettes

Insulin was first discovered through the efforts of Nobel Prize winners Banting and Best in the 1920s[23,24]. As a result of their efforts, exogenous insulin replacement therapy is and remains the mainstay treatment for T1DM[24]. However, even with strict regulation, there is a small subset of patients with "brittle diabetes" who are unable to achieve normoglycemia and suffer from life-threatening hypoglycaemic unawareness[1]. It is this group of patients who will benefit the most from cellular replacement therapy. Interestingly, attempts at cellular replacement actually preceded Banting and

Best's discovery of insulin by twenty years, whereby efforts were made to treat a 13-year-old diabetic child with fragments of sheep pancreata[25].

Given the clinical difficulties in managing patient complications through insulin replacement therapy alone, attention turned to transplantation with the hopes of offering a cure to diabetes. Kelly and Lillehei at the University of Minnesota were the first to attempt whole pancreas transplantation in 1966[26] carried out as a simultaneous pancreatic kidney (SPK) transplantation. Over the past several decades the surgical techniques have been refined, with most attention being directed towards exocrine drainage of the pancreas into the recipient intestine[27]. Since its introduction, over 35000 transplants have been carried out worldwide, mostly as SPK, with proven success in reversing diabetes and achieving insulin independence[28]. However, this involves major abdominal surgery with procedural techniques that are still undergoing refinement. Subsequently, it is primarily reserved for patients with end-stage renal disease associated with T1DM for whom dialysis and insulin independence can be achieved simultaneously.

An alternative to whole pancreas transplantation is islet transplantation. The ability to isolate islets evolved from the work of Best and Banting in their endeavours to isolate insulin[23]. The first to isolate islets was Polish Professor, Stanislaw Moskalewski, who prepared pancreatic islets in 1965 from a guinea pig for physiological study[29]. In 1972, Paul Lacy from Washington University was the first to demonstrate the ability to reverse diabetes through islet transplantation in an induced diabetic animal model[30]. Further advances in islet transplant research came from Kemp *et al*[31] who completed a major animal study demonstrating the superiority liver implantation *via* the portal vein compared to other sites, such as the renal capsule and subcutaneous space, in the size of the required cell mass to reduce hyperglycaemia. The first clinical attempt to translate these findings in a patient with type 1 diabetes led to one month of insulin independence, followed by cellular rejection attributed to inadequate immunosuppression[30]. Ricordi *et al*[30] at Pittsburgh University improved on these findings considerably with several clinical cases of prolonged diabetes reversal in 1990. Their use of the newly introduced FK-506 (tacrolimus) agent and steroid avoidance protocols together with cluster transplants after abdominal exenteration for abdominal malignancies (in the absence of T1DM), led to considerable success[30]. An essential contribution to clinical translation was the introduction of the "automated method" for islet extraction and the Ricordi chamber developed by Camillo Ricordi, which remains the mainstay technique for clinical islet isolation currently[32-34].

The first human trials aimed at treating autoimmune T1DM through islet allotransplantation began in 1974 under the direction of Sutherland *et al*[35] at the University of Minnesota. The included patients had all undergone previous kidney transplants and were already on immunosuppression regimens. This trial demonstrated the ability to reduce insulin requirements, but usually failed to achieve sustained insulin independence[35]. The inability to achieve sustained insulin independence also hampered subsequent clinical trials up until 2000. In 2000, Shapiro *et al*[2] at the University of Alberta demonstrated the ability to sustain insulin independence out to one year post-transplant in all seven of their initial patients. This is now considered one of the major milestones in the history of islet transplantation. This success allowed for large improvements in the current islet transplant protocol and led to international recognition of islet transplantation, with numerous new programs being developed worldwide demonstrating both reproducibility and further refinement and improvement of these results[18].

Longer-term follow-up of patients transplanted using the "Edmonton Protocol" demonstrates ongoing limitations of islet transplantation durability. An initial 5-year patient follow-up demonstrated graft loss all but 15% of patients in the program[17]. Furthermore, around 25% of patients required a second transplant after two to three years[36] in order to achieve sustained graft function. Even with these shortcomings, the "Edmonton Protocol" offered a benchmark for subsequent islet transplantation research. With redefined immuno-suppression therapy, islet transplantation is now able to match the results of pancreas-alone transplantation, with 5-year insulin independence rates of 50% now being observed[37,38].

Current protocol

The current islet transplant protocol begins with isolation of donor islet cells. Ideally, when a donor pancreas organ becomes available, the islets should be procured within 6-8 h. The isolation of islets involves both mechanical and enzymatic digestion. After digestion, the isolated islets must undergo purification in order to collect as much islet mass as possible (minimal requirement is ≥ 5000 islet equivalents per kilogram for initial transplants). Isolated cells are then kept in 250 cc of transplant media culture for 24-72 h, and must meet set product release criteria prior to being used for transplantation.

Islets are transplanted by gravity infusion into the portal vein. Percutaneous access is performed by an interventional radiologist under local anaesthesia, ultrasound and fluoroscopic guidance. The isolated islets [still in transplant media and now loaded with heparin (70 units/kg recipient weight)] are then subsequently infused[39]. A successive rinse solution is then given. As the catheter is removed, the created tract is sealed with radio-opaque thrombostatic material to prevent the risk of post procedural bleeding[40,41].

Review of the isolation process and subsequent transplantation has demonstrated a negative relationship on the ability of the cells to survive post-transplantation.

This is partially because the isolation process strips the islets of their inner vascular network[6]. Unlike whole organ transplantation, islets initially are not directly anastomosed to a blood supply and as such, remain markedly hypoxic within the portal venous terminal branches until they are able to establish a direct connection to a blood supply through p. This initial process may take up to 10-14 d to begin, and vascular remodelling ensues over several months thereafter. Although the portal vein does allow for diffusion of nutrients, including oxygen, into the islets, the lower oxygen tension of the liver compared to the pancreas places the islets in a relatively hypoxic environment. Chronic hypoxia then occurs due to a delay in engraftment, which ultimately leads to a large proportion of dead cells. The delay in engraftment is highly dependent upon stress-cell signalling between islet and surrounding hepatic arterial vasculature for stimulation of angiogenesis and remodelling[42,43].

It is quite remarkable that the entire metabolic regulation provided by the transplanted islets comes from just a small fraction (perhaps 30%-40%) of islets that eventually revascularize over time[5]. Another caveat, is that, even if the cells are able to engraft, their inner vascular density is not as robust as native islets[44,45], which may contribute to progressive graft failure due to ongoing relative hypoxia[46] (Table 1).

Immunosuppression

Allogeneic transplantation faces the challenges of allo-immunity. Immunological mechanisms underlying allo-immunity are complex and are related to both T-[47] and B-cell[48] mediated immune reactions. Without appropriate immunosuppression, this results in acute rejection and subsequent irreversible destruction of the donated tissue. While the risk of acute rejection may be lessened to a small degree through close tissue matching[49], long term graft rejection will occur if the immune system is not appropriately suppressed.

The autoimmune pathogenicity of T1DM poses a unique challenge to immunosuppression regimens. The destruction of pancreatic beta cells occurs in genetically susceptible individuals as a result of the formation of autoantibodies (anti-insulin, anti-GAD, and anti-IA-2)[50,51]. Theoretically, when pancreatic islet cells have been completely abolished, these autoantibody titres should decrease, but the autoreactive B cells that produce anti-islet antibodies remain quiescent. With the re-introduction of islet cells via transplantation, these autoreactive B cells undergo clonal expansion, such that the graft is exposed to a primed and more chronic immunological attack. This is supported by liver biopsies from patients undergoing transplantation under the "Edmonton Protocol" where beta-cells have been specifically destroyed[52].

The armamentarium of immunosuppressive drugs has expanded since the early days of transplantation. Initial drugs included high dose corticosteroid therapy and anti-metabolite compounds such as 6-mercaptopurine and azathioprine. The introduction of calcineurin inhibitors (cyclosporine and subsequently tacrolimus) in 1983 was a major turning point, as these agents are more selectively targeted to immune suppression with less off-target impact[19]. These are not without side effects and are known to increase the risk of developing de novo cancers, hypertension, dyslipidemia, diabetes and opportunistic infections[20,21]. Islet transplantation is a life-enhancing rather than life-saving therapy, and therefore these side effects remain of particular concern as they contribute significant morbidity with chronic use. In addition, many of the available immunosuppression drugs are toxic to the islets and interfere with islet function. While graft failure is likely multifactorial in its pathogenesis, exposure to diabetogenic immuno-suppressants (corticosteroids and calcineurin inhibitors) plays a negative role.

Current immunosuppression used in Edmonton and many other international sites for islet transplantation consists of a combination of induction therapy, anti-inflammatory therapy and maintenance therapy. Induction therapy is designed to deplete T-cells prior to transplantation and in clinical trials, has demonstrated superior long term results[53]. Following transplantation (up to post-transplant day 10), anti-inflammatory agents are given and include anti-TNF (etanercept) and anti-interleukin 1 receptor antagonist (anakinra). Patients are then placed on maintenance therapy. Currently the Edmonton group uses a combination of tacrolimus and MMF for maintenance[38]. Optimisation of maintenance therapy poses significant challenges as detailed above, including beta-cell toxicity and diabetogenicity[19]. However, large improvements have been observed with these redefined immuno-suppression regimens, with 5-year insulin independence rates of 50% being achieved[17,37,38].

Rationale for the use of porcine xenografts and human embryonic stem cells

The current limitations of islet transplantation place a tremendous burden on the system to obtain the needed donor cell populations. As detailed above poor survival post transplantation as well as progressive graft failure even with optimised immunosuppression regimens means that some patients will go on to require a subsequent transplantation. If islet transplantation is to be a sustained treatment option for all type 1 diabetic patients, alternative cells sources will be required. Currently two options are being explored as potential alternative cell sources. These include xenografts and bioengineered human embryonic stem cells.

The use of xenografts for islet transplantation has been studied extensively as an alternative cell source. As a result of this research, the international xenotransplantation association was established[54]. This association has been instrumental in developing consensus guidelines for the use of porcine xenografts

Table 1 Oxygen tension of alternative transplant sites and the ability to support islet transplantation

Site	Oxygen tension of native tissue (mmHg)	Oxygen tension of transplanted islets (mmHg)	Percent to pancreas	Vascular density of transplanted islets (vessel/mm²) (perfusion rate)	Ref.
Pancreas	Approximately 40	n/a	n/a	1074 ± 174 (6-7 mL/min per gram)	[44-46,61,75]
Portal vein	Approximately 40	Approximately 5	12.50%	< 100 TPU	
Spleen	No data	Approximately 5	CBD	> 100 TPU	
Kidney capsule	15	Approximately 5	12.50%	> 100 TPU	
Peritoneal lining	Approximately 50	No data	CBD	No data	[76]
Intramuscular space	15	25	63%	1162 ± 120	[77,78]
Subcutaneous site	8	No data	CBD	No data	[79]

CBD: Cannot be determined.

in all aspects of transplantation including islets. The rationale for porcine islets stems from the historical use of porcine insulin to treat T1DM, prior to the use of biosynthesised recombinant insulin[55]. Given the compatibility between porcine and human insulin, it is hoped is that similar compatibility will be seen with islets. However, transplantation of xenogenic tissue may represent a nearly insurmountable immunological barrier in humans. It has been possible to obtain sustained islet graft function in monkeys receiving human islets, but heavy (and risky) inductive and maintenance immunosuppression with agents usually considered too aggressive for routine clinical use, are required to achieve such function. Currently, two clinical trials are ongoing in New Zealand (DiaBCell) and in Russia. No subjects to our knowledge have been rendered insulin free with such approaches to date, and for these trials porcine islets have been encapsulated in alginate-based capsules as a mechanical barrier to immune cell engagement.

There have been several identified advantages of using xenografts as an alternative cell source. Firstly, pig islets represent a potential unlimited, on-demand source of islets. This would mean that patients could achieve insulin independence from one transplant as substantial islet mass could be infused at one time. Secondly, given that the islets can be harvested from young, healthy, living pigs with limited exposure to environmental hazards, theoretically, the quality of these islets would be superior to those harvested from deceased human donors. And thirdly, there is the potential to eliminate the requirement for immunosuppression by genetically modifying the source pigs[54].

However, safety concerns over using xenografts also need to be considered. One of the major concerns is the potential for zoonosis, which not only applies to be the recipient, but also to the population at large. Even with regulations to develop designated pathogen-free pig sources, long term follow-up of patients receiving xenografts still needs to be carried out to identify potentially yet unidentified pathogens[54]. The major issue with xenografts is that they carry a much higher immunological risk resulting in a more vigorous rejection reaction[56]. One reason for this is that humans

have pre-formed anti-Gal antibodies [Gal (galactose-α1,3-galactose) is an oligosaccharide expressed of pig endothelium]. This results in immediate complement activation as anti-Gal antibodies bind to the surface of the transplanted xenografts[56]. Another reason is that xenografts activate a more robust instant blood-mediated inflammatory reaction (IBMIR)[56]. Following transplantation platelets cause macroscopic coagulation of the islets leading to the recruitment of complement components as a secondary response. The resulting inflammatory response contributes to large islet losses. This taken together would mean that patients would have to be placed on intensive immunosuppressive regimens in order for xenograft survival. However, due to the associated morbidity of immunosuppression agents, this is far from an ideal option.

The other option for an alternative cell source is pancreatic endoderm derived human stem cells. Stem cell research has seen large innovations for cellular replacement therapy over the last few years. Two unique properties that stem cells possess are the ability to renew (proliferative) and the potential to differentiate into any tissue type (pluripotency). To date, *in vitro* propagation of pancreatic endoderm tissue from these pluripotent cells has been achieved successfully[57,58].

There are several advantages to using stem cells. Firstly, these cells (unlike human islets and porcine islets) do not have to be isolated from a whole organ. This has a two-fold advantage. One, this removes the requirement for specialized isolation centres and offers an "on-demand" reproducible and controlled cell source. And secondly, the bioengineered stem cells possess much higher tolerance for hypoxia and ability to neovascularize over time. As detailed above, the isolation process leads to a delay in engraftment as islet cells regenerate their inner vasculature. Theoretically, this means that these cells would be able to engraft more rapidly. Secondly, from a safety perspective, these cells are human derived and would therefore not carry the same pathogen risks or immunological barrier as xenografts.

Stem cell transplantation, however, is not without limitations. One of the current difficulties that stem cell

researchers face is the inability to fully mature the cells into functional insulin-secreting cells *in vitro*[57,58]. When these cells are transplanted, they do mature *in vivo*, but this maturation is delayed, and difficult to predict or control. Currently, the shortest time for *in vivo* maturation is eight weeks post-transplantation[58]. The delay in maturation presents an issue with monitoring since these stem cells, similarly to deceased donor islet transplantation, face the risk of early silent rejection at a time prior to functional maturation. However, this limitation is also seen in the current protocol, where direct monitoring of islet function post-transplantation is not yet possible. The current indirect methods of monitoring islet function through blood sugar levels and secreted C-peptide can be used to monitor for maturation of insulin-secreting stem cells.

Safety is the other major concern with use of embryonic derived stem cells for cellular replacement therapy. Teratoma development is the most well recognized risk. Classic teratomas are unique tumors that originate from stem cell populations and demonstrate tissue types from all three cell lines. They are usually detected when they cause morbidity either through a mass effect or through the release of hormones from functional endocrine tissue. The development of these classic teratomas in immune-compromised animal models implanted with monodermal propagated cells indicates a limitation in the purification protocol[58]. While the teratoma histogenesis is not fully understood, the intrinsic properties of pluripotency and self-renewal are risk factors for tumor formation[59]. To improve the safety of using *in vitro* differentiated stem cells these properties would need to be silenced. Another tumor concern is the development of embryonic carcinomas. These are teratomatous-like tumors that are monodermal in histology. These represent a proliferation of a single cell line and are thought to arise from mutations that occur during the differentiation process[59]. Furthermore, although tumorigenesis is largely influenced by the intrinsic properties of the cells, there are extrinsic factors within the microenvironment that appear to influence their development. As of yet, these features are not fully understood, but may influence where the cells can be transplanted. The site for transplantation will also be limited by retrievability if they do go on to develop tumors. A major interest in developing new beta cells from inducible pluripotential stem cells (iPSc) from the patient's own cells could change this dynamic. These cells would be entirely biocompatible from an alloimmune perspective, and not being of embryonic source may potentially be much less susceptible to teratoma or malignant transformation. There would still be a major barrier from recurrent autoimmune attack, which would require strategies for control.

Limitations of the current transplant site
Under the current "Edmonton Protocol", the hepatic portal vasculature is used as the site for islet transplantation. The portal vein offers a rich vascular environment for the newly infused islets. However, a large proportion of cells are initially lost, indicating the hostility of the environment. Some of the well-recognised factors that contribute to the hostility of the liver environment include the lower oxygen tension of the portal vasculature (compared to the pancreas), high exposure to immunosuppression drugs and toxins, and immunological destruction by both the innate and adaptive immune responses. In addition, a large initial loss is attributable to IBMIR (described above). In addition to poor survival outcomes, once the islets have been infused into the liver, they are not readily retrievable. The intraportal hepatic site has demonstrated that islet transplantation is beyond a proof-of-concept therapy, however due to the aforementioned limitations, the portal vein may not be the most ideal site, and indeed, may not be appropriate for more novel transplant technologies such as embryonic or iPScs.

Other transplant sites have been explored for islet transplantation and include: pancreas, spleen, gastric submucosal site, intraperitoneal site/omental pouch, kidney capsule, striated muscle, as well as immunoprivileged sites, including bone marrow, thymus, brain and testis. Review of the practicality of these transplantation sites was recently published by Vériter *et al*[8] and highlighted important criteria to consider when selecting a site for islet cell transplantation. The criteria included: (1) space of the site and the volume of the transplanted tissue; (2) contact to an abundant blood supply with a good oxygen supply; (3) access to physiological blood glucose levels; (4) ease of access and the potential for rapid retrieval; and (5) minimal early inflammatory reaction and promotion of long-term survival[8]. Given these requirements it is understandable why finding an ideal site has been so challenging thus far for islet cell transplantation.

The emphasis for a site with good vascular access has been well researched. Islets in the native pancreas have a rich glomerular-like vascular system (flow rate = 5-7 mL/min per gram)[60] that allows them to readily respond to changes in blood glucose and maintain a high partial pressure oxygen tension (pO_2 = 40 mmHg)[44]. Perhaps evolved from this, islets do not intrinsically possess a system to deal with hypoxic stress, with much lower anti-oxidant levels than any other tissue type. As such, irrespective of where islets are transplanted, they will be exposed to hypoxia due to the destruction of their inner vasculature by the isolation process[7,44]. The ability of the islets to regenerate their vascular densities will impact on their survival and functional outcomes. Studies have shown that when islets are transplanted under the kidney capsule there is a marked decrease in vascular density with an associated decrease in blood flow of around 25%-50% of endogenous islets[44,61]. This

is also associated with a decreased partial oxygen pressure of 5 mmHg[44,61]. Furthermore, vascular distribution was altered in transplanted islets with a higher density of capillaries being observed in the stromal connective tissue compared to the endocrine tissue[44,61]. Limited studies on the vascular densities of transplanted islets into alternative sites are available; however, comparison of native oxygen tension at these sites compared to the pancreas may shed light on the suitability of these sites (Table 1).

The subcutaneous site is one of the most extensively studied alternative sites for islet transplantation. The best recognized advantage being that it is readily accessible allowing for a minimally invasive monitoring, imaging and for biopsy/retrieval. Conversely, historical use of the subcutaneous site in both animal models and humans demonstrated an inability to completely reverse diabetes[7,62] due to the poor vasculature and oxygen tension of the site. However, numerous studies have since demonstrated the ability to manipulate the site to increase vasculature and oxygen tension. These methods included (and are not limited to) the use of polymers[12], meshes[14] and encapsulation devices[13,16]. In addition, angiogenic stimulation has been achieved through co-transplantation with growth factors (*e.g.,* fibroblast growth factor[63], hepatocyte factor, and vascular endothelial growth factor) and mesenchymal stem cells[64] (Table 1). These methods revealed the potential to manipulate the transplant site in order to create the ideal microenvironment for the islets to survive. They also highlight that native oxygen tensions alone are not suitable in predicting survival outcomes.

However, although studies have shown the ability to create a microenvironment in the subcutaneous site to support islet transplantation and reverse diabetes in an animal model, there were observed limitations in functional outcomes. In particular, islets transplanted into the modified subcutaneous site demonstrated an apparent delay in responding to changes in blood glucose levels[14]. This could be related to inefficiency in transporting insulin from the subcutaneous site into the blood stream[14] and/or a deficiency in responding due to decreased inner vascular density[44,61]. Again, limited studies are available to discuss the internal vascular density outcomes at the alternative sites. While it has been shown that the microenvironment is important for sustaining the islets during the engraftment period, it is unclear how the microenvironment impacts of vascular density outcomes and whether or not this could be further improved.

Some of the methods detailed above, in addition to manipulating the subcutaneous site, demonstrated the ability to transplant islets without the use immuno-suppression agents[13,14,16]. Clinical translation of this concept is predicted to dramatically change islet transplant outcomes as both patient morbidity and drug-related islet cell damage would be decreased markedly. Prototypic macroencapsulation devices consist of a semi-permeable membrane that allows nutrient exchange and insulin release, and prevents the immune cells from accessing the transplanted cells within. The biomaterial of the device stimulates angiogenesis around the device through an inflammatory reaction, but fail to provide a direct connection to a blood supply as vessel ingrowth is blocked. As such, one of the limitations of using this device is that the inner islets are hypoxic[65,66], similar to observations of large islet masses transplanted into the portal vein. In response, studies emerged with the aim of improving internal oxygenation of the devices. The approaches included changes in the size and shape of the devices[67], the size of the islet clusters, the material used[68,69], and the use of an external oxygen supply[70]. In addition, other groups looked at improving local oxygenation at the device through the use of electrochemical processes[71] or local photosynthesis[72]. Another limitation of the immune isolating device is the stimulation of the foreign body reaction to the biomaterial[73,74]. This inflammatory reaction persists for the *in vivo* lifespan of the device and ultimately leads to the formation of an avascular capsule around the device thereby limiting its function.

Expected outcomes with human embryonic and adult inducible pluripotential stem cells

Human embryonic stem cells, as detailed above, are an attractive alternative cell source for islet transplantation. The possibility of using human embryonic stem cells for islet transplantation has only been a reality in the last few years[57,58] and as such there are limited outcome studies available to report. However, knowledge of the bioengineered stem cell properties can be used to extrapolate on the potential outcomes. In addition, the use of stem cells in the context of the current protocol, will help to identify how this cell type can address some of the ongoing challenges. In addition, current research innovations can be synergized with the use of stem cells to enhance their translational application.

As has been previously noted, deceased donor islets have a poor engraftment rate. This has been largely attributable to destruction of their inner vasculature during the isolation process. Therefore, the advantage of using stem cells is that they already have a well-established vasculature. This may allow them to engraft at a more rapid rate and with a higher survival rate compared to donor islets. It could also be predicated that these stem cells will have a more robust vasculature than transplanted islets and therefore might function at a higher efficiency.

With current research focusing on the subcutaneous site and the development of immune isolating devices, a more adaptive cell type is required in order to withstand the relatively hypoxic environment of these devices. One property of stem cells is their ability to proliferate, which should convey a survival advantage when stressed. However, at noted above, these stem cells must undergo maturation after implantation prior to being functional and it is unknown whether or not

the proliferating cell type would be at just one stage of maturation or multiple. This poses safety concern as these cells may go on to develop into embryonic tumors. However, if the cells were enclosed within a device, then this concern would be limited.

As noted above, these previously studied immune isolating devices stimulate a robust foreign body reaction. While they remove the requirement for immunosuppression to protect against immune rejection, the devices are constantly under attack for their *in vivo* lifespan. Some proposed mechanisms for overcoming this reaction is to provide patients with lifelong anti-inflammatories and/or anti-proliferative agents. However, the limitation with using anti-proliferative agents is that they would interfere with the expansion and function of the stem cells. Alternatively, given that stem cells offer a ubiquitous source for transplantation, the other possibility is replace these devices at set time intervals. This would be less attractive for patients, but clearly attractive from the cell manufacturer's perspective.

CONCLUSION

Islet transplantation has been associated with remarkable research output and innovation in the last two decades. The introduction of the "Edmonton Protocol" ignited the possibility of providing all patients suffering from T1DM with a cure. One of the largest problems for islet transplantation, and transplant in general, is the limited supply of donor tissue. Insulin-secreting stem cells offer a potential solution to this problem and may in fact address some of the limitations that require large donor cell populations.

While using stem cells as an alternative source is still a novel idea for islet transplantation, it has promising potentials for the future. In particular, it may synergize well with other current innovations such as immune isolating devices and may open the door for using the subcutaneous site as an alternative transplant site. Further research on clinical outcomes is required but current speculations on outcomes are positive for the utility of stem cells in islet transplantation.

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MINIREVIEWS

Ultraviolet-induced alloantigen-specific immunosuppression in transplant immunity

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Abstract

After the first observation of the immunosuppressive effects of ultraviolet (UV) irradiation was reported in 1974, therapeutic modification of immune responses by UV irradiation began to be investigated in the context immunization. UV-induced immunosuppression is *via* the action of regulatory T cells (Tregs). Antigen-specific Tregs were induced by high-dose UV-B irradiation before antigen immunization in many studies, as it was considered that functional alteration and/or modulation of antigen-presenting cells by UV irradiation was required for the induction of antigen-specific immunosuppression. However, it is also reported that UV irradiation after immunization induces antigen-specific Tregs. UV-induced Tregs are also dominantly transferable, with interleukin-10 being important for UV-induced immunosuppression. Currently, various possible mechanisms involving Treg phenotype and cytokine profile have been suggested. UV irradiation accompanied by alloantigen immunization induces alloantigen-specific transferable Tregs, which have potential therapeutic applications in the transplantation field. Here we review the current status of UV-induced antigen-specific immunosuppression on the 40th anniversary of its discovery.

Key words: Alloantigen; Ultraviolet irradiation; Donor-specific immunosuppression; Interleukin-10; Regulatory T cells

Core tip: The perception of immunological changes induced by ultraviolet (UV) exposure has changed over the past several years. Although carcinogenesis and immunosuppression due to UV irradiation are regarded as detrimental, UV irradiation is also currently considered a useful tool to induce alloantigen-specific regulatory T cells (Tregs). There is great enthusiasm for the potential to develop strategies that can use Tregs for therapeutic interventions. Alloantigen-specific immunosuppression is an ideal therapy for allotransplant recipients. Although the

full mechanism has yet to be determined, UV irradiation accompanied by alloantigen immunization produces a beneficial effect in transplant immunity *via* the induction of alloantigen-specific transferable Tregs.

INTRODUCTION

Intermittent exposure to ultraviolet (UV) light, especially the mid-wave range (UV-B, 280-320 nm), is an important environmental factor affecting human health[1]. Although primary carcinogenesis is the most common problem[2], UV irradiation also impairs immune responses to oncogenic and infectious antigens[3,4]. Paradoxically, the immunosuppressive effects induced by UV irradiation may have therapeutic potential[5-8].

Immunosuppressants have revolutionized clinical transplantation, but have many side effects including pan-immunosuppression[9]. Infectious complications are mostly fatal for transplant recipients[10]. After organ transplantation, patients on immunosuppressants face a dilemma between infectious morbidity and graft rejection. Therefore, alloantigen-specific immunosuppression is an ideal therapy for transplant recipients[11,12]. Research has focused on the immune modulating effects of UV-B irradiation in conjunction with alloantigen immunization to induce donor alloantigen-specific immunosuppression.

Here, we review the application of UV irradiation accompanied by alloantigen immunization to induce alloantigen-specific immunosuppression and discuss the therapeutic potential of UV-induced regulatory T cells (Tregs) in the transplant immunology field.

HISTORY AND BACKGROUND

The initial observations on the immunosuppressive effects of UV irradiation were documented in 1974[13]. Thereafter, many researchers have pushed the frontiers of photopheresis and photoimmunosuppression. Two models of contact hypersensitivity and delayed-type hypersensitivity have been developed to clarify the immunological mechanisms involving UV irradiation[14-18]. The therapeutic capacity of UV irradiation to modify immune responses began to be investigated in the late 1970s[13,19,20]. By the late 1980s, many researchers had reported that antigen-specific Tregs were induced by high-dose UV-B irradiation before antigen immunization[15,21,22]. At this time it was thought that functional alteration and/or modulation of antigen-presenting cells (APCs) by

UV irradiation was required for the induction of antigen-specific immunosuppression[23].

METHODOLOGY FOR SUCCESSFUL UV-INDUCED IMMUNE EFFECTS

Many researchers have used mice in their studies on the immunosuppressive effect of UV irradiation. Animal care during and after UV irradiation is critically important for successful UV irradiation experiments[24,25]. Murine skin must be carefully shaved without any injuries. To prevent unevenness of UV irradiation, mice are anesthetized during UV exposure with their feet fixed to a metal plate. Thus, the shaved abdominal wall is sufficiently extended with even exposure to the UV lamps. Therefore careful shaving of the irradiation area and adequate anesthesia and restraint are very important for stable UV irradiation with even exposure. If challenge with antigen or graft beds for transplantation is required after UV irradiation, these sites should be protected from UV irradiation. High-dose UV-B exposure is very damaging, therefore post-irradiation care is also crucial. Adequate analgesic medication is thus a serious requirement after UV irradiation. UV-irradiated mice should be placed in separate cages to avoid scratching of irradiated skin by cage mates. They are also fed with a supply of Ringer's lactate solution. As irradiated skin undergoes contraction to become scar tissue, post-irradiated stiffening severely restricts movement and activity in mice. Therefore, some ingenuity to prevent unexpected death and post-irradiation dehydration (such as a raised floor for easy access to food and water and availability of gels containing sugar, water and dietary supplements to ensure a steady supply of nutrients and water) is required.

ALTERATION AND/OR MODULATION OF APC FUNCTION BY UV IRRADIATION

UV irradiation alters APC function[23]. UV-induced DNA damage has been recognized as the major molecular trigger for photoimmunosuppression[26-28]. Interleukin (IL)-12 reduces DNA damage and prevents the generation of UV-induced Tregs[28,29]. Langerhans cells (LCs) were initially regarded as the most important APC in the epidermis[18,30,31], and it was believed that LCs were killed by UV irradiation. However, it is now accepted that the primary APC in the skin is not LC but dermal dendritic cell (DC)[32-34], and UV irradiation destroys the DC network of LC in the skin[31]. LCs appear to be involved in down-regulating immune responses[35], and inducing and activating Tregs[36,37]. Recently, the functional role of LCs was redefined, and it was shown that UV-damaged LCs in the regional lymph nodes were required for Treg induction[28]. Damaged but viable LCs will present antigen in a nonprofessional manner, which will induce Tregs rather than effector T cells[27].

ANTIGEN-SPECIFIC IMMUNOSUPPRESSION

Many researchers have reported that antigen-specific Tregs were induced by high-dose UV-B irradiation prior to antigen immunization[15,21,22]. At the time it was thought that UV-induced functional alteration and/or modulation of APC function was required for the induction of antigen-specific immunosuppression[23]. This may explain why previous researchers documented that antigen immunization must follow UV-B irradiation and not *vice versa*[15,21,22]. However, the successful use of UV irradiation after antigen immunization has also been reported[24,25,38-41]. In both models, with UV irradiation before or after antigen immunization, antigen presentation in a nonprofessional manner is the key to inducing antigen-specific Tregs[23,27].

UV-induced Tregs and their phenotypes

UV-induced antigen-specific immunosuppression is attributable to T cells with suppressive activity (formerly called, "suppressor T cells")[42,43], and currently these T cells are referred to as Tregs[17,44,45]. A number of studies have investigated the phenotype and mechanism of UV-induced Tregs. UV-induced Tregs express CD4, CD25 and CTLA4[17,46,47] and the lymph node-homing receptor CD62L and therefore migrate into the lymph nodes[46,48].

The early inflammatory phase in the skin has been well studied[49]. When we investigate UV-induced Treg subsets, the role of natural killer T (NKT) cells and mast cells should also be considered. NKT cells are a unique class of T cells. They express T-cell receptor molecules and co-express surface antigens normally found on natural killer (NK) cells. NKT cells have a critical role in UV-induced tumor immune responses[37,50], and they appear to be dependent on IL-4[37]. Researchers have also focused on the role of mast cells in UV-induced immunosuppression[51,52]. Although mast cells were formerly ignored in the field of UV-induced immunosuppression, it has been suggested that they may have immunosuppressive potential[53]. The concept that LCs, mast cells and NKT cells can act in an unconventional manner is now well accepted in the communities of photobiology and immunology[26,27]. The LCs transmit an immunosuppressive signal from the skin to lymph nodes, where they activate NKT cells to secrete regulatory cytokines[26].

Dominant transferability of UV-induced Tregs

As described above, UV irradiation accompanied by antigen immunization induces antigen-specific Tregs. Moreover, these Tregs are dominantly transferable[19]. This transferability confirms that UV-induced immunosuppression is mediated by Tregs. Moreover, this transferability is an advantage for alloantigen-specific immunosuppression in the transplantation field as UV-induced Tregs dominantly have the same immune effect in recipients[24,25].

Role of cytokine milieu

CD4+ Th2 lymphocytes secrete pro-inflammatory cytokines (IL-4, IL-5 and IL-13)[54,55]. IL-4 is thought to promote the induction of transplantation tolerance and alloantigen-specific Tregs[56]. IL-4 also promotes both regulatory and effector T cells in the initial immune response. Moreover, IL-4 activation of effector cells can mediate rejection and will not support alloantigen-specific Tregs that could transfer specific tolerance[56]. Transforming growth factor (TGF)-β is a growth and differentiation factor that displays multiple functions[57]. It is known that the combined use of IL-10 and TGF-β effectively generates CD4+ Tregs[57,58].

Immunosuppression induced by UV irradiation and immunization is dependent on CD4+ Tregs[59-61] and cytokines play an important role[17,62]. The immunosuppressive effects induced by UV irradiation before immunization were explained by a shift in the activation of T cells from a Th1 to a Th2 immune response[63-67]. However, alloantigen-specific immunosuppression induced by UV irradiation after immunization depends not on IL-4, IL-5, IL-13 or TGF-β but on IL-10[24,25,38-41]. Thus, the mechanism of immunosuppression by UV irradiation after immunization cannot be simply explained only by a Th2 shift[24,25,38-41].

Role of IL-10

IL-10 is a well-known immunosuppressive cytokine[68,69], and is important for UV-induced immunosuppression[70-73]. The inhibitory capacity of UV-induced Tregs depends on IL-10 expression[46]. Antigen-specific activation of Tregs by APCs induces the release of IL-10[46,47], which mediates the inhibitory activity of UV-induced Tregs[47,74]. The source of IL-10 in UV-induced immunosuppression is therefore UV-induced Tregs themselves[17,71], although mast cell[52,75] and CD11b+ macrophages[76] have also been suggested. IL-10 is crucial for both the induction[25,72,77] and effector phases[46,78] of UV-induced Tregs, though some researchers reported that IL-10 is not required for Treg induction by UV irradiation[73].

CD4+ T cells with cytokine profiles displaying a large amount of IL-10, but no IL-4, are labelled regulatory T cell type 1 cells (Tr1)[79]. The presence of IL-10 gives rise to CD4+ T-cell clones with a low proliferative capacity that in turn produce high levels of IL-10, low levels of IL-2 and no IL-4[69,79]. These antigen-specific T-cell clones suppress the proliferation of effector CD4+ T cells in response to antigen[69,79]. Thus, IL-10 drives the generation of a CD4+ T-cell subset, designated Tr1, which suppresses antigen-specific immune responses and actively down-regulates pathological immune responses *in vivo*[69,79]. As described above, UV irradiation before immunization induces CD4+ Tregs, and resulted in a shift to Th2 immune response[63-67], and IL-10 plays an important role in this. However, in immunosuppression induced by UV irradiation after immunization, CD4+ Tr1-like cells with high expression of IL-10 are important for alloantigen-specific immunosuppression[24,25,38-41].

Figure 1 Schema illustrating the postulated reactions in achieving alloantigen-specific immunosuppression by ultraviolet-B irradiation accompanied with alloantigen immunization. APCs, such as mature DC and LC, capture alloantigen. UV-B irradiation and subsequent IL-10 secretion will cause antigen presentation in a nonprofessional manner to induce antigen-specific immunosuppression. Immature DC presents alloantigen to CD4^{+} T cell, and then, Treg induction and IL-10 secretion arise. LC presents alloantigen to NKT cell, and IL-4 secretion subsequently occurs. Also, LC presents alloantigen to Foxp3^{+} Treg, and thereafter, Foxp3^{+} Treg proliferation and Treg induction are triggered. Hence, alloantigen-specific Treg, Foxp3^{+} Treg, IL-10 and IL-4 will regulate allo-immune responses. MHC: Major histocompatibility complex; TCR: T cell receptor; CD: Cluster of differentiation; DEC: Dendritic and epithelial cells; APCs: Antigen-presenting cells; UV: Ultraviolet; DC: Dendritic cell; LC: Langerhans cell; IL: Interleukin; NKT: Natural killer T.

Panoptic finding in alloantigen-specific immunosuppressiton induced by UV-B irradiation

As described above, UV-B irradiation accompanied with alloantigen immunization is a useful tool to induce alloantigen-specific immunosuppression. Here, we reviewed previous documents which have described the possible mechanisms in achieving alloantigen-specific immunosuppression induced by UV-B irradiation[17,26,27,38,39,44,50,72], and summarized the postulated reactions in Figure 1.

In brief, APCs, such as mature DC and LC, capture alloantigen. UV-B irradiation and subsequent IL-10 secretion will cause antigen presentation in a nonprofessional manner to induce antigen-specific immunosuppression[23,27]. Immature DC presents alloantigen to CD4^{+}T cell, and then, Treg induction and IL-10 secretion arise. LC presents alloantigen to NKT cell, and IL-4 secretion subsequently occurs. Also, LC presents alloantigen to Foxp3^{+}Treg, and thereafter, Foxp3^{+}Treg proliferation and Treg induction are triggered. Hence, alloantigen-specific Treg, Foxp3^{+}Treg, IL-10 and IL-4 will regulate allo-immune responses.

So-called "bystander immunosuppression" or "linked suppression"

UV-induced Tregs will demonstrate unique behavior referred to as "bystander suppression". Antigen specificity appears to be restricted to the activation of UV-induced Tregs and not to the suppressive activity itself, as once activated by their cognate antigen, they release IL-10 and thereby suppress other immune reactions nonspecifically[46,80]. Previous researchers also demonstrated a rigor rule for activation of UV-induced Tregs[46,71,80]. Migratory behavior of UV-induced Tregs can be reprogrammed by APCs[81], and UV-induced Tregs switch APCs from a stimulatory to a regulatory phenotype[81]. This alteration of APC function may help to explain bystander suppression. In summary, once IL-10 is released upon antigen-specific activation by UV-induced Tregs, IL-10 suppresses other immune responses in a nonspecific fashion through bystander suppression[74]. The therapeutic potential for Tregs generated in response to antigens that are not necessarily the same antigen driving the pathogenic process has been reported in the literature[74,80].

Possibilities for clinical use, and some future perspectives in human

The view of photoimmunology has changed over the past several years[26,27]. The mechanisms involved are much more complex than those many researchers initially thought. The skin is an organ close to immunity,

and many autoimmune diseases affect the skin. One of the best routes to immunize is *via* the skin. The majority of these reactions are T cell-driven[82]. Therefore, many researchers focused on the tentative theory that UV-induced T cells may not always be beneficial, but more often harmful[26,27]. Nowadays, many researchers assume that a fine-tuned balance is optimal[26,27]. Hence, suppression may be as relevant as induction, and replacing the negatively perceived term "suppression" with "regulation" is preferable[17].

Clinical physicians recognized that UV-induced immunosuppression has a therapeutic potential in human, and therefore, UV-irradiation itself have been already applied for actual clinical use[5-8]. Experimental studies demonstrated that UV-induced immunosuppression supports the exacerbation of skin infections and the suppression of T-cell reactions against microbial antigens[83]. However, the clinical experience differs. The risk for infections, in particular bacterial infections, after UV-B exposure is low[27]. Atopic dermatitis is frequently superinfected with Staphylococcus aureus, but can be improved by UV-B irradiation even without antiseptic or antibiotic measures.

A strong association of UV-susceptible and UV-resistant phenotypes in humans with single-nucleotide polymorphisms in the tumor necrosis factor region was found, suggesting this region to contain genes that determine the outcome of an UV response[84]. Human volunteers developed tolerance when the hapten was initially painted onto UV-treated skin[85]. UV-B irradiation not only depleted LCs but also induced CD11b$^+$ macrophages, which released IL-10[76].

Experimental studies demonstrated that high-dose UV-B irradiation accompanied with antigen immunization is required for antigen-specific Tregs. From the viewpoint of transplant immunity, a simple question arises. How do we establish an actual regimen without severe rejection and intractable infection? Moreover, the development of tolerance versus suppressed contact hypersensitivity appears to correlate with the timing of antigen application after UV-B exposure[27]. How do we consolidate the timing of alloantigen immunization, in an emergent case of available allograft from a deceased-donor. Hence, in current status, translational researched and clinical trials are seriously required, and we should carefully attempt those studies for the further developments.

CONCLUSION

Paradoxically, although high-dose UV exposure is toxic, it is suggested that photopheresis and photoimmuno-suppression may have therapeutic potential. The perception of UV-induced immunological changes has thus changed over the past several years[26,27]. Carcinogenesis and immunosuppression due to UV irradiation were regarded as detrimental; however, a finely-tuned therapeutic dose may be possible[26,27]. To induce alloantigen-specific transferable CD4$^+$

Tregs, UV irradiation is a very useful tool[15,19,21,22,24, 25,39-41]. Clinically, there is great enthusiasm for the potential to develop strategies that can use Tregs for therapeutic interventions[71]. Alloantigen-specific immunosuppression is an ideal therapy for transplant recipients[86-88]. Although the full mechanism has not yet been determined, UV irradiation accompanied by alloantigen immunization to induce alloantigen-specific Tregs may have great benefits in the transplant immunology field.

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ORIGINAL ARTICLE

Observational Study

Conversion from calcineurin inhibitors to mTOR inhibitors stabilizes diabetic and hypertensive nephropathy after liver transplant

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Abstract

AIM: To investigate if conversion to the mammalian target of rapamycin inhibitors (mTORi) improves renal function in diabetic and/or hypertensive liver transplant patients immunosuppressed with tacrolimus or cyclosporine.

METHODS: The study included 86 liver graft recipients immunosuppressed with mTORi treatment after orthotopic liver transplantation (OLT), including all liver recipients with worsening renal function before conversion to mTORi (n = 55 patients) and recipients with normal renal function who converted to mTORi for other reasons (n = 31 patients). We identified patients with diabetes mellitus (n = 28), arterial hypertension (n = 27), proteinuria (n = 27) and all three factors (n = 8) (some patients have hypertension and diabetes and no proteinuria). The primary endpoint was evolution in renal function defined as the development in plasma creatinine as a function of diabetes mellitus (DM), hypertension (HT) or proteinuria. We required elevated serum creatinine for at least two weeks to define renal dysfunction.

RESULTS: Only patients that converted because of renal failure with plasma creatinine levels > 1.5 mg/dL showed an improvement of renal function (2.14 to 1.77 mg/dL) (P = 0.02). Patients with DM showed no improvement of serum creatinine levels (1.31 mg/dL to 1.37 mg/dL) compared with non DM patients (1.31 mg/dL to 1.15 mg/dL) (P = 0.01), HT patients (1.48 mg/dL to 1.5 mg/dL) with non HT patients (1.21mg/dL to 1.08 mg/dL) and patients with proteinuria (1.44 mg/dL to 1.41 mg/dL) and no proteinuria (1.31 mg/dL to 1.11 mg/dL).

CONCLUSION: In OLT recipients with diabetes or hypertensive nephropathy, conversion to mTORi does not improve renal function but stabilizes plasma levels

of creatinine. Proteinuria is not a contraindication to conversion to mTORi; it also stabilizes renal function. Conversion to mTORi should only be avoided in patients with diabetes, hypertension and proteinuria.

Key words: Mammalian target of rapamycin inhibitors; Liver transplant; Renal dysfunction; Hypertension; Diabetes

Core tip: These results could be useful in choosing an immunosuppressant regimen in liver transplant recipients, especially in patients with diabetes mellitus and/or arterial hypertension with proteinuria and possibly renal dysfunction.

INTRODUCTION

Survival after orthotopic liver transplantation (OLT) is getting better because of improvement in surgical techniques and better management in immuno-suppressant therapy. This important survival leads to more side effects from immunosuppression agents so it is very important to identify the best drug regimen for each patient to reduce toxicity[1]. Calcineurin inhibitors (CNI) tacrolimus and cyclosporine have a common (18%-25%) side effect of chronic renal dysfunction[2] and some of these patients will need hemodialysis with the possibility that this renal failure could be the cause of death[3]. Immunosuppressive therapies that reduce or eliminate CNI based treatment should preserve renal function after OLT.

mTOR inhibitors, sirolimus and everolimus (mTORi), block cell proliferation based on interleukin-2 pathway interacting kinases called the mammalian target of rapamycin[4]. CNI inhibit production of cytokines as interleukin-2 in the first phases of the lymphocyte cell cycle[5]. These days, mTORi is being studied more in renal transplant patients and less in liver transplant patients. There are some studies that show that elimination or reduction of CNI and inclusion of mTORi preserve renal function[6-12]. However, there are no controlled studies of the effect of mTORi exposure in liver transplant patients with well-known chronic renal insufficiency because of diabetes and/or hypertension associated with worsening urinary protein excretion and renal function. It is probable that improvement in renal function is reduced in patients with diabetes

mellitus (DM), hypertension (HT) and/or proteinuria.

The potential side effects of mTORi, such as hyperlipidemia, hepatic artery thrombosis and a bad wound cicatrization, have been investigated in these patients[13]. No controlled studies have examined these potential effects in the OLT population.

This study attempts to compare outcomes of renal function in cohorts treated with mTORi with diabetes mellitus, hypertension or/and proteinuria.

MATERIALS AND METHODS

Study cohorts

We studied 86 liver recipients immunosuppressed with mTORi treatment after OLT at our center from March 2007 to June 2013. Renal dysfunction was defined as serum creatinine $\geq$ 1.2 mg/dL for at least two weeks (whenever it occurred at least two months after OLT). We included all liver recipients who were diagnosed with renal dysfunction before conversion to mTORi (n = 55 patients) as well as patients with normal renal function who converted to mTORi for other reasons (n = 31 patients). We identified patients with diabetes mellitus (n = 28), arterial hypertension (n = 27), proteinuria (n = 27), and all three factors (n = 8) (some patients had hypertension and diabetes and no proteinuria).

Definition of variables

Baseline creatinine was determined as plasma creatinine level at the moment of switching to mTORi, then at 6, 12 and 18 mo, and actual creatinine (last drawn serum creatinine) when collected.

DM patients were defined by the American Diabetes Association criteria [*Diabetes Care* 2005; 28 (suppl 19): 37-42]. HT patients were catalogued as patients with systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg. Proteinuria was defined as the appearance of proteins in urine. There was no difference in severity.

Endpoint

The main endpoint was evolution of renal function, determined by serum creatinine and according to the presence of DM, HT and/or proteinuria. Renal dysfunction was defined by elevated serum creatinine for at least two weeks.

Administration of immunosuppression

The immunosuppressant regimen after OLT was administered inside a wide protocol at our center. Induction drugs at time of OLT were given in cases of well-known renal dysfunction before transplant. Post OLT, tacrolimus was given to obtain serum levels between 7 and 10 ng/mL for 180 d after OLT and levels between 5 and 8 ng/mL for the next 180 d. Cyclosporine was only used in cases of neurotoxicity because of tacrolimus. If cyclosporine was used, the serum level was between

Variable	DM (28)	HT (27)	Prot (27)	DM + HT + prot (8)	*P*-value
Table 1 Features of patients converted to mammalian target of rapamycin inhibitors					
Age (yr)	54.3	55.1	54.8	55.2	0.61
Male	21	23	22	7	0.43
DM prior to OLT	28	8	8	8	0.52
Hypertension prior to OLT	5	19	5	5	0.34
Proteinuria prior to OLT	7	6	19	6	0.42
Etiology of liver disease					
Hepatitis C	11	11	10	3	0.32
Alcohol	14	13	13	4	0.67
Other	3	3	4	1	0.56
Hepatocellular carcinoma	6	5	5	1	0.48
Initial creatinine	1.31	1.48	1.44	1.35	0.23

DM: Diabetes mellitus; HT: Hypertension; Prot: Proteinuria; OLT: Orthotopic liver transplant.

250-350 lg/L after OLT with a maintenance cyclosporine level of 50-100 lg/L. Prednisone was administered after OLT and generally stopped within the first two months, except in autoimmune, primary biliary and primary sclerosing cholangitis cirrhosis. Mycophenolate mofetil was used in all patients, one gram/day, except in CMV infection.

mTORi has been used in liver grafts recipients with renal dysfunction, patients with tacrolimus and cyclosporine neurotoxicity, in high risk hepatocellular carcinoma (HCC) liver transplant patients to avoid its recurrence, and in patients with "*de novo*" neoplasia after OLT, adjusting the dosage to obtain levels between 5 and 8 ng/mL. After two to four weeks of double immunosuppressant treatment with tacrolimus, this is usually discontinued. mTORi use is stopped for an elective surgical procedure.

After hospital discharge, patients are visited and blood samples taken every week and after three/four months, patients are visited monthly for laboratory testing. One hundred and eighty days after OLT, visits were every 60 d.

Elevation in serum creatinine, blood pressure and blood sugar or the appearance of proteinuria were registered.

Data collection
Patient information is prospectively registered in an SPSS electronic register on all OLT patients at our center. The database is available only for clinical studies. For this study, data were extracted on mTORi treated patients from this clinical register. Clinical and demographic information contained sex, age, donor age, cause of liver cirrhosis, graft quality, existence of HCC, OLT date, complications, cause of CNI treatment being converted to mTORi, retransplantation and presence of diabetes mellitus and/or hypertension before OLT. Biochemistry and hematological data included baseline plasma creatinine levels (just to conversion to mTORi), at 6, 12, 18 mo, and the last serum creatinine level while taking mTORi treatment. One independent investigator audited 10% of the

results and found > 99% data congruity.

Statistical analysis
This analysis used means for parametric data and medians for non-parametric data. We used Fisher's exact tests for comparisons of categorical variables. We analyzed non-normally distributed variables with Mann-Whitney U-tests and two-sided *t*-tests were used to compare normally distributed variables.

Linear regression was applied to examine the effect of mTORi exposure on the last serum creatinine at the end of follow-up. MTORi exposure was examined as a continuous and a dichotomous variable.

We applied only confounders which influenced the point estimate by $\geq$ 10% for adjusted models (19). We considered *P value* < 0.05 as significant; two-sided tests were used.

RESULTS
mTORi was started at a median 48 mo (DT: 56.8, range = 0-241) after OLT. Recipients were followed on mTORi for a median of 40.6 mo (DT: 18.0, range = 18-76). Reasons for switching to mTORi were avoiding HCC recurrence (*n* = 27), neurotoxicity because of tacrolimus (limb tremors, headaches, paresthesia) (*n* = 3), prevention of renal insufficiency (*n* = 28), acute rejection with tacrolimus/cyclosporine (*n* = 6), and "*de novo*" neoplasia (*n* = 22).

No mTORi patient developed serious adverse effects and there was no hepatic artery thrombosis. The clinical characteristics of the patients converted to mTORi are described in Table 1.

Initial plasma creatinine levels of patients at the moment of initiating mTORi treatment (median 48 mo after OLT) were 1.31 mg/dL. Creatinine was (mg/dL) 1.19, 1.19, 1.22 at 6, 12 and 18 mo and 1.23 mg/dL at the follow-up after the mTORi switch. There was an improvement between the initial and final creatinine levels while taking mTORi, but without statistical significance: 1.31 mg/dL and 1.22 mg/dL (*P* = 0.92), although this is a global analysis in all patients,
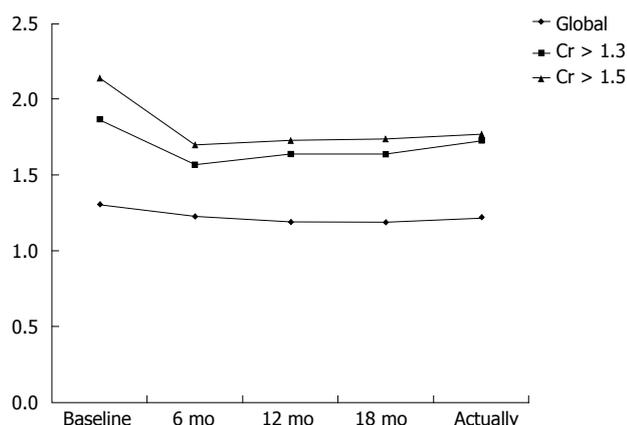
Figure 1 Improvement of serum creatinine (mg/dL) after conversion to mammalian target of rapamycin inhibitors. Cr: Serum creatinine mg/dL; Baseline: Serum creatinine just before conversion.

converting because of renal dysfunction or for other reasons. We can observe the same low difference when we analyze converted patients with plasma creatinine levels > 1.3 mg/dL (1.87 mg/dL and 1.73 mg/dL, $P = 0.78$). Only patients converted because of renal dysfunction with plasma creatinine levels > 1.5 mg/dL show a statistically significant improvement of renal function, with initial levels of 2.14 and final ones of 1.77 mg/dL ($P = 0.02$) (Figure 1).

We next investigated whether the mTORi effect is less in recipients with diabetes mellitus and/or high blood pressure (HT) (Figure 2). Subgroup analysis of only those mTORi patients with DM shows no improvement of serum creatinine levels (1.31 mg/dL to 1.37 mg/dL) compared with non DM patients (1.31 mg/dL to 1.15 mg/dL) ($P = 0.01$) and it is the same when comparing HT patients (1.48 mg/dL to 1.5 mg/dL) with non HT patients (1.21 mg/dL to 1.08 mg/dL) and patients with proteinuria (1.44 mg/dL to 1.41 mg/dL) and no proteinuria (1.31 mg/dL to 1.11 mg/dL).

Finally, we considered patients with DM, HT and proteinuria (Figure 3). Although converting to mTORi, these patients have worsening renal function (1.35 mg/dL to 2.07 mg/dL) compared with patients when only one of these factors is present ($P = 0.04$).

DISCUSSION

Our study shows retrospectively that mTORi conversion resulted in an improvement in renal function in patients with plasma creatinine levels above 1.5 mg/dL. In patients with better renal function, conversion therapy involves no improvement. This improvement has been described in several published studies but none have shown that the worse the renal function, the greater the improvement after conversion[9,10,14-17].

MTORi was started a median of eight months after OLT for a variety of reasons. Plasma levels of creatinine at the start of the study were comparable in both mTORi and CNI cohorts. A personal history

of risk factors for renal damage, such as diabetes mellitus and arterial hypertension, was comparable in both mTORi and CNI cohorts and considered in a multivariate model. Patients with hepatocellular carcinoma were adjusted in the mTORi cohort because treatment with chemotherapy may have affected serum creatinine. Despite this, it could be possible that some confounders are distributed unevenly in both groups. Further randomized trials may be necessary to avoid this problem.

Furthermore, we have segregated groups of patients with DM, hypertension and proteinuria and patients with all three diseases. We have seen how renal function does not improve after conversion to mTORi in these patients but it stops the progressive deterioration secondary to calcineurin inhibitors. However, in patients with DM, hypertension and proteinuria, renal function worsens despite conversion to mTORi.

Nephropathy is a major complication of type 1 and type 2 diabetes mellitus, along with CNI toxicity, end-stage renal dysfunction and hemodialysis[18]. Chronic nephropathy is also worsened by arterial hypertension. Diabetic nephropathy is first characterized by microal-buminuria and later by glomerular sclerosis. Podocytes play an important role in preventing proteinuria. Podocyte damage and reduction in the number of these cells contribute to the development of diabetic nephropathy[19]. mTOR plays a very important role in podocyte growth and size control. This molecule forms two different functional complexes, mTORC1 and mTORC2. Sirolimus and everolimus selectively inhibit mTORC1 but not mTORC2. In the first stages of diabetic damage in the kidney, an increased mTORC1 activity and podocyte hypertrophy can be observed. Moreover, there are some studies that report mTORi treatment to prevent diabetic nephropathy in animal models. Paradoxically, sirolimus and everolimus cause proteinuria and glomerular sclerosis in some patients[19,20]. In our study, we observed that these experimental findings are corroborated clinically in liver transplant patients with diabetic nephropathy.

There are no studies linking mTORi effectiveness in patients with hypertensive nephropathy. In our series, we showed how renal function, although not improved after conversion to mTORi, stabilizes after this change in immunosuppression regimen.

Proteinuria is a frequent side effect after switching from CNI to mTORi treatment in another solid tran-splant patient as a kidney graft recipient[21-26]. Wadei *et al*[21] shows that patients who developed massive proteinuria had a 3.3-fold increased risk of further renal insufficiency after mTORi conversion and proteinuria less than 1000 mg/d do not present with this association. This author indicates that a higher mTORi level after OLT diabetes and a lower eGFR at time of mTORi switching were observed with the appearance of very important urinary protein excretion after mTORi treatment. This study is in concordance with other articles that show a dose-dependent effect of mTORi on proteinuria and

Figure 2 Improvement of serum creatinine (mg/dL) after conversion to mammalian target of rapamycin inhibitors in different groups. Baseline: Serum creatinine just before conversion; Cr: Serum creatinine mg/dL; DM: Diabetes mellitus; HT: Hypertension.

Figure 3 Improvement of serum creatinine (mg/dL) after conversion to mammalian target of rapamycin inhibitors in different groups. Baseline: Serum creatinine just before conversion; DM: Diabetes mellitus; HT: Hypertension; Prot: Proteinuria.

podocyte protein expression[12,27-31]. Higher proteinuria before mTORi treatment has also been correlated with massive proteinuria after switching.

Our results do not support these studies as we have shown that in patients with proteinuria, mTORi conversion leads to a stabilization of this proteinuria as well as serum levels of creatinine.

We recognize some limitations in this study. We do not routinely measure eGFR levels with Modification of Diet in Renal Disease or Cockcroft-Gault equations because this measurement is not very precise and not validated in OLT recipients.

In conclusion, we observed that, after OLT, switching from a CNI-based immunosuppression regimen to mTORi-based treatment improves renal function, when compared with recipients who did not switch, when creatinine levels are ≥ 1.5 mg/dL. In patients with diabetes or hypertensive nephropathy, conversion to mTORi does not improve renal function but stabilizes plasma levels of creatinine. Proteinuria is not a contraindication to conversion to mTORi, it also stabilizes renal function. Only patients with diabetes, hypertension and proteinuria should avoid conversion to mTORi because it worsens. Complete understanding of the effects of mTORi in liver transplant recipients derived from randomized, controlled trials will help better use of this immunosuppression regimen after OLT.

COMMENTS

Background
This study shows how mammalian target of rapamycin inhibitors (mTORi) based immunosuppression therapy in liver transplant recipients with diabetic

and/or hypertensive renal dysfunction, even in patients with proteinuria, preserves renal function and plasma levels of creatinine.

Research frontiers
MTORi based immunosuppression therapy in liver transplant patients and renal chronic disease.

Innovations and breakthroughs
Observational study in diabetic and hypertensive liver transplant patients and those with proteinuria.

Applications
This study helps to choose immunosuppression treatment in patients with renal dysfunction after liver transplant.

Terminology
mTORi (mTOR inhibitors like sirolimus and everolimus, immunosuppression drugs for transplanted patients).

Peer-review
The manuscript observed the effect of mTORi-based immunosuppression therapy on diabetes mellitus, arterial hypertension and proteinuria for analysis of the potency of mTORi to renal function. This may be useful for clinical therapy.

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ORIGINAL ARTICLE

Observational Study

Underestimation of chronic renal dysfunction after liver transplantation: ICEBERG study

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Abstract

AIM: To compare prevalence of chronic renal dysfunction (CRD) according to serum creatinine (sCr) *vs* estimated glomerular filtration rate (eGFR) among maintenance liver transplant patients.

METHODS: The ICEBERG study was an observational, retrospective, cross-sectional, and multicenter study. Consecutive adult patients (aged 18 years or older) with liver transplantation (LT) performed at least two years previously were recruited. Multi-organ transplant recipients were excluded. Chronic renal dysfunction was defined according to sCr based criteria in routine clinical practice ($\geq$ 2 mg/dL) and eGFR using MDRD-4 equation (< 60 mL/min per 1.73 m^2). Agreement between sCr definition and eGFR assessment was evaluated using the Kappa index. Cox regression analysis was applied to identify predictive factors for developing CRD after LT.

RESULTS: A total of 402 patients were analyzed (71.6% males). Mean ± SD age at transplant was 52.4 ± 9.8 years. Alcoholic cirrhosis without hepatocellular carcinoma was the most common reason for LT (32.8%). Mean time since LT was 6.9 ± 3.9 years. Based on sCr assessment, 35.3% of patients (95%CI: 30.6-40.0) had CRD; 50.2% (95%CI: 45.3-55.1) according to eGFR. In 32.2% of cases, sCr assessment had underestimated CRD. Multivariate analysis showed the following factors associated with developing CRD: eGFR < 60 mL/min per 1.73 m^2 at three months post-transplant [hazard ratio (HR) = 4.76; 95%CI: 2.78-8.33; *P* < 0.0001]; calcineurin inhibitor use (HR = 2.31; 95%CI: 1.05-5.07; *P* = 0.0371); male gender (HR = 1.98; 95%CI: 1.09-3.60; *P* = 0.0260); and $\geq$ 10 years post-transplantation (HR = 1.95; 95%CI: 1.08-3.54; *P* = 0.0279).

CONCLUSION: Seven years after LT, CRD affected half our patients, which was underestimated by sCr. An eGFR < 60 mL/min per 1.73 m² three months post-LT was predictive of subsequent CRD.

Key words: Calcineurin inhibitor; Glomerular filtration rate; Chronic renal dysfunction; Liver transplantation; Prevalence

Core tip: We aimed to compare the prevalence of chronic renal dysfunction (CRD) according to serum creatinine (sCr) *vs* that based on estimated glomerular filtration rate (eGFR) among maintenance liver transplant patients. According to eGFR assessment, after seven years of post-transplant follow-up, half of patients have CRD, suggesting that the occurrence of renal dysfunction is significantly under-estimated by sCr assessment in routine practice. The study outlines the importance of early CRD detection using more sensitive tools. In this sense, eGFR at 3-mo post-transplantation provides a powerful independent predictive factor for the development of CRD in liver transplant recipients.

INTRODUCTION

Chronic renal dysfunction (CRD) is a common and dangerous complication following liver transplantation (LT)[1-3]. The majority of liver transplant recipients who survive beyond the first six months post-transplant develop CRD[4,5]. The reported incidence varies widely, from 20% to 80%[5-7], depending on the definition of CRD and the methodology used in studies[8].

The key causative factor for renal disorders in nonrenal transplant recipients has been attributed to calcineurin inhibitor (CNI) nephrotoxicity[2,9]. Nevertheless, other risk factors-including older age, hepatitis C virus (HCV) infection, the presence of diabetes mellitus or hypertension before transplantation, and pre-transplant renal dysfunction-are known to be independent predictors of CRD after LT[10-16].

Development of CRD after nonrenal organ transplantation is associated with a greater than 4-fold increase in the risk of death[12]. Therefore, early detection of CRD following LT is essential to delay the progression of renal disease and reduce its associated morbidity/mortality.

Serum creatinine (sCr) is the most established tool for estimating renal function. However, sCr alone may not be an accurate indicator of the degree of renal dysfunction. Not only it is a delayed marker of decreased kidney function[17], but it is also influenced by such nonrenal factors as gender, age, race, weight or protein intake and, additionally, is significantly decreased in patients with chronic liver disease[9,17]. Consequently, estimated glomerular filtration rate (eGFR) using a prediction equation that takes into account the sCr level and some of these independent factors has been recommended as a method for measuring renal function in these patients[18]. A number of creatinine-based equations have been developed for estimating GFR[19-23]. In adults, the modification of diet in renal disease (MDRD) equation[20] provides a clinically useful estimate of GFR[18].

This is a descriptive study primarily aiming to evaluate a national cohort of liver transplant patients still alive after a median follow-up of seven years and to assess CRD prevalence by comparing two measurements currently employed in routine practice: sCr and GFR estimated by MDRD-4. Secondary objectives were to analyze how renal function evolved, identify potential risk factors for developing CRD and assess to what extent the clinical diagnosis of CRD leads to a change in immunosuppressive therapy.

MATERIALS AND METHODS

The ICEBERG study was an observational, retrospective, cross-sectional, multicenter study conducted in 21 LT outpatient clinics in Spain. Patients eligible for inclusion were consecutive patients seen at the clinic aged 18 years or older at transplantation, with at least two years of post-transplant data on renal function to better ensure stable renal function. Multi-organ transplant recipients were excluded. The study was approved by the ethics committee at Hospital Clinic of Barcelona (Spain). Signed informed consent was obtained from all patients prior to their inclusion.

Patients fulfilling the selection criteria were consecutively enrolled by the participating investigators, resulting in the inclusion of 409 patients between September and November 2009. Patient profiles consisted of current clinical and analytical data and medical records.

CRD diagnosis was recorded based on sCr and, alternatively, estimating GFR using the abbreviated MDRD-4 equation[20,21]: estimated GFR (mL/min per 1.73 m²) = 186 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) × (1.210 if African-American). The cut-off point to define CRD was ⩾ 2 mg/dL for sCr and < 60 mL/min per 1.73 m² for eGFR based on Kidney Disease Outcome Quality Initiative (K-DOQI) guidelines[18,24].

McNemar's test was used to compare frequencies between subgroups for qualitative variables. Agreement between sCr definition and eGFR assessment was evaluated using the Kappa index. Cox regression

Table 1 Demographics and clinical characteristics of the 402 liver transplant recipients

Variables	*n* (%)
Age at transplant (yr), mean ± SD	52.4 ± 9.8
Gender (male)	288 (71.6)
Ethnicity (Caucasian)	400 (99.5)
Donor age, mean ± SD	47.0 ± 18.9
Time since transplantation (yr), mean ± SD	6.9 ± 3.9
Pre-transplant comorbidities	
Diabetes mellitus	71 (17.7)
Hypertension	36 (9.0)
Dyslipidemia	13 (3.2)
Coronary heart disease	8 (2.0)
Reason for transplantation	
Alcoholic cirrhosis without hepatocellular carcinoma	132 (32.8)
Hepatocellular carcinoma (in HCV or HBV-related liver cirrhosis, alcoholic cirrhosis or non-cirrhotic liver)	92 (22.9)
HCV-related liver cirrhosis without hepatocellular carcinoma	74 (18.4)
Cholestatic liver disease	24 (6.0)
HBV-related liver cirrhosis without hepatocellular carcinoma	23 (5.7)
Acute liver failure	9 (2.2)
Others	45 (11.2)
Induction therapy	68 (16.9)
Immunosuppressive treatment (at discharge)	
Monotherapy	34 (8.5)
Cyclosporine	14 (3.5)[1]
Tacrolimus	20 (5.0)[1]
Combined therapies	368 (91.5)
Cyclosporine-based	155 (38.6)[1]
Tacrolimus-based	149 (37.1)[1]
mTOR inhibitor-based	63 (15.7)[1]
Others	1 (0.3)[1]

[1]Percentages with respect to the total population. HCV: Hepatitis C virus; HBV: Hepatitis B virus; mTOR: Mammalian target of rapamycin.

Figure 1 Prevalence of chronic renal dysfunction based on serum creatinine (A) and estimated glomerular filtration rate (modification of diet in renal disease-4) (B) in 402 liver transplant recipients.

analysis was applied to determine the predictors of CRD after LT. A *P*-value < 0.05 was considered significant. Statistical analyses were performed with SPSS (version 12.0, SPSS Inc., Chicago, Illinois, United States).

Statistical analysis

The statistical methods of this study were reviewed by Daniel Mosteiro (Senior Biostatistician) from TFS.

RESULTS

A total of 402 patients were included in the analysis. Seven patients with missing values for sCr were excluded. Table 1 shows the main characteristics of the study sample. The vast majority of patients were male Caucasians, with a mean age of 52.4 ± 9.8 years at transplant and an average Model for End-Stage Liver Disease (MELD) score during the transplant evaluation of 15.9 ± 6.1 (125 patients were lacking data). Mean time post-transplantation was 6.9 ± 3.9 years (range: 2-20 years). At the time of transplantation, 17.7% of patients had diabetes mellitus and 9.0% hypertension. The main indication for LT was alcoholic cirrhosis without hepatocellular carcinoma (32.8%),

while hepatocellular carcinoma was the impetus for transplantation in 22.9% of patients. Antibody induction therapy was used in 16.9% of patients (mainly anti-CD25). At the time of discharge, the most commonly used immunosuppressants were CNI (either cyclosporine or tacrolimus), prescribed as monotherapy (8.5%) or in combination with other immunosuppressive treatments (91.5%). Biopsy-confirmed acute rejection was diagnosed in 94 patients (23.4%) and, during the maintenance phase, diabetes and hypertension were diagnosed in 135 (33.6%) and 208 (51.7%) patients respectively. Additionally, 48 patients (11.9%) developed a malignancy following transplantation.

Based on sCr, CRD was diagnosed in 142 out of 402 patients (35.3%, 95%CI: 30.6 to 40.0) whereas, according to MDRD-4, CRD was diagnosed in 202 patients (50.2%, 95%CI: 45.3 to 55.1; *P* < 0.0001) (Figure 1). Of the 202 patients with eGFR below 60 mL/min per 1.73 m^2, 63 (31.2%) had creatinine levels ≥ 2 mg/dL but 139 (68.8%) had creatinine < 2 mg/dL (Table 2). When examining the concordance between the sCr-based definition and eGFR, diagnosis of CRD according to the former was established in 98.4% of patients with laboratory values of sCr ≥ 2 mg/dL and eGFR < 60 mL/min per 1.73 m^2. However, 46.0% of CRD patients with sCr < 2 mg/dL and eGFR < 60 mL/min per 1.73 m^2 were not correctly diagnosed. In this patient subgroup, 56.3% of patients had creatinine values above 1.25 mg/dL but below 2 mg/dL (Table 2). Among 31 patients with creatinine < 1.25 mg/dL and eGFR < 60 mL/min per 1.73 m^2, only 3 cases (4%) were adequately diagnosed by the sCr. In summary, there was moderate agreement between the two definitions Kappa coefficient: 0.65 (95%CI: 0.58-0.72); with 32.2% of patients with eGFR < 60 mL underdiagnosed using the sCr based assessment (Table 3).

The mean time point when CRD was clinically diagnosed according to sCr was 2.5 ± 3.7 years after transplantation; the time from transplantation to CRD diagnosis was less than 2 years in 62.7% of patients, from 2-5 years in 20.4% and over 6 years later in 16.9% of patients.
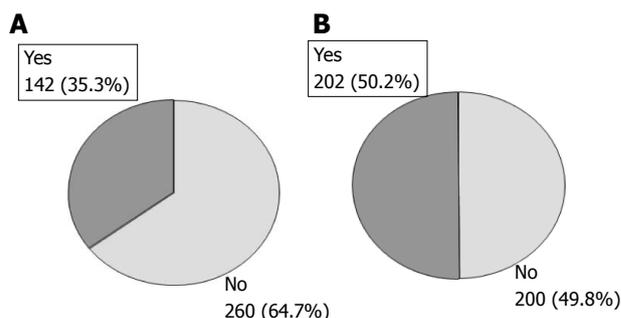
Table 2 Chronic renal dysfunction based on serum creatinine definition and estimated glomerular filtration rate assessment (modification of diet in renal disease-4) *n* (%)

| | | CRD diagnosis according to serum creatinine definition | | |
		Yes	No	Total
CRD diagnosis according to eGFR (MDRD-4)	Creatinine ≥ 2 mg/dL and eGFR < 60 mL/min per 1.73 m[2]	62 (98.4)[1]	1 (1.6)[1]	63 (31.2)[2]
	Creatinine < 2 mg/dL and eGFR < 60 mL/min per 1.73 m[2]	75 (54.0)[1]	64 (46.0)[1]	139 (68.8)[2]
	Creatinine < 1.25 mg/dL	3 (4.0)[3]	28 (43.8)[3]	
	Creatinine 1.25 - < 1.50 mg/dL	19 (25.3)[3]	30 (46.9)[3]	
	Creatinine 1.50 - < 2.0 mg/dL	53 (70.7)[3]	6 (9.4)[3]	
	Total	137 (67.8)[2]	65 (32.2)[2]	202 (100)

[1]Row percentages; [2]Percentages with respect to the total number of patients with CRD diagnosis according to eGFR; [3]Column percentages. CRD: Chronic renal dysfunction; eGFR: Estimated glomerular filtration rate; MDRD: Modification of diet in renal disease.

Table 3 Chronic renal dysfunction: concordance between serum creatinine definition and estimated glomerular filtration rate assessment (modification of diet in renal disease-4) *n* (%)

| | | CRD diagnosis according to serum creatinine definition | | |
		Yes	No	Total
CRD diagnosis according to eGFR assessment (MDRD-4)	Yes	137 (67.8)[1]	65 (32.2)[1]	202 (50.2)[2]
	No	5 (2.5)[1]	195 (97.5)[1]	200 (49.8)[2]
	Total	142 (35.3)[2]	260 (64.7)[2]	402 (100)

[1]Row percentages; [2]Percentages with respect to the total population. CRD: Chronic renal dysfunction; eGFR: Estimated glomerular filtration rate; MDRD: Modification of diet in renal disease.

Figure 2 shows the changes in sCr levels and eGFR in liver recipients with and without clinical diagnosis of CRD one year post-transplant. Thereafter, patients with diagnosis of CRD showed higher levels of sCr and lower estimated GFR compared to those patients without CRD.

Multivariate Cox regression analysis showed that the following factors were associated with an increased risk of CRD: an eGFR value below 60 mL/min per 1.73 m[2] at 3 mo post-transplant; CNI-based immunosuppressive therapy at discharge; recipient male gender; and time since transplantation (Table 4).

Following a diagnosis of CRD based on sCr, renal biopsy was performed in only four patients (2.8%). Renoprotective treatment [angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers] was introduced in 43 out of 142 patients (30.3%), while 7 patients (4.9%) needed renal replacement therapy (5 hemodyalisis, 1 renal transplant and 1 both). When CRD was diagnosed, changes in immunosuppressive therapy were initiated in 128 out of 142 patients (90.1%). All such changes were based on a reduction in CNI therapy. In addition, modifications to mycophenolic acid (MPA) therapy or introduction of mammalian target of rapamycin (mTOR) inhibitor therapy were undertaken in 45.1% and 12.0% of patients with CRD respectively.

DISCUSSION

Early identification of renal dysfunction after LT is essential to delay the progression of chronic kidney disease and improving long-term patient health[11,16]. In our study of LT patients, CRD was a common post-transplant complication with a prevalence ranging from 35.3% to 50.2% depending on the criteria applied. It is worth noting that the study shows how CRD is markedly underestimated by the sCr based assessment still used in clinical practice. Three out of ten patients with criteria for CRD based on eGFR using the MDRD-4 equation[21] had been underdiagnosed. It is important to note that sCr values from 1.25 to 2 mg/dL can frequently be misinterpreted despite concomitant abnormal eGFR values. Our results indicate that clinical diagnosis of renal dysfunction in routine clinical practice relies frequently on the less sensitive measurement of increased sCr concentration when, in fact, the eGFR may provide a better tool for detecting early renal dysfunction[24]. However, our findings are within the range of CRD prevalence reported by previous studies that had already shown CRD to be a common post-LT complication[5-7,12]. For instance, in the adult Finnish LT population, almost 40% of patients had an eGFR below 60 mL/min at three years post-transplantation[7], and according to Gayowski *et al*[6], 28% of liver transplant recipients developed late-onset renal failure, defined as sCr levels persistently exceeding 2.0 mg/dL six months post-transplantation. However the lack of a standard definition for CRD explains differences in prevalence among studies.

We performed a retrospective analysis of liver recipients to identify risk factors for the development

Table 4 Predictive factors associated with developing chronic renal dysfunction in liver transplant recipients

Variables	HR (95%CI)	*P*-value
Three months post-transplant eGFR (< 60 *vs* ≥ 60 mL/min per 1.73 m²)	4.76 (2.78-8.33)	< 0.0001
CNI treatment at discharge (CNI *vs* non-CNI)	2.31 (1.05-5.07)	0.0371
Recipient gender (male *vs* female)	1.98 (1.09-3.60)	0.026
Year of transplantation (≤ 1999 *vs* > 1999)	1.95 (1.08-3.54)	0.0279

Other analyzed but non-significant variables were: donor gender (male or female), reason for transplantation (hepatitis C virus-related or not), antibody induction therapy at transplantation (yes or no), biopsy-confirmed graft rejection (yes or no), post-transplant diabetes mellitus (yes or no), post-transplant hypertension (yes or no), and pre-transplant eGFR (< 60 or ≥ 60 mL/min per 1.73 m²). eGFR: Estimated glomerular filtration rate; CNI: Calcineurin inhibitors.

Figure 2 Changes in serum creatinine levels (A) and estimated glomerular filtration rate (B) in 402 liver recipients with or without clinical diagnosis of chronic renal dysfunction from one year post-transplantation (grey and black lines respectively). CRD: Chronic renal dysfunction; eGFR: Estimated glomerular filtration rate.

of CRD. Several studies have reported that eGFR, either at the time of LT or during the early stages following transplantation, is an independent predictor of post-transplant chronic kidney disease[10,11,25-28]. Our study validated these results and showed that a low eGFR three months post-transplant was associated with an increased risk of CRD [hazard ratio (HR) = 4.76 for eGFR < 60 mL/min per 1.73 m² *vs* eGFR ≥ 60 mL/min per 1.73 m²]. This finding is particularly interesting since it suggests that it may be possible to identify those patients at high risk of developing CRD within the first three months after transplantation with an easy-to-use tool such as the MDRD-4 equation.

After examining a variety of demographic and clinical variables, in contrast to previous studies, we found that male gender was a predictive factor of CRD (HR = 1.98 for male *vs* female). Curiously, other studies have reported just the opposite, with female gender being associated with a higher risk for developing CRD after LT[10,12,29]. However, Ojo *et al*[12] defined chronic renal failure as an eGFR ≤ 29 mL/min per 1.73 m², instead of using a CRD cut-off point (eGFR < 60 mL/min per 1.73 m²), which could explain the different outcomes discussed above.

In the current study, time since transplantation was also significantly associated with the risk of developing

CRD (HR = 1.95 for transplantations performed prior to 1999 *vs* those carried out after that date), as had previously been reported by other authors[12]. In fact, this might largely be explained by the more persistent nephrotoxic effects of immunosuppression in those patients with better survival rates and longer follow-up available[9].

In contrast to previous studies[12,15,16], we found that such comorbidities as hypertension or diabetes mellitus prior to transplantation were not predictors of CRD. HCV-related disease has also been reported to be a risk factor affecting renal function[10,12], though this did not prove significant in our study. Differences in comorbidity profile and therapeutic management among different patient cohorts may account for the disparities in the results.

The introduction of ACE inhibitors and angiotensin receptor blockers may be of particular benefit in liver transplant recipients due to the renoprotective effects they confer[30,31]. Nevertheless, based on routine clinical practice criteria, the introduction of renoprotective treatment after clinical diagnosis of CRD was moderately low (approximately 30% of patients). Moreover, renal biopsy was performed in a low percentage of patients (2.8%) and few patients (4.9%) required renal replacement therapy, similar to what has been previously reported[32].

CNI-associated chronic nephrotoxicity has been widely reported[9,30,31] and CNI-based regimens at discharge have already been identified as independent predictors of CRD following transplantation[12,15], which is consistent with our own results (HR = 2.31 for CNI *vs* non-CNI). Moreover, CNI reduction in combination with MMF has been shown to improve eGFR in *de novo* LT, as well as in patients with moderately impaired renal function[33-35]. In our study, a strategy based on the reduction or withdrawal of CNI therapy was carried out in approximately 80% of liver recipients with diagnosis of CRD based on the sCr definition, while MPA therapy modification was undertaken in nearly half of them.

The present study has several strengths. Firstly, the relatively large sample size of a country-based cohort and secondly, the patients are representative of routine clinical practice in Spain. Several indicators, such us the high percentage of changes in immunosuppressive therapy and the low percentage of patients requiring renal replacement therapy among patients with CRD, demonstrate adequate clinical management in current practice. Thirdly, patients were enrolled by consecutive sampling. All this should outweigh the limitations inherent to retrospective studies which can lead to patient selection bias and inaccurate data collection. Moreover, we were able to compile data over a prolonged time period (almost 20 years), which allowed us to examine long-term changes in renal function. However, the laboratory criteria used to define CRD were arbitrarily established using a cut-off point of 2 mg/dL that has been used in other studies in solid organ transplantation[36]. Furthermore, local creatinine assessment techniques were not analized. Thus, heterogeneity in diagnosis cannot be ruled out. In addition, the use of creatinine secretion inhibitors was not an exclusion criterion. Also, the use of a simplified MDRD equation for GFR estimation also carries some limitations[37,38] although it has been validated in liver transplant patients[39]. Additionally, the study focused only on CRD defined two years after liver transplant and did not differentiate between other common functional renal disorders such as hepatorenal syndrome. Nevertheless, we have been able to provide detailed independent data on eGFR and creatinine in order to better understand the interpretation of these parameters in the clinic-based liver transplant setting. Another constraint worth mentioning is the lack of MELD scores, which have been used since 2002, in a third of the patients. Consequently, we were not able to evaluate how the introduction of these prioritization criteria might have influenced worsening of renal function in these patients[40]. Lastly, data on the effects of immunosuppression could only be analyzed on the basis of drug class; once the CRD diagnosis according to sCr was established, we could not assess whether or not these therapeutic interventions had any effects on renal function.

In conclusion, our study corroborates that CRD is a prevalent condition following LT and that the occurrence of renal dysfunction is significantly under-assessed in routine practice. The significant divergence between a currently used sCr based definition and an eGFR assessment of CRD may stem from the absence of broadly accepted criteria among physicians, thus hindering their ability to accurately identify the disorder. In this sense, estimated GFR at 3-mo post-transplantation provides a powerful and independent predictive factor for the development of CRD in LT patients. The use of more accurate diagnostic measurements will not only permit earlier detection of renal dysfunction, but also facilitate appropriate therapeutic intervention, which could yield significant benefits for long-term renal function and patient survival.

ACKNOWLEDGMENTS

COMMENTS

Background

Chronic renal dysfunction (CRD) is a common complication following liver transplantation. Serum creatinine is the most established tool for estimating renal function. However, serum creatinine alone may not be an accurate indicator of the degree of renal dysfunction. The abbreviated modification of diet in renal disease equation could provide a clinically useful estimate of glomerular filtration rate.

Research frontiers

Serum creatinine not only is a delayed marker of decreased renal function, but it is also influenced by nonrenal factors. Consequently, estimated glomerular

filtration rate (eGFR) using a prediction equation that takes into account the serum creatinine level and some of these independent factors, such as gender, age or race, has been recommended as a method for measuring renal function.

Innovations and breakthroughs

The study results suggest that there is a significant divergence between the diagnosis of CRD based on a serum creatinine assessment and the eGFR, under daily practice conditions. According to eGFR assesment, CRD is present in almost half percent of liver recipients after approximately seven years of post-transplant follow-up. However, the rate of CRD is significantly under-estimated according to serum creatinine assessment in daily practice.

Applications

Overall, this study outlines the importance of early CRD detection among liver transplant recipients *via* the use of more sensitive tools. In this sense, eGFR at 3-mo post-transplantation is a powerful independent predictive factor for the development of CRD in liver transplant recipients.

Terminology

Chronic renal dysfunction is defined as kidney damage or glomerular filtration rate < 60 mL/min per 1.73 m^2 for three months or more, irrespective of the cause.

Peer-review

The data provided show that CRD is more prevalent than expected in liver transplants, and that a change from calcineurin Inhibitors to mammalian target of rapamycin inhibiting drugs may alleviate the renal damage.

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CASE REPORT

Diagnostic dilemma of coagulation problems in an HIV-positive patient with end-stage liver disease undergoing liver transplantation

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Abstract

Human immunodeficiency virus (HIV) may result in devastating multi-organ complications, including cirrhosis. Consequently, liver transplantation is often required for these patients. We report a case of a 43-year-old female with cryptogenic cirrhosis and HIV on highly active antiretroviral therapy, presenting for non-related living donor liver transplantation. The intraoperative course was complicated by hepatic artery and portal vein thrombosis, requiring thrombectomy. On postoperative day-3, the patient required re-transplantation with a cadaveric donor organ due to primary graft failure.

Key words: Hypercoagulation; Liver transplant; Highly active antiretroviral therapy; Human immunodeficiency virus

Core tip: Liver transplantation is a technically complicated procedure associated with both predictable and unpredictable coagulation abnormalities. The surgeons are more concerned about bleeding than thrombotic complications in cirrhotic patients undergoing liver transplant, but the reality these patients are equally at risk of both complications. The risk of a thrombotic event is even higher in human immunodeficiency virus (HIV) patients on highly active antiretroviral therapy (HAART) both during and after the surgical procedure. This fact should be ranked high in the differential diagnosis of liver allograft failure in liver transplant recipients who are HIV positive and receiving HAART.

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INTRODUCTION

Human immunodeficiency virus (HIV) is a devastating illness with an estimated incidence of 40000 annual cases in the United States. Treatment with highly active antiretroviral therapy (HAART) has allowed for significant immunologic recovery, resulting in a notable decrease in opportunistic infections and prolonged life expectancy. However, treatment with HAART has resulted in the emergence of other complications such as severe hepatotoxicity. We present a case involving a patient with cryptogenic liver disease and HIV whose liver transplantation was complicated by unanticipated hepatic artery and portal vein thrombosis as well as subsequent graft failure.

CASE REPORT

A 43-year-old female with cryptogenic cirrhosis, presented for non-related living donor liver transplantation. Her medical history was notable for HIV, diagnosed in 1994, and HIV-related complications including pneumocystis carinii pneumonia and malabsorption syndrome. Her Model for End-Stage Liver Disease score on the date of the transplant was 24 and her pre-transplant laboratory test results were as follows: serum bilirubin 3.5 mg/dL, INR 1.4, platelet count 70000, and hemoglobin 11 gm/dL; acid-base and electrolytes were within normal range. Due to malabsorption and severe emaciation, she was put on total parenteral nutrition (TPN) was initiated in 2006 and resulted in significant weight gain. Preoperative viral load was undetectable and CD4 count was acceptable at 204/μL. Antiviral therapy included efavirenz (sustiva), a non-nucleoside reverse transcriptase inhibitor, and zidovudine and lamivudine (3TC), which are both nucleoside reverse transcriptase inhibitors. The patient underwent non-related living donor liver transplantation using a right lobe graft donated by a friend. The patient had the standard of care invasive monitoring which include, 2 arterial lines (one radial and one femoral), pulmonary artery catheter, and 18 FG cannula for veno-venous bypass both were established in the right internal jugular vein while another 9 FG cannula was inserted in the left internal jugular and connected to electrical-powered rapid infuser to be available in case of requirement for massive transfusion. As standard of care at our Institution TEE probe was used for continuous cardiac monitoring. All lines were established after induction of general anesthesia and performed by senior anesthesiologist without any complications. As a standard of care for liver transplant recipients, arterial blood gas and thromboelastograph tracing are tested on hourly basis or as the clinical situation demands.

The case progressed smoothly until completion of the vascular anastomosis, when thrombosis of the hepatic artery and portal vein was noticed. The diagnosis of vascular thrombosis was confirmed by Doppler ultrasound monitor. Immediate thrombectomy and heparin treatment was initiated to allow adequate graft perfusion. The thromboelastograph tracing revealed a hypercoagulable state throughout the majority of the case. The only blood products she had received were three units of red blood cells.

On post-operative day 2 (POD 2), the patient's condition deteriorated, with significant elevations in ammonia levels and liver enzymes. Doppler ultrasound examination demonstrated no blood flow through the main vessels. Graft biopsy revealed a submassive ischemic hepatic necrosis, portal vein and hepatic artery branch thrombosis, and non-occlusive hepatic vein thrombosis. Patient was re-listed as a "category 1" for orthotropic liver transplantation (OLT). Although she developed primary graft failure with a clinical picture similar to fulminant hepatic failure, her coagulation profile by TEG remained hypercaogulable and she was kept on heparin infusion during POD 1 and no fresh frozen plasma or platelets were given. A cadaveric organ became available on POD 3. The re-transplantation was completed in less than 8 h without complication during which the patient was placed on continuous Ⅳ prostacycline infusion prophylactically to prevent intravascular thrombosis. Her TEG during the second OLT was within normal range and she did not receive any coagulation products until stage Ⅲ (the neohepatic phase). Postoperatively, the patient's course was complicated with deep vein thrombosis and a new piece of the patient' past medical history that emerged: a questionable hypercoagulable syndrome with possible heparin-induced thrombocytopenia (HIT) that was considered the cause of the problem.

Heparin was discontinued secondary to continuous suspicion for HIT type Ⅱ and patient was anticoagulated with bivalirudin after her second OLT. The HIT panel and PF4 antibody testing were performed on POD 1 of her first transplant and became available on POD 7, which revealed negative HIT antibody, methylenetetrahydrofolate reductase gene mutation, and factor Ⅴ leiden mutation with a negative lupus anticoagulant test. After five weeks in the ICU and nine weeks in the hospital, she was discharged for inpatient rehabilitation facility in stable condition.

DISCUSSION

The patient's complicated course raised questions regarding the etiology of the unexpected thrombotic events. The primary concern was heparin-induced thrombocytopenia or HIT type Ⅱ, which is an antibody-mediated prothrombotic thrombocytopenia. While HIT

type 1 is a non-immune reaction, characterized by self-resolved thrombocytopenia even with continued heparin administration, HIT type II is caused by an IgG antibody that recognizes platelet factor 4 (PF4) and heparin. PF4/heparin complexes bind to platelet surfaces, forming HIT-IgG/PF4/heparin[1] that lead to platelet aggregation and vascular endothelial injury. The typical HIT course results in thrombocytopenia within 4-5 d after exposure to heparin, and patient may develop thrombocytopenia within 10 h of heparin re-exposure[1].

The temporal course is uncharacteristic of HIT type II and there was no documentation that our patient had received heparin. However, she did have a pre-existing PICC line for TPN and heparin may have been used to flush the peripherally inserted central catheter (PICC line). It is known that even the smallest amounts of heparin in the form of coated catheters and line flushes can initiate the cascade of HIT type II in susceptible individuals. Early diagnosis of HIT type II is critical, as it may be associated with serious thrombotic complications with high morbidity and mortality rate related to stroke or amputation. In addition to clinical presentation, laboratory tests are useful in the diagnosis of HIT; however, negative results do not always exclude its diagnosis. These tests can be classified as functional assays and immunlogical assays. Functional assays include heparin-induced platelet aggregation and serotonin release assay, with specificity and sensitivities of 40% and 88% respectively[1]. The immunoassays (used at our institution), which measure IgG, IgM, and IgA antibodies that bind PF4 to heparin such as enzyme-linked immunosorbent assay, have an 86% specificity and a 97% sensitivity. Since HIT type II was ruled out due to the timing of thrombosis and the negative HIT panel, HIV and HAART were considered the main etiology of thrombosis and thrombocytopenia. Primary HIV associated thrombocytopenia is commonly seen in 40% of HIV positive individuals during the course of the disease. Thrombocytopenia may occur during any part of the illness with fluctuating severity based upon the levels of immunosuppression. Other causes of thrombocytopenia in this patient population include opportunistic infections and malignancy[2]. HIV related thrombosis is ten times more common in HIV positive patients than in the general population. The clinical studies reveal that the incidence of venous thromboembolism in HIV positive patients ranges from 0.25%-0.96%, however, this incidence increases to 17% when based on autopsy results[3]. Copur *et al*[4] concluded that the increased frequency of venous thromboembolism in HIV positive individuals is only applicable to those who are $\leqslant$ 50 years of age[4] and our patient age falls in this age bracket. Potential risk factors that place these individuals at higher risk include cytomegalovirus infection, Kaposi sarcoma, intravenous drug use, and medications like erythropoietin, megesterol

acetate, and protease inhibitors.

Newly emerging mechanisms continue to emphasize the correlation between antiretroviral therapies and hypercoagulability and antiretroviral therapy may be considered an independent cause of thromboembosis in HIV patients. *In vitro* studies showed that HIV might irritate vascular endothelial cell, thus altering storage and excretion of key proteins such as Von Willebrand factor and antithrombin III and decreased quantities of proteins C and S with possibly of disruption of the fibrinolytic pathway. Pro-inflammatory cytokines which activate the hemostatic system are unregulated during HIV infection and might trigger the coagulation system[5]. In HIV-positive patients and even under well-controlled viral levels, they remain at risk for inflammatory-associated complications such cardiovascular diseases and cancers. It is vital to acknowledge that immune activation results in inflammation and thrombosis, and conversely, inflammation and thrombosis induce immune activation[6]. These cumulative changes may result in a prothrombotic condition, even in well-controlled viral loads as in our patient. Autoantibodies, like lupus anticoagulant may appear in many HIV positive patients (as in our patient), while anticardiolipin antibodies that are associated with the hypercoagulable state have been found in in 45%-50% of HIV positive patients[7]. Interestingly, some of these autoantibodies are higher in HIV-infected women than in HIV-infected, putting women at a higher risk for thrombotic complications[8]. Lijfering *et al*[9] noted a higher risk of venous and arterial thrombosis for those on combinations of antiretroviral therapy, an effect that was amplified for those on protease inhibitor (PI). Possible mechanisms may include PI-induced pleiotropic effects such as alterations in blood lipids with increase in plasminogen activator inhibitor-1 and fibrinogen. It was found that HIV can lead to impairment in vascular endothelial-dependent vasodilatation[10] and may induce dyslipidemia and hyperlipidemia with an increased risk of thrombosis. However, some studies have found that antiviral therapy may be a contributing factor to endothelial dysfunction[11]. No matter which offending agent, the development of vascular endothelial dysfunction will affect all endothelial functions and lead to abnormal vascular relaxation, activation of coagulation, and abnormal immune response. HAART has significantly improved the outcome and prognosis of HIV patients. However, the potentially serious cardiovascular complications that may be implicated with the use of protease inhibitors cannot be ignored. Still, fear of these complications should not prohibit their use.

In conclusion, liver transplantation is a technically complicated procedure associated with both predictable and unpredictable coagulation abnormalities. In HIV-positive patients on HAART regimens, risk of a thrombotic event is high both during and after any surgical procedure. Thus, prophylactic anticoagulation may be justifiable. During OLT, the administration of

small doses of heparin (≤ 3000 units) and frequent monitoring of coagulation by TEG to prevent life-threatening thrombosis should be considered.

COMMENTS

Case characteristics
The authors presented a patient with history of human immunodeficiency virus (HIV) and on highly active antiretroviral therapy (HAART) who underwent orthotropic liver transplantation (OLT) that was complicated by intraoperative thrombosis of the hepatic artery and portal vein. The possible etiologies of the hypercoagulability in this patient were HIV and HAART.

Clinical diagnosis
The hypercoagulability was presented by an immediate intravascular thrombosis and prothrombotic thromboelastograph during the OLT.

Differential diagnosis
The differential diagnosis included: Heparin-induced thrombocytopenia (HIT): Hypercoagulability induced by HIV and HAART and the presence of undiagnosed lupus antibodies.

Laboratory diagnosis
The HIT panel and platelet factor 4 antibody test to exclude the diagnosis of HIT as the etiology for the intravascular thrombosis.

Treatment
Due to ischemic liver graft failure patient was re-transplanted within the 1st 72 h after the diagnosis of primary graft failure.

Related report
Although there are scientific evidences that documented the changes in the coagulation functions in HIV patients, the authors are unaware of such complication in OLT recipient.

Experiences and lessens
It is important when taking care of HIV patients to understand the complicated interaction of the pathological process of the disease itself and the anti-HIV medications. As both the disease and the medications have complicated effects on multiple organ systems such as the effects on the immune and the coagulation systems that can make the clinical presentation quite confusing.

Peer-review
This is an interesting case with a good discussion that this reviewer recommends for to be published only after a series of small issues are fixed.

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