ORIGINAL ARTICLE

Renal safety of high-dose, sucrose-free intravenous immunoglobulin in kidney transplant recipients: an observational study

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SUMMARY

High-dose intravenous immunoglobulin (IVIg) is commonly used during kidney transplantation. Its nephrotoxicity has been attributed to sucrose stabilizers. We evaluated the renal safety of newer formulations of sucrose-free IVIg. We retrospectively studied clinical and histological data from 75 kidney recipients receiving high-dose, sucrose-free IVIg courses. This group was compared with 75 matched kidney recipients not treated with IVIg. Sucrose-free IVIg treatment was not associated with any acute kidney injury episode at 3 months, but an increased frequency of tubular macrovacuoles (28% vs. 2.8%, $P < 0.001$) was observed. Among IVIg-treated patients, the presence of macrovacuoles at 3 months was associated with increased IF/TA scores at 3 months (1.7 ± 1 vs. 1 ± 1, $P = 0.005$) and was more often observed in kidneys with higher IF/TA scores on day 0 (0.6 ± 0.9 vs. 0.3 ± 0.8, $P = 0.03$) at 3 months. Finally, patients treated with amino-acid-stabilized formulations developed fewer macrovacuoles at 3 months (12% vs. 60%; $P < 0.001$) than those treated with carbohydrate-stabilized IVIg. Our study shows that high-dose, sucrose-free IVIg use in early kidney recipients is clinically well tolerated. Among sucrose-free IVIg, amino-acid-stabilized formulations are associated with less tubular toxicity than carbohydrate-stabilized IVIg.

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Key words
intravenous immunoglobulin, kidney transplantation, osmotic nephrosis, safety, stabilizers, tubular damage

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Introduction

Intravenous immunoglobulin (IVIg) indications have dramatically increased recently in many medical fields as antibody replacement therapy or for immunomodulatory purposes [1,2]. High-dose IVIg is increasingly used in solid organ transplantation [3] for their immunomodulatory properties.

In kidney transplantation, sensitization against HLA antigens is a barrier for obtaining a compatible renal graft and decreases graft survival. IVIg is used at high doses before or after transplantation in desensitization protocols [3,4]. Two randomized controlled trials investigated the use of IVIg or IVIg associated with plasma exchanges for HLA desensitization [5,6], and more recently, it has been shown that rituximab, in addition to IVIg, reduces desensitization time from 16 to 5 weeks and transplant glomerulopathy (cg) 1 year after transplantation [7,8]. Other teams have also used IVIg for preventing humoral rejection in patients with high immunological risk [9]. In addition, IVIg is widely used to treat humoral rejection combined with plasma exchange and rituximab [10]. Additionally, IVIg has also been chosen to treat certain infectious complications in renal transplantation such as BK virus nephropathy [11] or parvovirus B19 infection [12].

However, the nephrotoxicity of IVIg has been well described since the 1990s and has been explained by tubular toxicity related to osmotic nephrosis [13]. The use of IVIg expanded in the years 1980–1990, and many studies dating from the 1990s described IVIg therapy-associated moderate-to-severe acute renal failure [14–16]. The incidence of AKI related to high-dose IVIg infusion (1–2 g/kg) was estimated to be 7% in patients with normal renal function treated for inflammatory or infectious diseases [17] and approximately 1% in another recent study [18]. In some reports, renal replacement therapy was necessary in approximately 40% of cases of AKI due to IVIg therapy [19]. Histological analysis of the kidneys showed osmotic nephrosis lesions with tubular vacuolization in almost all patients. Sucrose, an excipient contained in some formulations, has been implicated as one of the main causes of IVIg-related nephropathy [20,21], which has histological similarities with the "sucrose-induced nephropathy" described in the 1930s [22,23]. In rodents and in humans, histological findings have shown the vacuolar degeneration of proximal tubular cells associated with acute tubular necrosis [24]. The initial lesions are composed of apical microvacuoles that grow in size and finally aggregate into macrovacuoles. These macrovacuoles are identified by immunohistochemistry as lysosomes [25] and have been associated with a poorer renal prognosis in IVIg-treated kidney recipients [26].

From the mid-2000s forward, some new IVIg formulations without sucrose or any carbohydrate excipient have been used to decrease AKI incidence. Currently, high-dose, sucrose-free IVIg is used in many transplant units and nephrology departments, but no evidence has been reported regarding their safety profiles in kidney recipients.

The aim of this study was to evaluate the clinical and histological kidney safety of high-dose, sucrose-free IVIg early in the course of kidney transplantation. Our hypothesis, supported by a decrease in IVIg-associated AKI reports in the literature, was that sucrose-free IVIg, particularly carbohydrate-free formulations, is safe in kidney recipients.

Patients and methods

Study design and patient population

This study was an observational retrospective monocenter study on the clinical and histological consequences of high-dose, sucrose-free IVIg therapy early in kidney transplantation.

We included seventy-five high immunological risk patients transplanted between 2006 and 2011 who received prophylactic, high-dose IVIg courses during the 3 first months after kidney transplantation from a deceased donor. Patients were considered to be at high immunological risk when they had current or historical donor-specific anti-HLA antibodies. The IVIg treatment indication was antibody-mediated rejection prevention, as previously described [4]. Kidney recipients without a screening biopsy performed the day of transplantation and at 3 months post-transplantation were excluded.

The control group consisted of 75 patients who did not receive IVIg but who were matched with respect to the date of transplantation, type of kidney donor, type of immunosuppressive regimen (i.e., triple drug CNI-based therapy), and availability of a 3-month screening biopsy.

Immunosuppressive regimen

All included patients received an induction therapy with polyclonal antibodies or basiliximab, a calcineurin inhibitor, mycophenolic acid, and corticosteroids. Acute T-cell-

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mediated rejection episodes were treated with corticosteroids, and acute antibody-mediated rejection episodes were treated with corticosteroids in association with plasma exchange therapy and rituximab.

All IVIg-treated patients received 2 g/kg courses of IVIg administered over a 96-h period. The first course was started before reperfusion, with subsequent courses given 3, 6, and 9 weeks after transplantation. The type of IVIg differed according to availability and included Endobulin® (glucose-stabilized; Baxter, Vienna, Austria), Octagam® (maltose-stabilized; Octapharma, Boulogne Billancourt, France), Clairyg® (mannitol-stabilized; LFB, Courtaboeuf, France), Kiovig® (glycine-stabilized; Baxter), and Privigen® (L-proline-stabilized; CSL Behring, Marburg, Germany). None of these IVIgs contained sucrose as an excipient.

Histological analysis

All screening biopsies were reviewed by a renal pathologist (M.R.) and a nephrologist (Y.L.) who were blinded to the clinical information. Biopsy specimens were stained with Masson's trichrome stains. The IF/TA score was graded according to the Banff 2007 update of the Banff 1997 classification. Biopsy specimens were also examined for the presence of tubular vacuolizations. The morphologic features of the vacuolizations were evaluated as previously described [26]. The extent of vacuolization was scored according to the fraction of tubules with vacuoles at 10× magnification (0, absent; 1, <25%; 2, 25–50%; 3, >50%).

Statistical methods

Continuous variables are presented as medians ± interquartile and counts as percentages. Characteristics between patients with and without vacuolization are compared using Mann–Whitney–Wilcoxon's rank sum test, and Pearson chi-square test or the Fisher's exact test, as appropriate, for continuous and categorical variables, respectively. Factors associated with vacuolization occurrence were assessed by a multiple logistic regression. Variables with P values <0.20 in univariate analysis were introduced into multivariate logistic regression analysis through a backward selection. Interactions were searched by introducing an interaction factor in the multivariate model. Linearity of continuous variables was verified. Discriminations were assessed by Hosmer–Lemeshow test.

To deal with missing data, multiple imputation (MI) by chained equation was performed as recommended [27]. Briefly, data were imputed using an imputation model repeated 10 times. An analysis model was fitted in each of the 10 imputed datasets separately, and these 10 datasets were therefore pooled and give an overall set of estimates and corresponding standard errors.

Characteristics of patients with or without tubular macrovacuoles at 3 months biopsies are resumed at Table 3.

The linear correlation of phosphate Tm and PTH levels was analyzed when available at 3 months and 1 year in each group, and slope differences were tested by ANCOVA.

All statistical tests will be two-sided using a type I error of 0.05 unless otherwise mentioned. Analyses will be performed using GRAPHPAD PRISM 5® and R® software (R Foundation for Statistical Computing Vienna, Austria).

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

Results

Sucrose-free IVIg formulations are clinically well tolerated in early kidney recipients but can induce subclinical tubular damage

We first compared the clinical course of the 75 IVIg-treated patients with the matched control group. The demographic characteristics, the immunosuppression, and graft outcomes are shown in Table 1. There was no significant difference in patient or graft survival during the first 3 months between the two groups. Two patients in the group treated with sucrose-free IVIg and three patients in the control group experienced graft loss or death before 3 months of transplantation and were not included in the study. Cold ischemia time, delayed graft function rates, and recipient age were similar between the two groups, but donor age was higher in the control group (57.1 ± 12.4 vs. 51.3 ± 17 years, $P = 0.02$). Calcineurin inhibitor blood concentrations were similar between the IVIg-treated and control patients (Table 1).

The IVIg-treated patients received 3.2 ± 1.0 courses of IVIg in the first post-transplantation trimester, with a mean IVIg cumulative dose of 369 ± 142 g (6 ± 2 g/kg). A total of 239 courses were administered to 75 kidney recipients, and the IVIg stabilizers used were glycine ($n = 147$, 61.5%), glucose ($n = 63$, 26.4%), maltose ($n = 15$, 6.3%), L-proline ($n = 11$, 4.6%), and mannitol ($n = 3$, 1.3%).

Table 1. Baseline characteristics, immunosuppression, and graft outcomes in IVIg-treated kidney recipients and controls.

	Controls (n = 75)	Missing data	IVIg-treated group (n = 75)	Missing data	P value
Transplant characteristics					
Donor age (years, mean ± SD)	57.1 ± 12.4	0	51.3 ± 17	0	0.02
Deceased donor (n, %)	75 (100)	0	75 (100)	0	1
Cold ischemia time (h, mean ± SD)	21.4 ± 6.4	0	22.4 ± 7.6	0	0.37
Delayed graft function [n (%)]	23 (30.7)	0	24 (32)	1	1
Recipient characteristics and graft outcome					
Recipient age (years, mean ± SD)	52.6 ± 11.2	0	50.1 ± 13.3	0	0.21
Male (n, %)	54 (72)	0	39 (52)	0	0.02
Cyclosporine C_2 at 3 month (ng/ml, mean ± SD)	684 ± 318	3	940 ± 416	8	0.08
Tacrolimus C_0 at 3 month (ng/ml, mean ± SD)	9.2 ± 3.5	3	9.4 ± 2.8	8	0.65
Serum creatinine at 3 month (µmol/l, mean ± SD)	143 ± 50	0	134 ± 48	0	0.24
eGFR at 3 month (ml/min/1.73 m^2, mean ± SD)	51.2 ± 16.5	0	49.3 ± 17.1	0	0.50
Proteinuria at 3 month (g/day, mean ± SD)	0.2 ± 0.3	0	0.3 ± 0.3	0	0.37
Donor-specific anti-HLA antibodies on day 0 (n, %)	0 (0)	4	35 (46.7)	1	<0.001
Acute rejection during the first 3 months (n, %)	4 (5.3)	0	15 (20)	0	0.007

No AKI episode, defined by RIFLE criteria, related to IVIg administration was observed. The estimated GFR (51.2 ± 16.5 vs. 49.3 ± 17.1 ml/min/1.73 m^2, P = 0.55), serum creatinine (143 ± 50 vs. 134 ± 48 µmol/l, P = 0.21), and proteinuria levels were similar in the IVIg-treated and control patients at 3 months post-transplantation. As anticipated by their high immunological risk, the IVIg-treated group had a higher incidence of acute rejection (20.0% vs. 5.3%, P = 0.007) and of donor-specific anti-HLA antibodies on day 0 (46.7% vs. 0%, P < 0.001).

As previously described by our team [26], histological analysis of the kidney transplant screening biopsies revealed two different patterns of tubular vacuolization: macro- and microvacuoles (Fig. 1a) mainly localized in the proximal tubular cells. The macrovacuoles were focally distributed in <25% of the tubular sections and usually localized in fibrotic areas. The macrovacuolized tubular cells had higher cell volumes, were clarified, and had lost the brush-border epithelial phenotype. The nuclei of these cells had a picnotic aspect upon microscopic observation (Fig. 1b).

The microvacuoles were frequently but similarly observed in pre-implantation biopsies in both the IVIg-treated (53.3%) and control (73.3%) patients (P = 0.083) (Fig. 2a). Tubular macrovacuoles were never found in pre-implantation biopsies in either group (Fig. 2b). At 3 months post-transplantation, a higher rate of tubular vacuolization was observed in the IVIg-treated group (18.6% vs. 48.0%, P < 0.001) (Fig. 2c). Specifically, macrovacuoles were observed in 28% of the IVIg-treated patients and in only 2.8% of the control patients (P < 0.001) (Fig. 2b). Microvacuolizations were also

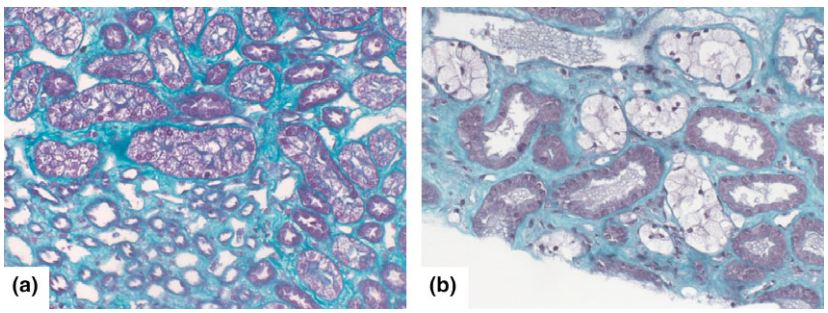

Figure 1 Vacuole patterns in kidney transplant biopsies at 3 months post-transplantation (Masson's trichrome staining). (a) Some tubular epithelial cells have small isometric vacuoles termed microvacuoles that were found in pre-implantation kidney biopsies, at 3 months and 1 year. (b) In sucrose-free IVIg-treated patients, some tubules exhibit voluminous vacuoles called macrovacuoles, characterized by the cytoplasmic swelling of epithelial cells. Magnifications: 40× in a and b.

observed more frequently in the IVIg-treated patients (37.3% vs. 18.6%, $P = 0.01$), and their frequency decreased 3 months post-transplantation compared with pre-implantation biopsies in both groups (Fig. 2a). Tubular micro- and macrovacuolization scores related to the percentage of tubular sections with vacuoles in each biopsy were also higher in the IVIg-treated patients compared with the controls (Table 2). However, IF/TA scores were similar in the IVIg-treated and control patients at 3 months post-transplantation (0.9 ± 1.0 vs. 1.2 ± 1.1, $P = 0.06$) (Fig. 2d).

Risk factors for and consequences of macrovacuoles in IVIg-treated patients

We then compared IVIg-treated patients with ($n = 21$) or without ($n = 54$) macrovacuoles at 3 months post-transplantation to determine the risk factors for IVIg-associated tubular damage and its consequences on IF/TA progression and renal function.

As shown in Table 3, macrovacuoles were more frequent in donors with higher IF/TA scores on day 0 (0.6 ± 0.9 vs. 0.3 ± 0.8, $P = 0.03$), but no association was found with diabetes mellitus history, cold ischemia time, delayed graft function, acute rejection rate or recipient, and donor age. While the cumulative IVIg doses were similar in both groups, macrovacuoles were highly significantly associated with the type of stabilizer, with an increased frequency in carbohydrate-stabilized IVIg compared with amino-acid-stabilized IVIg (71.4 vs. 18.5%, $P < 0.001$). Higher tacrolimus trough concentrations at 3 months (11.2 ± 2.7 vs. 8.8 ± 2.6 ng/ml, $P = 0.003$) were also associated with macrovacuoles. In the multivariate analysis, the use of a carbohydrate-stabilized IVIg was independently associated with the presence of tubular macrovacuoles [HR = 19.48 (3.86–144), $P = 0.008$], and marginally associated with donor age [HR = 1.04 (0.99–1.09), $P = 0.10$] and IF/TA scores at day 0 [HR = 1.89 (0.85–4.3), $P = 0.13$]. Interestingly, we did not observe an independent association between macrovacuoles and tacrolimus trough concentrations at 3 months [HR = 1.01 (0.76–1.33), $P = 0.59$].

IF/TA scores in the 3-month (1.7 ± 1.0 vs. 1.0 ± 1.0, $P = 0.005$) and 1-year screening biopsies (2.1 ± 1.1 vs. 1.3 ± 1.1, $P = 0.02$) and the frequency of tubular vacuoles were higher in the IVIg-treated patients with macrovacuoles at 3 months (100.0% vs. 27.8%, $P < 0.001$) (Fig. 3a and b). When present at 3 months, the macrovacuoles persisted in 47.4% of cases at 1 year (Fig. 3c).

With regard to renal function, macrovacuoles were not associated with a reduced eGFR (44.9 ± 15.8 vs. 51.1 ± 17.4 ml/min/1.73 m^2, $P = 0.13$, and 48.8 ± 20.3 vs. 53.4 ± 18.8 ml/min/1.73 m^2, $P = 0.32$, at 3 months and 1 year, respectively) or elevated serum creatinine (149 ± 56 vs. 128 ± 44 μmol/l, $P = 0.12$; and 176 ± 137 vs. 141 ± 75 μmol/l, $P = 0.27$, at 3 months and 1 year, respectively). To further examine the functional consequences of macrovacuoles, we studied the

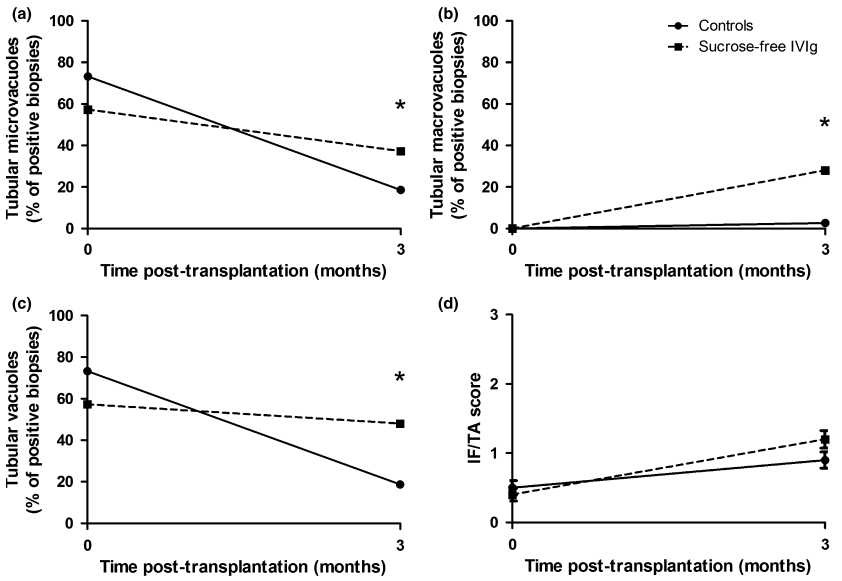

Figure 2 Percentage of day 0 and 3-month screening biopsies showing microvacuoles (a), macrovacuoles (b), or total vacuoles (c) in sucrose-free IVIg-treated kidney recipients and controls unadjusted for other factors. IF/TA score (mean ± SEM) at day 0 and 3 months post-transplantation in IVIg-treated kidney recipients and controls (d). *$P < 0.05$.

Table 2. Histological characteristics of the protocol biopsies at day 0 and at 3 months of IVIg-treated kidney recipients and controls.

	Controls (n = 75)	Missing data	IVIg-treated group (n = 75)	Missing data	P value
Day 0 biopsy					
Total vacuoles (n, %)	33 (44)	30	43 (57.3)	0	0.08
Macrovacuoles (n, %)	0	30	0	0	1
Microvacuoles (n, %)	33 (44)	30	43 (57.3)	0	0.08
Microvacuoles score (mean ± SD)	1.5 ± 1.2	30	1 ± 1.1	0	0.02
IF/TA score at day 0 (mean ± SD)	0.5 ± 0.7	30	0.4 ± 0.8	0	0.36
3 months screening biopsy					
Total vacuoles (n, %)	14 (18.7)	0	36 (48)	0	<0.001
Microvacuoles (n, %)	14 (18.6)	0	28 (37.3)	0	0.01
Macrovacuoles (n, %)	2 (2.7)	0	21 (28)	0	<0.001
Total vacuoles score (mean ± SD)	0.2 ± 0.5	0	0.6 ± 0.7	0	<0.001
Microvacuoles score (mean ± SD)	0.2 ± 0.5	0	0.4 ± 0.6	0	0.02
Macrovacuoles score (mean ± SD)	0.03 ± 0.16	0	0.4 ± 0.6	0	<0.001
IF/TA score (mean ± SD)	0.9 ± 1	0	1.2 ± 1.1	0	0.06

phosphate maximal reabsorptive capacity (PhTm) of patients with (n = 13) and without (n = 35) macrovacuoles as a marker of proximal tubular function. Because PhTm is dependent on the PTH value, we compared the relationship between the PhTm and PTH levels in both groups. Figure 3d illustrates an altered relationship between the PhTm and PTH levels in patients with macrovacuoles, with a lower PhTm at the same level of PTH, suggesting a proximal tubular dysfunction at 1 year post-transplantation.

Decreased tubular injury with amino-acid-stabilized IVIg

Because we found an association between carbohydrate stabilizers and the presence of macrovacuoles in 3-month screening biopsies (Table 3), we compared the clinical and histological outcomes from patients who received carbohydrate-stabilized IVIg (n = 25) versus amino-acid-stabilized IVIg (n = 50). The patient characteristics of these two groups are shown in Table 4. The donor age, sex ratio, histological characteristics including IF/TA and tubular microvacuolizations of the kidney graft at day 0, delayed graft function rate and IVIg cumulative dose, and number of courses were similar between groups. Carbohydrate-stabilized and amino-acid-stabilized IVIg were sequentially used in our unit, and several characteristics of these two groups of patients reflected the evolution of our program over time, including a shorter cold ischemia time (20.7 ± 6.6 h vs. 25.8 ± 8.4 h, P = 0.01), a lower tacrolimus exposure at 3 months (8.5 ± 2.5 ng/ml vs. 11.2 ± 2.6 ng/ml, P < 0.001), and older recipients (52.4 ± 14.0 years vs. 45.5 ± 10.8 years, P = 0.02) in the amino-acid-stabilized IVIg-treated patients. The difference in tacrolimus exposure disappeared at 1 year post-transplantation (6.9 ± 2.6 vs. 7.9 ± 1.9 ng/ml, P = 0.07). Besides, in the amino-acid-stabilized IVIg-treated group, we observed a higher frequency of patients with donor-specific anti-HLA antibodies at day 0 (64% vs. 38%, P = 0.05); however, acute rejection rates at 3 and 12 months were similar in both groups.

Compared with carbohydrate-stabilized IVIg, amino-acid-stabilized IVIg formulations were associated with a decreased frequency of tubular vacuolizations at 3 months (30% vs. 84%, $P \leq 0.001$) and 1 year post-transplantation (36.4% vs. 64.0%, P = 0.06) (Fig. 4a), a difference that was mainly due to a decreased frequency of macrovacuoles in this group at 3 months (12% vs. 60%, P < 0.001) and 1 year (6% vs. 40%, P < 0.003) (Fig. 4b and c). In accordance with this result, patients who received amino-acid-stabilized IVIg experienced better proximal tubular function, as determined by the PhTm/PTH relationship at one year post-transplantation, than patients who received carbohydrate-stabilized IVIg (Fig. 4d). However, this result did not translate into short-term increased IF/TA scores (1.2 ± 1.0 vs. 1.2 ± 1.1, P = 0.88, and 1.5 ± 1.3 vs. 1.5 ± 1.1, P = 0.89, at 3 months and 1 year, respectively) or decreased kidney allograft function (50.0 ± 15.8 vs. 49.1 ± 17.9 ml/min/1.73 m^2, P = 0.87, and 50.7 ± 18.9 vs. 52.9 ± 19.5 ml/min/1.73 m^2, P = 0.63, at 3 months and 1 year, respectively).

Table 3. Baseline characteristics and immunosuppression of IVIg-treated kidney recipients with or without macrovacuoles in their 3-month screening biopsies.

	Macrovacuoles at 3 months				Univariate analysis *P* value	Multivariate analysis *P* value
	Yes (*n* = 21)	Missing data	No (*n* = 54)	Missing data		
Transplant characteristics						
Donor age (years; mean ± SD)	54.4 ± 12.3	0	50.1 ± 18.5	0	0.32	0.10
Cold ischemia time (min; mean ± SD)	25.2 ± 8.6	0	21.3 ± 7	0	0.08	
Delayed graft function [*n* (%)]	9 (42.9)	1	15 (27.8)	0	0.27	
IF/TA score in pre-implantation biopsy (mean ± SD)	0.6 ± 0.9	0	0.3 ± 0.8	0	0.03	0.13
Tubular vacuolization in pre-implantation biopsy [*n* (%)]	13 (61.9)	0	30 (55.5)	0	0.79	
Recipient characteristics and immunosuppressive regimen						
Age (years; mean ± SD)	48.4 ± 13.2	0	50.7 ± 13.5	0	0.67	
Male [*n* (%)]	13 (61.9)	0	26 (48.1)	0	0.31	
Diabetes mellitus (*n*, %)	4 (19)	0	7 (13)	1	0.49	
Body surface area (m^2; mean ± SD)	1.68 ± 0.2	0	1.71 ± 0.2	0	0.54	
Weight (kg; mean ± SD)	61.5 ± 11.4	0	64.5 ± 13.5	0		
Height (cm; mean ± SD)	166 ± 12	0	166 ± 10	0		
IVIg cumulative dose (g, mean ± SD)	358 ± 162	0	373 ± 134	0		
IVIg per body weight (g/kg, mean ± SD)	5.8 ± 2.4	0	5.8 ± 2	0	0.85	
Carbohydrate-stabilized IVIg [*n* (%)]	15 (71.4)	0	10 (18.5)	0	<0.001	0.008
Tacrolimus at 3 month (*n*, %)	15 (71.4)	4	45 (83.3)	4	0.34	
Cyclosporine at 3 month (*n*, %)	2 (9.5)	4	5 (9.3)	4		
Tacrolimus C_0 at 3 month (ng/ml; mean ± SD)	11.2 ± 2.7	4	8.8 ± 2.6	4	0.003	0.59
Cyclosporine C_2 at 3 month (ng/ml; mean ± SD)	1276 ± 204	4	806 ± 412	4	0.19	
Tacrolimus C_0 at 1 year (ng/ml; mean ± SD)	7.7 ± 2.5	1	7.1 ± 2.3	4	0.21	
Cyclosporine C_2 at 1 year (ng/ml; mean ± SD)	1880	1	767 ± 307	4		
Donor-specific anti-HLA antibodies on day 0 (*n*, %)	13 (61.9)	0	22 (40.7)	1	0.12	
Acute rejection during the first 3 months (*n*, %)	6	0	9	0	0.34	
Acute rejection rate from D0 to Y1 (*n*, %)	5	0	10	0	0.75	

Discussion

Our study analyzes the safety profile of high-dose, sucrose-free IVIg treatment in kidney transplant recipients in the first 3 months post-transplantation. We confirm the progressive improvement of IVIg with regard to their renal safety over time. Our study demonstrates that high-dose, sucrose-free IVIg are clinically well tolerated in the early post-transplantation period, with no graft dysfunction. The tubular damage characterized by tubular macrovacuoles appears to be reduced with the use of amino-acid-stabilized IVIg. Our study also suggests that recipients of a fibrotic kidney may be more susceptible to tubular toxicity induced by IVIg.

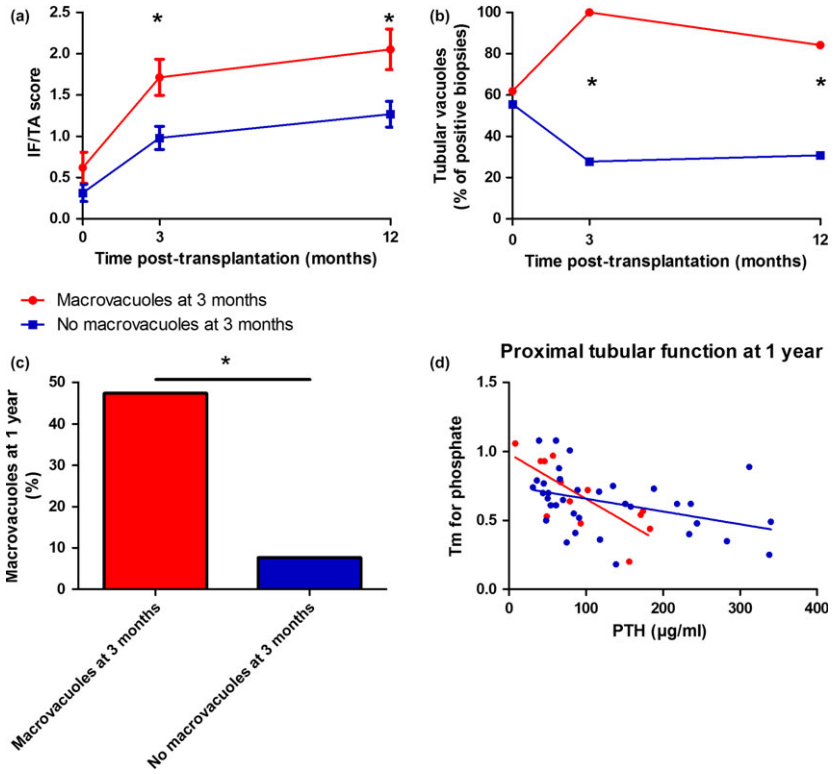

Figure 3 (a) IF/TA scores (mean ± SEM) and the presence of tubular vacuoles (b) in day 0, 3 month, and 1 year post-transplantation screening biopsies in sucrose-free IVIg-treated kidney recipients with or without macrovacuoles in their 3-month biopsies unadjusted for other factors. Frequency of macrovacuoles in 1-year biopsies in sucrose-free IVIg-treated kidney recipients with or without macrovacuoles in their 3-month biopsies (c). *$P < 0.05$. Proximal tubular function was estimated by the correlation between the Tm for phosphate and PTH levels at 1 year post-transplantation (d) in sucrose-free IVIg-treated kidney recipients with or without macrovacuoles in their 3-month biopsies. Slopes are significantly different ($P = 0.03$, ANCOVA analysis).

The use of IVIg formulations without sucrose as a stabilizer has been widespread among patients with altered renal function since the mid-2000s. The increasing use of these products, especially in patients with impaired renal function, including kidney recipients, is associated with lower reported cases of AKI, which emphasizes the good clinical tolerance of these products. Our study confirmed this observation, as high-dose IVIg therapy in the early post-transplantation period was not associated with AKI episodes or graft dysfunction, and the clinical safety of sucrose-free IVIg has improved compared with previous formulations [28].

Although we did not observe any AKI episodes after the IVIg courses, we investigated the histological impact of IVIg by analyzing protocol biopsies at 3 months and 1 year after transplantation. Interestingly, high-dose sucrose-free IVIg infusion was not associated with any histological change in the majority of cases at 3 months. However, in 28% of cases, we found subclinical nephrotoxicity features, as revealed by osmotic nephrosis and tubular lesions defined by cytoplasmic vacuolization of the tubular cells. Our results thus confirm that sucrose is not the only IVIg stabilizer associated with tubular damage. Indeed, some studies have reported that maltose-stabilized IVIg [29] or excessive glucose loading [25] can also induce similar lesions. Interestingly, we found fewer tubular vacuoles in screening biopsies and better proximal tubular function in patients treated with amino-acid-stabilized IVIg, which suggests an improved renal safety of these new formulations. Although amino-acid-stabilized IVIg has been associated with other rare side effects, such as hemolytic anemia [30], we did not observe any adverse events in our study.

Tubular macrovacuoles are associated with IVIg treatment and with fibrosis progression in kidney transplantation. A previous study about IVIg treatment in early kidney recipients showed that donor age was a risk factor for tubular macrovacuolization [26]. In our multivariate analysis, we observed a marginal association between the older donors, the higher IF/TA scores at

Table 4. Baseline characteristics and immunosuppression of kidney recipients treated with amino-acid-stabilized or carbohydrate-stabilized IVIg.

	Amino-acid-stabilized IVIg (n = 50)	Missing data	Carbohydrate-stabilized IVIg (n = 25)	Missing data	P value
Transplant characteristics					
Donor age (year; mean ± SD)	53.6 ± 17.6	0	46.6 ± 15	0	0.10
Cold ischemia time (min; mean ± SD)	20.7 ± 6.6	0	25.8 ± 8.4	0	0.01
Delayed graft function [n (%)]	13 (26)	0	11 (44)	1	0.13
IF/TA score in pre-implantation biopsy (mean ± SD)	0.4 ± 0.9	0	0.3 ± 0.6	0	0.85
Tubular vacuolization in pre-implantation biopsy [n (%)]	27 (54)	0	16 (64)	0	0.47
Recipient characteristics and immunosuppressive regimen					
Age (years; mean ± SD)	52.4 ± 14	0	45.5 ± 10.8	0	0.02
Male [n (%)]	24 (48)	0	15 (60)	0	0.46
Diabetes mellitus (n, %)	7 (14)	0	4 (16)	1	0.74
Body surface area (m^2; mean ± SD)	1.7 ± 0.2	0	1.7 ± 0.2	0	0.09
Weight (kg; mean ± SD)	65.8 ± 13.5	0	59.4 ± 10.7	0	0.03
Height (cm; mean ± SD)	166 ± 10	0	166 ± 11	0	
IVIg cumulative dose (g, mean ± SD)	375 ± 129	0	357 ± 166	0	
IVIg per body weight (g/kg, mean ± SD)	5.8 ± 1.9	0	6 ± 2.5	0	0.36
IVIg courses per patient (mean ± SD)	3.2 ± 0.9	0	3.1 ± 1.2	0	
Tacrolimus at 3 month (n, %)	39 (78)	8	21 (84)	0	
Cyclosporine at 3 month (n, %)	3 (6)	8	4 (16)	0	
Tacrolimus C_0 at 3 month (ng/ml; mean ± SD)	8.5 ± 2.5	8	11.2 ± 2.6	0	<0.001
Cyclosporine C_2 at 3 month (ng/ml; mean ± SD)	669 ± 404	8	1144 ± 328	0	0.23
Tacrolimus C_0 at 1 yr (ng/ml; mean ± SD)	6.9 ± 2.6	5	7.9 ± 1.9	0	0.07
Cyclosporine C_2 at 1 year (ng/ml; mean ± SD)	607 ± 296	5	1298 ± 509	0	0.10
Donor-specific anti-HLA antibodies on day 0 (n, %)	19 (38)	1	16 (64)	0	0.05
Acute rejection from D0 to M3 (n, %)	8 (16)	0	7 (28)	0	0.24
Acute rejection from D0 to Y1 (n, %)	13 (26)	0	9 (36)	0	0.43

day 0 and macrovacuoles in their 3-month biopsies. Both studies suggest that IVIg clearance may be decreased in older and fibrotic kidneys, which may promote IVIg-associated tubular injury. Further studies are needed to confirm this association with the new sucrose-free IVIg formulations used nowadays.

We also confirmed that tubular macrovacuoles appear to be a major histological toxicity feature of IVIg therapy, which is associated with increased IF/TA scores at 3 months and 1 year post-transplantation. Interestingly, macrovacuoles observed at 3 months persisted in 1-year biopsies in approximately 50% of cases. Thus, IVIg toxicity may not be reversible and may explain differences in proximal tubular function and long-term kidney fibrosis after IVIg discontinuation. Cases of drug-associated osmotic nephrosis and tubular macrovacuoles have already been described and can also persist 1 year after transplantation [31]. This finding suggests that macrovacuoles might represent an irreversible tubular lesion that is accompanied by the loss of epithelial phenotype and is associated with ongoing fibrogenesis.

Our study has some methodological limitations due in part to its retrospective design. First, the control group without IVIg treatment had a lower immunological risk and consequently a lower rejection rate than the group receiving IVIg. Even if there is no evident relationship between tubular vacuolizations

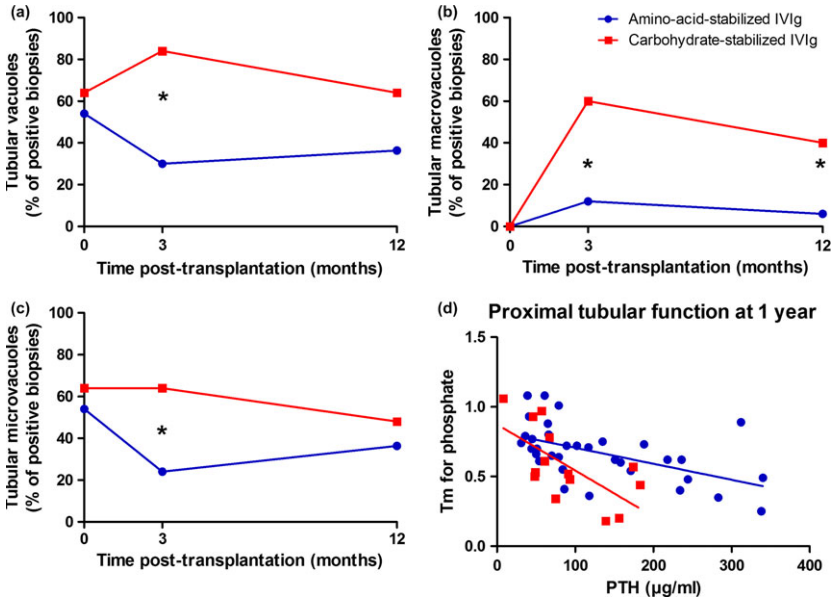

Figure 4 Percentage of day 0, 3-month and 1-year screening biopsies of patients treated with carbohydrate or amino-acid-stabilized IVIg showing tubular vacuoles (a), macro- (b), or microvacuoles (c) unadjusted for other factors. *$P < 0.05$. The proximal tubular function was estimated by the correlation between the Tm for phosphate and PTH levels at 1 year post-transplantation (d) in patients treated with carbohydrate or amino-acid-stabilized IVIg. Slopes are significantly different ($P = 0.04$, ANCOVA analysis).

and alloimmune injury, we cannot exclude that difference in immunological risk could have affected IF/TA progression. Second, as carbohydrate-based and amino-acid-based IVIg formulations were sequentially used, we cannot exclude that other factors could explain the observed differences. Finally, other factors associated with tubular toxicity, as colloid and mannitol therapy or IV contrast administration, were not available for the study. However, a previous study showed no association between iodinated contrast agents before transplantation and macrovacuoles [26]. To conclude, our results show that high-dose, sucrose-free IVIg treatment in the early post-transplantation period is clinically safe and is not associated with histological lesions in the majority of kidney recipients. Among sucrose-free IVIg, amino-acid stabilizers appear to induce less tubular damage than carbohydrate stabilizers. Our data confirm the progressive improvement in the renal safety of the new IVIg formulations.

Authorship

YL: participated in research design, in the writing of the paper, in the performance of the research, and in data analysis. DA and CL: participated in research design, in the writing of the paper, in the data analysis, and in the performance of the research. MR and L-HN: participated in the performance of the research and in research design. KEK and MJ: participated in the performance of the research and in data analysis. RC, MM, GB, AS, HK and M-OT: participated in the performance of the research.

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Conflict of interest

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