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Open Randomized Multicenter Study to Evaluate Safety and Efficacy of Low Molecular Weight Sulfated Dextran in Islet Transplantation

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Background. When transplanted human pancreatic islets are exposed to blood during intraportal infusion, an innate immune response is triggered. This instant blood-mediated inflammatory reaction (IBMIR) activates the coagulation and complement cascades and leads to the destruction of 25% of all transplanted islets within minutes, contributing to the need, in most patients, for islets from more than 1 donor. Low molecular dextran sulfate (LMW-DS) has been shown in experimental settings to inhibit IBMIR. **Methods.** The Clinical Islet Transplantation consortium 01 study was a phase II, multicenter, open label, active control, randomized study. Twenty-four subjects were randomized to peritransplant intraportal and systemic treatment with either LMW-DS or heparin, targeting an activated partial thromboplastin time of 150 ± 10 seconds and 50 ± 5 seconds, respectively. C-peptide response was measured with a mixed meal tolerance test at 75 and 365 days after transplant. **Results.** Low molecular dextran sulfate was safe and well tolerated with similar observed adverse events (mostly attributed to immunosuppression) as in the heparin arm. There was no difference in the primary endpoint (stimulated C-peptide 75 ± 5 days after the first transplant) between the 2 arms (1.33 ± 1.10 versus 1.56 ± 1.36 ng/mL, $P = 0.66$). Insulin requirement, metabolic parameters, Clarke and HYPO score, quality of life, and safety were similar between the 2 treatments groups. **Conclusions.** Even with low dosing, LMW-DS showed similar efficacy in preventing IBMIR to promote islet engraftment when compared to "state-of-the art" treatment with heparin. Furthermore, no substantial differences in the efficacy and safety endpoints were detected, providing important information for future studies with more optimal dosing of LMW-DS for the prevention of IBMIR in islet transplantation.

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Beta cell replacement with islet transplantation is a therapeutic alternative for selected patients with unstable type 1 diabetes (T1D), and reports have shown improved results over the years.[1] Even so, most patients require islets from more than 1 donor, and both early and late loss of islet function contribute to suboptimal results.[2] In contrast to solid organ transplants, the small-volume endocrine graft is infused into the portal vein and subsequently widely dispersed throughout the liver. We have demonstrated by dynamic positron emission tomography that 18F-fluorodeoxyglucose-labeled pancreatic islets can be readily visualized after an intraportal infusion. Islets were heterogeneously distributed in the liver, and 25% of the transplanted islets were lost within the first few minutes after transplantation.[3] When the islet surface is exposed to blood an innate immune response is triggered which is an important cause of partial graft loss.[4] This instant blood-mediated inflammatory reaction (IBMIR) is characterized by a rapid binding and activation of platelets to the islet surface and activation of the coagulation and complement systems. We detected a peak in thrombin-antithrombin (TAT) complex just 15 minutes after islet infusion, reflecting a clotting process. In vitro studies have demonstrated that this reaction is triggered by tissue factor (TF) and generation of FXIIa-AT and FVIIa-AT complexes soon after infusion, peaking after 60 minutes, underscoring the involvement of both the contact system and the TF-pathways of coagulation later in the thrombo-inflammatory reaction. The increase in TAT complex levels was concomitant with an increase in C-peptide, indicating release from damaged islets.[5,6]

The ability to monitor function, injury or cell death after islet transplantation is limited, and the search for suitable biomarkers is ongoing.[7] It has long been known that blood contains small fragments of cell-free DNA that originate from dead cells. One method identifies cell-type-specific DNA methylation to identify damaged beta cells in patients recently diagnosed with T1D or after islet transplantation.[8] Higher concentrations of soluble donor DNA immediately after islet infusion are shown to correlate with a higher likelihood of graft failure.[2]

Low molecular weight dextran sulfate (LMW-DS) inhibits activation of the complement cascade and contact activation of the coagulation system,[9,10] and acts directly on cell-cell interactions, for example by inhibition of E-selectin-mediated adhesion of neutrophils to endothelial cells.[11] Low molecular weight dextran sulfate has therefore been identified as a more powerful inhibitor of IBMIR,[4,12] and notably even at the same activated partial thromboplastin time (APTT), LMW-DS confers a significantly lower risk of bleeding compared with heparin.[13] Besides its capacity to counteract IBMIR, LMW-DS also promotes intrahepatic islet engraftment via a hepatocyte growth factor-mediated mechanism.[13,14] This study, conducted within the Clinical Islet Transplantation (CIT) consortium, aimed to evaluate the safety and efficacy of LMW-DS to enhance engraftment and prevent IBMIR in the setting of clinical islet transplantation.

MATERIALS AND METHODS

Methods

The CIT-01 study was a phase II, multicenter, open label, active control, randomized study. Once a compatible islet preparation became available, eligible subjects were randomized with a web-based system 1:1 to either of 2 peritransplant treatment arms: the experimental arm (LMW-DS) or the control arm ("state-of-the art" heparin arm). The primary efficacy endpoint was the level of stimulated C-peptide at 90 minutes after a mixed-meal tolerance test (MMTT) performed 75 ± 5 days after the first islet infusion. Safety and secondary endpoints are specified in the study synopsis provided as online **Supplemental Digital Content (SDC,** http://links.lww.com/TP/B631). Regular safety summaries were prepared for the National Institute of Diabetes and Digestive and Kidney data safety monitoring board and were used to monitor the overall safety profile of the study.

Subjects and Randomization

Three centers participated in the trial: 2 sites in Sweden (University Hospital in Uppsala and Karolinska University Hospital in Stockholm) and 1 in Norway (Oslo University Hospital). Enrolled subjects aged, 18 to 65 years (actual age range, 27.5 to 63.8 years), had confirmed T1D of more than 5 years duration, absent stimulated C-peptide, were involved in intensive diabetes management, had a history of at least 1 episode of severe hypoglycemia in the last year, experienced reduced awareness of hypoglycemia and/or marked glycemic lability as evidenced from the Clarke/HYPO scores (see below). Subjects were enrolled between July 2008 and January 2012. The last subject's last study visit was in August 2014, 1 year after the subjects last transplant. Thirty-nine subjects were enrolled, and 29 subjects were eligible for randomization. Five subjects became ineligible while on the waitlist resulting in a total of 24 subjects that received at least 1 islet transplant. Ten subjects were randomized to the experimental arm and 14 to the control arm. Three subjects, all randomized to the experimental arm, left the study before day 365 leaving 21 subjects who remained in the study through the final transplant assessment at day 365 after final transplant.

Once a compatible islet donor preparation was available and the reevaluation of eligibility criteria was confirmed, subjects were randomized using a web-based electronic data entry system. The second planned interim analysis included 24 subjects who had reached day 75 after the first islet transplant. The analysis showed no safety concerns but indicated that no efficacy difference would be

patients and edited the study. B.N. designed the study, performed experiments, analyzed the data and wrote the article. O.K. conceived, designed and directed the study, performed islet isolation, analyzed the data and wrote the article.

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seen with full accrual of the planned 36 subjects. The data safety monitoring board therefore recommended ending the study early.

LMW-DS and Heparin

Low molecular weight dextran sulfate was provided by TikoMed AB (S-263 65 Viken, Sweden) in compliance with EU-GMP, and was delivered with an average molecular mass of 5000 Da in 50-mL glass vials at a concentration of 20 mg/mL and stored at +2°C to 8°C. The study drug was mixed with saline to obtain the desired concentration. Subjects randomized to the experimental arm received LMW-DS administered as follows: a bolus of 1.5 mg/kg body weight given intravenously (IV), a second dose of 3.0 mg/kg body weight administered intraportally in the same bag with the islets, and a continuous intravenous infusion that started immediately after islet infusion and was maintained for 5 hours, adjusted to target a concentration of 30 µg/mL LMW-DS in peripheral blood (corresponding to APTT of 150 ± 10 seconds).

A standard heparin regimen was given to subjects randomized to the control treatment arm. No bolus of heparin was given intravenously before islet infusion, but 70 U/kg body weight was administered intraportally in the same bag with the islets, and immediately after completion of the islet infusion a continuous intraportal infusion of heparin targeting an APTT of 50 ± 5 seconds was administered for 5 hours.

Islet Isolation

The organs used for production of the islet transplants were allocated through Scandiatransplant to the Nordic Network for Clinical Islet transplantation. Pancreases were obtained from brain-dead donors with the same acceptance criteria as for kidney transplantation, with donors aged 25 to 69 years. The organs were transported to the Rudbeck laboratory in Uppsala Sweden for processing. Islet isolation and culture procedures have previously been described in detail and all procedures are available at http://nordicislets.medscinet.com.[15,16] Once a pancreas became available and successfully isolated, the islets were selected to go to the next suitable person on the Nordic Network waiting list, whether that person was enrolled in this study or not.

Islet Transplantation

Isolated islets (> 5000 IEQ/kg body weight) were transported in transfusion bags and transplanted by gravity infusion through a percutaneous transhepatic cannula in the portal vein over a time period of about 30 minutes.[17] As for all CIT studies, a second islet transplant was not allowed before 75 days after the first transplant and a third islet transplant was not allowed before 28 days after the second islet transplant.[18] The subsequent transplants were required to take place within 8 months of the first islet transplant and contain >4000 IEQ/kg body weight. Seven of 10 in the LMW-DS arm and 7 of 14 in the heparin arm received a second islet infusion. One patient in the LMW-DS arm and 1 patient in the heparin arm received a third islet infusion.

Induction therapy for the initial transplant consisted of rabbit antithymocyte globulin (ATG) except for the first patient enrolled in the study who received the monoclonal antibody IL-2 receptor blocker basiliximab, 20 mg intravenously on the day of transplant and repeated on day 4 posttransplant. Antithymocyte globulin was given intravenously over 5 days starting 2 days before the transplant, with a total dose of 6 mg/kg. Premedication included methylprednisolone 1 mg/kg IV 1 hour before the first ATG, as well as paracetamol 1000 mg and clemastine 1 mg orally half hour before every ATG infusion. At the second and third transplants basiliximab was used for induction at the dose referred to above. Maintenance immunosuppression was continued throughout the study with mycophenolate mofetil (MMF) (500–1000 mg orally bid) or sirolimus (trough level 10–15 ng/mL the first 90 days and 7–10 ng/mL thereafter), together with a calcineurin inhibitor. The preferred regimen was MMF and tacrolimus and the protocol allowed adaption of the immunosuppressive treatment in response to adverse events (AE) or tolerability. Because of the problems with islet graft rejections and similar experiences at other CIT centers tacrolimus trough levels in combination with MMF were increased after July 2010. The early tacrolimus regimen until July 2010 was 3 to 8 ng/mol for the first transplant days 1 to 365, and for second or third transplant 6 to 10 ng/mol the first 90 days and thereafter 3 to 8 ng/mol. After July 2010, tacrolimus trough levels were 10 to 12 ng/mL the first 90 days, 8 to 10 ng/mL on month 3 through month 6 and 6 to 8 ng/mL thereafter. Throughout the study cyclosporine trough levels were 200 to 250 ng/mL the first 90 days, 150 to 200 ng/mL month 3 through month 6 and 100 to 150 ng/mL thereafter.

All subjects received 2 mg IV of clemastine (Tavegyl) within 1 hour before transplant. Concomitant medication included enoxaparin sodium 30 mg subcutanously (SC) BID through day 7 with the first dose given 2 hours after the removal of the intraportal catheter, acetylsalicylic acid 75 mg orally starting 24 hours posttransplant and continued throughout the study and etanercept at a dose of 50 mg IV 1 hour before transplant and 25 mg SC on day 3, day 7, and day 10. Infection prophylaxis included cefuroxime 1.5 mg IV immediately before each transplant; either trimethoprim-sulfamethoxazole 80/400 mg orally once daily for a period of 6 months or pentamidine inhaled at a dose of 300 mg every 4 weeks for 6 months for *Pneumocystis jiroveci* prophylaxis; valganciclovir 900 mg orally for 3 months, adjusted dose for renal function, for cytomegalovirus prophylaxis; and nystatin 1 mL orally 4 times daily during the first posttransplant month, for fungal prophylaxis. All subjects received intravenous insulin infusion at least 2 hours before initiation of islet transplantation and treatment continued for the first 2 days to maintain plasma glucose levels in the target range 4 to 8 mmol/L. Additional exogenous insulin was given subcutaneously for 4 to 8 weeks to achieve partial beta cell rest and facilitate islet engraftment and aiming at the same glucose levels.

Mixed Meal Tolerance Test

Subjects were instructed not to eat or inject short-acting insulin after 8 PM the night before the test. Evening or bedtime administration of long-acting insulin was permitted as well as consumption of water. Insulin glargine was, however, avoided within 24 hours of the test. If the

blood glucose was less than 70 mg/dL or greater than 136 mg/dL upon arrival at the testing center, the test was rescheduled. Immediately after obtaining a basal blood sample, the subjects received 6 mL/kg (to a maximum of 360 mL) of Boost® High Protein Drink, to consume in 5 minutes. Basal (fasting) and stimulated glucose and C-peptide levels were determined before (ie, 0 minute) and at 15, 30, 60, 90, and 120 minutes after this carbohydrate intake. Each blood sample was drawn per University of Washington standard protocol and shipped frozen to the University of Washington for measurement by the CIT core laboratory.

Metabolic Parameters

Methods for the metabolic parameters, which included mean amplitude of glycemic excursions (MAGE), lability index (LI), β-score, Clarke survey, HYPO score and insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) have been previously described.[18]

Measurement of TAT Complexes, C3a and C-peptide

Thrombin-antithrombin complexes were assessed using a commercially available kit containing monoclonal antibodies specific for thrombin and antithrombin (HRP-conjugated; Enzyme Research Laboratories, South Bend, IN). The assay was performed according to the manufacturer's recommendation. C3a was analyzed using a previously-described ELISA[19] and C-peptide was assessed using an EIA kit from Mercodia (Uppsala, Sweden).

Quality of Life

Quality of life (QOL) was assessed with a Swedish or Norwegian translated version of the Short form 36 health survey (SF36). Validity was confirmed with back-translation forms. This questionnaire has been widely used for assessment of health-related QOL.[20]

Statistical Analysis

The primary analysis was based on an independent samples 2-sided *t* test. A difference was declared statistically significant if $P \leq 0.05$. The estimated effect size and a 95 % confidence interval are reported. With compelling evidence that the normal distribution was absent, a logarithmic or square root transformation was done before using the *t* test. For secondary endpoints dichotomous variables were analyzed using logistic regression, continuous variables were analyzed by a *t* test and regression models for longitudinal data were used to examine the differences between groups. With the original selected sample size of 18 subjects in each of the 2 arms the study would have 90% power to detect a difference in the primary endpoint of 1.0 nmol/L between the mean for the LMW-DS treatment group and the mean for the control group.

Ethics

The final study protocol, including any substantial amendments and the final version of the informed consent documents, were reviewed and approved by an Independent Ethics Committee (IEC), The Norwegian Medicines Agency and the Swedish Medical Products Agency. The study was conducted in compliance with the protocol, regulatory requirements, good clinical practice and the ethical principles of the latest revision of the Declaration of Helsinki as adopted by the World Medical Association. All subjects received written and verbal information regarding the study.

RESULTS

Recipient characteristics at baseline are shown in Table 1. The differences in age, body mass index, duration of diabetes, insulin requirement, glycosylated hemoglobin (HbA1c) and Clarke score between the 2 groups were not significant.

Efficacy

There was no significant difference between the 2 arms in the primary endpoint results, ie, the level of stimulated C-peptide at 90 minutes derived from a MMTT (see Figure 1 and Table 2). The proportion of subjects with a functioning islet graft, ie, C-peptide >0.3 ng/mL, at day 75 after the first transplant were 80.0% in the experimental arm and 85.7% in the control arm. At day 365 after the first and final transplantation, the proportion of subjects with a functioning islet graft was 60.0% and 71.4% in the experimental arm and 50% and 71.4% in the control arm, respectively.

The TAT complexes and C-peptide values during insulin infusion with plasma glucose aimed in the range 4 to 8 mmol/L increased immediately after islet transplantation in both treatment arms. Extreme values greater than 250 mg/mL were regarded as sampling errors (in total 8 samples). After 24 hours, however, levels of both factors were similar and even lower than the pretransplant levels (see Figure 2).

TABLE 1.

Baseline demographic and metabolic data described as mean values and standard deviation (SD) were applicable

	LMW-DS	Heparin	*P*
n	10	14	
Sex: male/female	4/6	6/8	1.000
Age mean and range	47.4 (27.5–59.7)	51.8 (36.7–63.8)	0.27
Weight mean and range	74.2 (56–93)	66.5 (51–86)	0.15
BMI: mean (range), kg/m^2	24.9 (20.1–28.7)	22.8 (18.6–29.1)	0.11
Duration of diabetes: mean (range), y	33.0 (18–49)	33.6 (16–46)	0.86
Insulin requirement U (SD)	42.2 (20.9)	36.3 (12.3)	0.88
Clarke score (SD)	5.3 (1.5)	6.1 (1.1)	0.13

BMI, body mass index; LMW-DS, low molecular dextran sulfate.

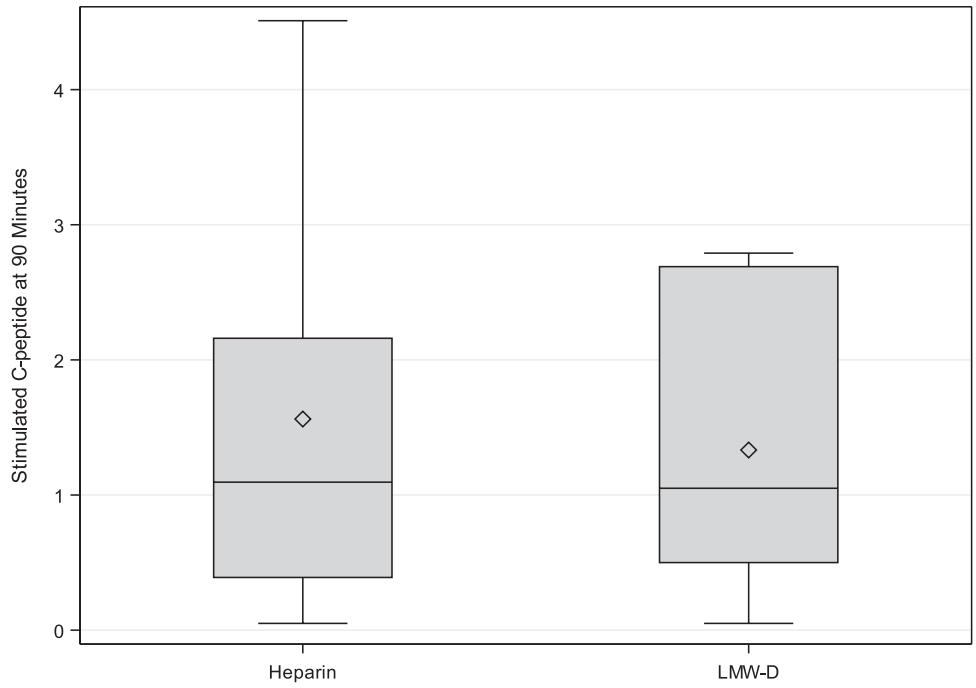

FIGURE 1. The distribution of stimulated C-peptide (ng/mL) at 90-minutes derived from a mixed-meal tolerance test (MMTT) at 75±5 days after the first transplantation. Heparin in the left box and low molecular dextran sulfate (LMW-DS) in the right box with range, median, mean and quartiles with no significant difference (*P*=0.66).

The daily insulin requirements decreased markedly in both the LMW-DS and heparin arm at day 75 after the first transplantation (by 30.7% ± 14.9% and 27.8% ± 30.2%, respectively) and at day 365 after the first (47.7% ± 39.0% and 53.3% ± 31.3%, respectively) and final transplantation (52.5% ± 39.6% and 45.3% ± 41.1%, respectively). Insulin requirement in U/kg body weight are shown in Table 3. No significant difference in the HbA1c levels was detected between the 2 arms at any time point. The proportion of subjects meeting the day 365 endpoint (HbA1c < 7.0% and absence of severe hypoglycemic events from day 28 to day 365) was 20% in the experimental arm and 57% in the control arm. No significant difference in hypoglycemia (Clark score and HYPO score) was detected between the treatment arms at baseline or at day 365 after the first transplant. At day 365 after the final transplant, however, the Clarke score was significantly higher (worse) for subjects included in the experimental arm compared to the control arm, whereas no significant difference was reported for the HYPO score. There were no significant differences in β-score or glycemic lability (MAGE or LI) detected between the 2 arms at any time point.

Compared with baseline, the SF36 mental component score was significantly increased (better) in subjects included in the experimental arm 1 year after both the first (see Table 3) and final transplantation compared with baseline, whereas no significant difference was detected for subjects included in the control arm. No significant treatment related difference was detected for the SF36 physical component score.

Safety

Intraportal islet transplantation did not alter the portal pressure in either treatment arm during or after the first, second or third transplant. The most frequent AE from randomization to day 75 after the initial transplant were urinary tract infections and transient hepatic enzyme increase in subjects receiving LMW-DS, whereas headache and transient hepatic enzyme increase were most frequent in subjects receiving heparin. From randomization to day 365 after the final transplant, a total of 113 AEs were reported in subjects in the experimental arm (LMW-DS) and 137 AEs in the heparin arm. In the later period, stomatitis and hemoglobin decrease were the most frequently occurring AEs in subjects receiving LMW-DS, whereas neutropenia and headache were the most frequently reported AEs in subjects receiving heparin. The majority of the AE were attributed to immunosuppressive medications. Two patients in the experimental arm with pretransplantation normal retinal photography developed retinopathy at day 365 after the initial transplant.

TABLE 2.

Level of stimulated C-peptide (ng/mL) at 90 minutes derived from a MMTT at 75 ± 5 days in the intention to treat population

	n	$N_{missing}$	Mean	SD	Standard error	Min	Max	95% CI mean	*P*
Heparin	14	2	1.56	1.36	0.36	0.05[a]	4.51	(0.78–2.35)	0.66
LMW-D	10	1	1.33	1.10	0.35	0.05[a]	2.79	(0.55–2.12)	

[a] The lowest observed value, regardless of treatment arm was 0.05 (ng/mL). This value was imputed for all 3 subjects that had missing primary endpoint values.
CI, confidence interval; MMTT, mixed-meal tolerance test.

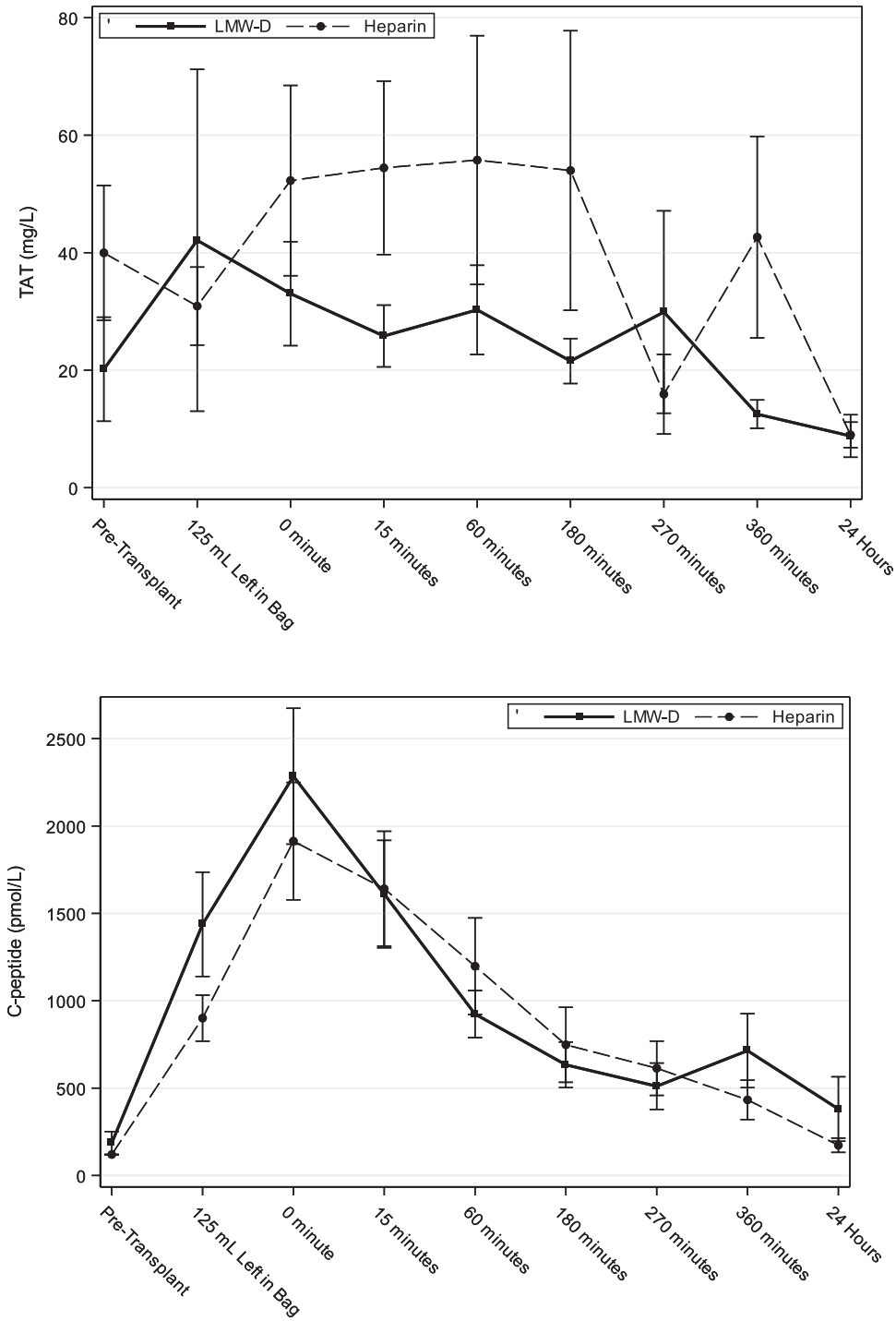

FIGURE 2. The thrombin-antithrombin (TAT) complexes (upper panel) and C-peptide (lower panel) increased immediately after islet transplantation in both treatment arms after the first and second transplantations. After 24 hours, however, levels of both factors were similar or even lower than pretransplant levels. Low molecular dextran sulfate (LMW-DS) with solid line and squares and heparin with dashed line and circles.

There were 31 serious AEs (SAEs) during the study (12 prerandomization and 19 after randomization). Only 1 was attributed to the study intervention (unintended puncture of a branch of the hepatic artery). The majority of the SAEs were attributed to the concomitant immunosuppression and granulocytopenia was the most commonly reported SAE. There were no reported deaths. All SAEs resolved within 35 weeks and were without sequelae.

Three subjects, all in the heparin arm, whose pretransplant panel-reactive antibodies (PRA) was zero, developed post-transplant sensitivity from baseline to final visit with peak PRA values of 2, 10 and 34 % PRA, respectively. In 5 of 8 subjects with a positive pretransplant PRA (1–73%), at least 1 occasion with lower peak PRA during the study period was detected. Safety and secondary endpoints are specified more in detail in the study synopsis provided as online Supplemental Digital Content (SDC, http://links.lww.com/TP/B631).

TABLE 3.

Insulin requirement, HbA1c, β-score, MAGE, and LI and QOL SF-36 at baseline, 75 ± 5 days, 365 ± 5 days after initial transplant, and 365 ± 5 days after final transplant expressed as mean values and SD

	LMW-DS (n = 10)	Heparin (n = 14)	*P*
Insulin baseline, U/kg	0.55 (0.20)	0.54 (0.13)	0.39
Insulin day 75, U/kg	0.36 (0.13)	0.34 (0.25)	0.60
Insulin day 365 initial, U/kg	0.27 (0.24)	0.29 (0.22)	0.53
Insulin day 365 final, U/kg	0.26 (0.23)	0.32 (0.24)	0.74
HbA1c baseline, %	7.6 (0.8)	7.5 (1.8)	0.84
HbA1c day 75%	5.5 (0.4)	5.6 (0.8)	0.68
HbA1c day 365 initial, %	5.9 (0.7)	6.1 (1.2)	0.62
HbA1c day 365 final, %	6.1 (0.7)	6.3 (1.3)	0.70
β -score from FSIGT baseline	0.5 (0.7)	0.7 (0.9)	0.54
β -score from FSIGT day 75	4.7 (1.6)	4.4 (1.7)	0.62
β -score from FSIGT day 365 initial	4.3 (2.1)	4.7 (2.1)	0.64
β -score from FSIGT day 365 final	3.9 (2.2)	4.0 (2.0)	0.88
MAGE baseline, mmol/L	9.7 (4.6)	8.2 (3.2)	0.38
MAGE day 75, mmol/L	4.6 (1.6)	4.8 (3.0)	0.85
MAGE day 365 initial, mmol/L	4.3 (2.8)	5.0 (3.1)	0.58
MAGE day 365 final, mmol/L	4.2 (2.3)	4.6 (3.0)	0.80
LI baseline	351.0 (116.4)	383.2 (207.4)	0.69
LI day 75	176.9 (122.5)	240.3 (186.0)	0.36
LI day 365 initial	96.4 (114.4)	155.0 (108.6)	0.44
LI day 365 final	77.1 (85.6)	141.4 (97.5)	0.27
Clarke score baseline	5.3 (1.5)	6.1 (1.1)	0.13
Clarke score day 75	4.4 (2.0)	3.5 (2.1)	0.33
Clarke score day 365 initial	2.9 (2.8)	2.7 (2.1)	0.87
Clarke score day 365 final	4.3 (2.3)	1.8 (2.0)	0.03
Mental component SF36 difference from baseline at day 365	12.3 (13.7) $P = 0.04$	4.6 (8.7) $P = 0.11$	
Physical component SF36 difference from baseline at day 365	6.4 (9.4) $P = 0.08$	2.4 (10.8) $P = 0.54$	

LMW-DS, low molecular dextran sulfate; SD, standard deviation.

DISCUSSION

Peritransplant treatment with LMW-DS versus heparin showed no difference in the primary endpoint, that is, mean levels of stimulated C-peptide at 90-minutes derived from the MMTT at 75 + 5 days after the first islet infusion. Furthermore, secondary efficacy and safety endpoints were similar and there were no substantial differences in the efficacy and safety endpoints between groups.

It should be noted that the main objective of all CIT studies is protection from hypoglycemic events and maintenance of near-normal HbA1c levels, and not independence from requirement of exogenous insulin. In this respect, the outcome of both treatment arms is comparable to that of the previously reported Protocol CIT-07.[18]

This is the first study involving LMW-DS in clinical islet transplantation. Although no clinically relevant safety issues were reported in the prior studies in healthy volunteers, the dosing of LMW-DS in CIT-01 was carefully adjusted during a period of 5 hours to avoid risks of bleeding from the portal vein.[12] It should be noted that the risk for bleeding after administration of LMW-DS cannot be directly determined by APTT due to the different mechanism of action when compared with heparin. The primary aim of the present study was to obtain additional safety results enabling dose adjustments of LMW-DS in future studies. Notably, even with suboptimal dosing, LMW-DS showed no inferiority in efficacy when compared with heparin administration.

Several mechanisms are involved in the IBMIR. When the islets, which are normally not exposed to blood, face the recipient's blood-borne innate immune system complement and coagulation activation occur.[21,22] A key factor in coagulation and islet destruction is the expression of TF on the endocrine cells of pancreas. Blocking the active site of TF with antibodies or FVIIa has been shown to abrogate the clotting reaction in vitro.[5] LMW-DS has previously been shown to be a potent inhibitor of both the complement and the coagulation systems without activation of the fibrinolytic system.[12,23]

There have been several previous attempts to reduce the impact of IBMIR. Peritransplant intensive heparin and insulin infusions improved single-donor success.[24] Coating islets with conjugated, preformed heparin complexes showed protection against IBMIR in a porcine model.[25] In a xenotransplantation model with pig islets transplanted to monkeys, a cobra venom-derived complement inhibitor and the anticoagulant dextran sulfate together (but not individually) reduced the early loss of islets.[26]

Based on data from in vitro and in vivo experimental studies, it was anticipated that if IBMIR could be prevented, subjects undergoing clinical islet transplantation would have a clinically relevant response to the transplant with significantly fewer islets than needed in current practice. The dose of LMW-DS tested in this study was determined based on available safety results from a phase I study in healthy volunteers.[13] Based on the outcome and safety

results obtained in this study and in additional recently conducted studies in healthy volunteers, a follow-up study applying a dose of 18 mg LMW-DS given as a bolus dose just before islet infusion is planned. Also, to promote engraftment via mobilization of stem cells and hepatocyte growth factor mediated mechanisms, additional doses of 3 mg LMW-DS on days 3 and 6 will be administered.

In conclusion, even with low dosing, LMW-DS showed similar efficacy in preventing IBMIR to promote islet engraftment when compared to "*state-of-the art*" treatment with heparin. Furthermore, no substantial differences in the efficacy and safety endpoints were found, providing important information for future studies with more optimal dosing of LMW-DS for the prevention of IBMIR in islet transplantation.

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REFERENCES

1. Shapiro AM. Islet transplantation in type 1 diabetes: ongoing challenges, refined procedures, and long-term outcome. *Rev Diabet Stud*. 2012;9:385–406.
2. Gadi VK, Nelson JL, Guthrie KA, et al. Soluble donor DNA and islet injury after transplantation. *Transplantation*. 2011;92:607–611.
3. Eriksson O, Eich T, Sundin A, et al. Positron emission tomography in clinical islet transplantation. *Am J Transplant*. 2009;9:2816–2824.
4. Bennet W, Sundberg B, Groth CG, et al. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? *Diabetes*. 1999;48:1907–1914.
5. Moberg L, Johansson H, Lukinius A, et al. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*. 2002;360:2039–2045.
6. Nilsson B, Ekdahl KN, Korsgren O. Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant*. 2011;16:620–626.
7. Jiang L, Brackeva B, Ling Z, et al. Potential of protein phosphatase inhibitor 1 as biomarker of pancreatic β-cell injury in vitro and in vivo. *Diabetes*. 2013;62:2683–2688.
8. Lehmann-Werman R, Neiman D, Zemmour H, et al. Identification of tissue-specific cell death using methylation patterns of circulating DNA. *Proc Natl Acad Sci U S A*. 2016;113:E1826–E1834.
9. Wuillemin WA, te Velthuis H, Lubbers YT, et al. Potentiation of C1 inhibitor by glycosaminoglycans: dextran sulfate species are effective inhibitors of in vitro complement activation in plasma. *J Immunol*. 1997;159:1953–1960.
10. Fiorante P, Banz Y, Mohacsi PJ, et al. Low molecular weight dextran sulfate prevents complement activation and delays hyperacute rejection in pig-to-human xenotransplantation models. *Xenotransplantation*. 2001;8:24–35.
11. Matsumiya A, Yamaguchi M, Nakano H, et al. Dextran sulfate inhibits E-selectin-mediated neutrophil adhesion to endotoxin-activated vascular endothelial cells. *Life Sci*. 1999;64:PL9–PL17.
12. Johansson H, Goto M, Dufrane D, et al. Low molecular weight dextran sulfate: a strong candidate drug to block IBMIR in clinical islet transplantation. *Am J Transplant*. 2006;6:305–312.
13. Schmidt P, Magnusson C, Lundgren T, et al. Low molecular weight dextran sulfate is well tolerated in humans and increases endogenous expression of islet protective hepatocyte growth factor. *Transplantation*. 2008;86:1523–1530.
14. Nakano M, Yasunami Y, Maki T, et al. Hepatocyte growth factor is essential for amelioration of hyperglycemia in streptozotocin-induced diabetic mice receiving a marginal mass of intrahepatic islet grafts. *Transplantation*. 2000;69:214–221.
15. Friberg AS, Ståhle M, Brandhorst H, et al. Human islet separation utilizing a closed automated purification system. *Cell Transplant*. 2008;17:1305–1313.
16. Goto M, Eich TM, Felldin M, et al. Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. *Transplantation*. 2004;78:1367–1375.
17. Baidal DA, Froud T, Ferreira JV, et al. The bag method for islet cell infusion. *Cell Transplant*. 2003;12:809–813.
18. Hering BJ, Clarke WR, Bridges ND, et al. Phase 3 trial of transplantation of human islets in type 1 diabetes complicated by severe Hypoglycemia. *Diabetes Care*. 2016;39:1230–1240.
19. Nilsson Ekdahl K, Nilsson B, Pekna M, et al. Generation of iC3 at the interface between blood and gas. *Scand J Immunol*. 1992;35:85–91.
20. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care*. 1992;30:473–483.
21. Johansson U, Olsson A, Gabrielsson S, et al. Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation. *Biochem Biophys Res Commun*. 2003;308:474–479.
22. Tjernberg J, Ekdahl KN, Lambris JD, et al. Acute antibody-mediated complement activation mediates lysis of pancreatic islets cells and may cause tissue loss in clinical islet transplantation. *Transplantation*. 2008;85:1193–1199.
23. Goto M, Johansson H, Maeda A, et al. Low-molecular weight dextran sulfate abrogates the instant blood-mediated inflammatory reaction induced by adult porcine islets both in vitro and in vivo. *Transplant Proc*. 2004;36:1186–1187.
24. Koh A, Senior P, Salam A, et al. Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success. *Transplantation*. 2010;89:465–471.
25. Cabric S, Sanchez J, Lundgren T, et al. Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. *Diabetes*. 2007;56:2008–2015.
26. Rood PP, Bottino R, Balamurugan AN, et al. Reduction of early graft loss after intraportal porcine islet transplantation in monkeys. *Transplantation*. 2007;83:202–210.