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Original Article

Changes in lipid and carbohydrate metabolism under mTOR- and calcineurin-based immunosuppressive regimen in adult patients after liver transplantation

Anca Zimmermann [a,*], Christina Zobeley [b], M.M. Weber [a], H. Lang [c], Peter R. Galle [b], Tim Zimmermann [b]

[a] Dept. of Endocrinology and Metabolic Diseases, 1st Medical Clinic, University Medical Center, Langenbeckstr. 1, 55131 Mainz, Germany
[b] Dept. of Gastroenterology and Hepatology, Transplant Hepatology, 1st Medical Clinic, University Medical Center, Langenbeckstr. 1, 55131 Mainz, Germany
[c] Dept. for General, Visceral and Transplantation Surgery, University Medical Center, Langenbeckstr. 1, 55131 Mainz, Germany

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ABSTRACT

Background: Cardiovascular disease is a leading cause of long-term mortality after liver transplantation (LT). Life long immunosuppression harbors the risk of metabolic alterations. We aimed to analyze the impact of calcineurin (CNI)-only containing regimen (group A) compared to mTOR-containing regimen (group B) on lipid and carbohydrate metabolism.

Patients/methods: 92 adult patients after LT, University of Mainz (group A—78 patients, group B—14 patients; 65 M/27 F; mean age 59 +/− 10.2 years; mean time from LT 5.8 +/− 5 years). Clinical data, comorbidities, and medication were assessed. Fasting lipid profile including small dense LDLs (sdLDL) and oral glucose tolerance tests were performed.

Results: Group B had significantly higher levels of total cholesterol (TC), LDL-cholesterol (LDL-C), triglycerides (TG) and sdLDL, with persistence of higher TC, TG, sdLDLs (mg/dl) after exclusion of patients under lipid lowering medication. Concentrations above the upper limits of normal were found: for LDL-C in 9% of group A/35.7% of group B ($p = 0.016$); for TG: in 32.1% of group A/92.9% in group B ($p = 0.0001$). A positive correlation between time since LT (years) and sdLDL (mg/dl) was found in group B ($p = 0.018$). In patients without previously known diabetes, NODAT and impaired glucose tolerance developed in 27.9% of group A/44.4% of group B (n.s.).

Conclusion: Patients under mTOR-containing regimen are at higher risk to develop dyslipidemia with increased atherogenic sdLDLs compared to patients under CNI-only-containing regimen and display more frequently a dysglycemic status, with uncertain relevance for long-term cardiovascular risk. A careful monitoring after LT is needed to identify early metabolic risk and manage this appropriately.

1. Introduction

Cardiovascular events are the second most frequent cause of death after liver transplantation [1]. Therefore, an individual monitoring of metabolic and cardiovascular risk factors under life long immunosuppression becomes mandatory.

Various immunosuppressive drugs are associated with metabolic alterations with potential atherogenic risk. Dyslipidemia, which is more pronounced under mammalian target of rapamycin (mTOR)-inhibitors than under calcineurin-inhibitors (CNI), is one potential risk factor. Under mTOR inhibitors, total cholesterol (TC) levels are increased in kidney and liver transplant recipients [2]. However, other data suggest that mTOR inhibitors may offer a cardioprotective effect, with delayed progression of atherosclerosis after heart transplantation under everolimus (Ev) versus cyclosporine (CsA). Preliminary data also indicate that mTOR inhibitors may improve arterial stiffness, may reduce ventricular remodeling and decrease left ventricular mass through an anti-fibrotic effect [2–4].

Among CNIs, (CsA) is associated with higher levels of serum TC, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and triglycerides (TG) compared to tacrolimus (Tac) in patients after heart transplantation [5]. On the other hand, an increased risk for the development of diabetes mellitus (DM) under Tac by impairment of ß-cell function is discussed [6]. Risk factors of post-transplant DM are also male sex, pretransplant DM, alcohol abuse and mycophenolate mofetil (MMF) [7]. CsA and Tac increase isoproterenol-stimulated lipolysis, inhibit lipid storage by 20–35% and enhance isoproterenol-stimulated hormone-sensitive lipase Ser552 phosphorylation in adipose tissue, which may contribute to the development of dyslipidemia and insulin resistance associated with immunosuppressive therapy [8].

Abbreviations: LT, liver transplantation; CNI, calcineurin-inhibitors; mTOR, mammalian target of rapamycin; TC, total cholesterol; Ev, everolimus; CsA, cyclosporine; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; TG, triglycerides; Tac, tacrolimus; DM, diabetes mellitus; sd-LDL, small-dense LDL; Sir, sirolimus; HOMA-IR, homeostasis model assessment-insulin resistance; IRI, insulin resistance index; HOMA-B, homeostasis model assessment.

* Corresponding author at: Tel.: +49 6131 170; fax: +49 6131 17 5615.
E-mail address: zimmeran@uni-mainz.de (A. Zimmermann).

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Based on these data, the question arises if different immunosuppressive substances or combinations lead to a metabolic profile with potential cardiovascular risk in contrast to others, which could be neutral or protective. Since weight gain is relatively common after LT, the question becomes more relevant related to the need of an individualized immunosuppressive therapy and follow-up of patients who already display cardiovascular disease and/or DM.

Beyond variable changes in the routinely analyzed lipid parameters of uncertain prognostic significance, no study addressed the issue of possible discrete alterations of lipid metabolism which might contribute to an accelerated atherogenesis in adults, in comparison between CNI- and mTOR-containing regimen. A previous study reported features of dysmetabolic syndrome after kidney and liver transplantation in a pediatric population, showing a negative correlation between body mass index (BMI) and the diameter of low density lipoproteins [9]. Recent data point out to increased small dense LDL concentrations in adult liver transplant recipients compared to healthy controls [10]. Several studies have shown that particularly small-dense LDLs (sd-LDL) are atherogenic and are linked to the progression of coronary artery disease [11–16].

The present study aimed to analyze changes in lipid metabolism and insulin resistance in patients after LT, in relation to the immunosuppressive regimen and the underlying disease, in order to identify potential surrogate markers of atherogenic risk and hence select patients who may need a more careful individual metabolic surveillance.

2. Patients and methods

2.1. Patients

We included 92 adult patients who underwent LT at the University Medical Center Mainz, at the Department for General, Visceral and Transplantation Surgery between 1989 and 2011: 65 males, 27 females, mean age 59 +/− 10.2 years (range 23–78 years). Mean time from LT was 5.8 +/− 5 years. Patients were identified from an administrative transplant database and all data were retrieved from patients' charts and reports. Patients with less than 6 months of follow-up were excluded from the study. Patients with missing clinical data or retransplanted patients were also excluded. Patients who experienced one or more acute rejection episodes (biopsy proven with a rejection activity index >4) and who needed therefore steroids (methylprednisolone 500 mg/d i.v. for 3 days) have also been excluded.

In addition to usual patient demographics, we reviewed the etiology of liver failure and pretransplant comorbidities. Patients treated with oral hypoglycemic agents and/or insulin before transplantation were diagnosed as pretransplant diabetes mellitus. Patients obtained immunosuppression according to individual risk factors and comorbidities [17]. A common immunosuppressive regimen was the combination of mycophenolate mofetil (MMF) with a calcineurin inhibitor (CsA or Tac). For cyclosporine, target trough levels were 70–90 ng/ml during the first year and 40–60 ng/ml thereafter. Target trough levels for Tac were 5–7 ng/ml during the first year and 3–5 ng/ml after one year. All patients received steroids until 3 months post LT. Methylprednisolone was reduced from 1.5 mg/kg on day one and two post LT to 1.0 mg/kg on day three and four, 0.5 mg/kg on day five to 14 and 0.2 mg/kg on day 15 up to three months. No patient received chronic glucocorticoid treatment during the observation period.

NODAT was diagnosed (in case of missing pretransplant diabetes) according to international consensus guidelines: casual plasma glucose ≥200 mg/dl plus symptoms of diabetes, or fasting plasma glucose ≥126 mg/dl or 2 h plasma glucose ≥200 mg/dl during an oral glucose tolerance test [18]. Therapy of diabetes was initiated according to international guidelines [19].

3. Methods

3.1. Lipid metabolism

A lipid profile analysis was performed in each patient after a minimum 12-hour overnight fast. TC, LDL-C, HDL-C, TG and LDL subfractions were measured.

TC, LDL-C, HDL-C and TG were obtained by homogeneous assays carried out on an Abbott ARCHITECT c8000 Clinical Chemistry Analyzer (Abbott, Wiesbaden, Germany).

The LDL subfractions were determined from −75 °C frozen plasma aliquots using a density gradient ultracentrifugation method, as previously described [11,20]. Briefly, dry solid KBr was added to the plasma to achieve a density of 1.21 g/ml. A discontinuous density gradient was constructed by 2 ml of a NaCl/KBr solution (d = 1.26 g/ml), 3 ml plasma (d = 1.21 g/ml), 2 ml of a NaCl/KBr solution (d = 1.063 g/ml), 2.5 ml of another NaCl/KBr solution (d = 1.019 g/ml) and 2 ml of a NaCl solution (d = 1.006 g/ml). All solutions contained NaN3 (0.1%) and EDTA (0.04%). Densities were measured by a precision density meter (Anton Paar DMA 38, Graz, Austria). Ultracentrifugation was performed in a Beckmann SW 40 Ti rotor (Palo Alto, Calif., USA) at 40.000 rpm for 48 h at 15 °C. Fifteen fractions were collected successively by aspiration of 0.5 ml with an Eppendorf pipette beginning at the top of each gradient. After centrifugation, total LDL was separated in seven LDL subfractions and cholesterol concentrations of large-buoyant (lb-LDL), intermediate (id-LDL) and sd-LDL were determined. Intra- and interassay variability was less than 5%. Density limits were determined by a standard curve derived from control gradients constructed with a NaCl/KBr solution (d = 1.21 g/ml) instead of plasma.

3.2. Carbohydrate metabolism

Every patient underwent an oral glucose tolerance test (OGTT), according to the standard protocol. Additionally, basal values for C-peptide were determined. All patients and controls were given a 25% solution with 1.75 g glucose/kg body weight with a maximum of 75 g. Venous blood samples were obtained at fasting, 30, 60, 90 and 120 min for plasma glucose and serum insulin concentrations. Glucose (mg/dl) was measured by a colorimetric method with a commercial kit from Diagnostikum (Hungary) on a Cobas Mira plus analyzer (Roche, Switzerland). The intra- and interassay variation were less than 2.3 and 5.6%, respectively. The sensitivity was 0.04 mg/dl. Insulin was detected in serum by an electrochemiluminescence method (Insulin Elecsys; Roche Diagnostics, Boehringer Mannheim, Germany) on an Elecsys 1010 automatic analyzer (Boehringer Mannheim). Sensitivity of the assay was 0.2 μU/ml, within-run variation was less than 1.33%, between-run variation was less than 1.85%.

C-peptide was measured in serum using a similar electrochemiluminescence method (C-Peptide Elecsys; Roche Diagnostics, Boehringer Mannheim), on the Elecsys 1010 automatic analyzer. Sensitivity was 0.01 ng/ml, intra- and interassay coefficients of variation were 1.6 and 2.6%, respectively.

Impaired glucose tolerance and diabetes were defined according to current guidelines [19].

Data from the OGTT were used to assess the following indexes to evaluate insulin resistance [21,22]:

- HOMA-IR (homeostasis model assessment-insulin resistance): fasting insulin (μU/ml) X fasting glucose (mmol/l) divided by 22.5.
- IRI (insulin resistance index): log10 [fasting insulin (μU/ml) x fasting glucose (mg/dl)].

Plasmatic ß-cell function was assessed by calculating:

- HOMA-B (homeostasis model assessment — insulin secretion ß-cell): [20 X fasting insulin (μU/ml)/fasting glucose (mmol/l) — 3.5]. This reflects insulin secretion in basal conditions.