Effect of aspirin on renal disease progression in patients with type 2 diabetes: a multicentre, double-blind, placebo-controlled, randomised trial. The LEDA (renaL disEase progression by aspirin in Diabetic pAtients) trial. Rationale and study design.

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Conflicts of interest: none

Abstract

Background

Type 2 diabetes mellitus (T2DM) is one of the most common causes of chronic kidney disease (CKD) and kidney failure. It has been estimated that the annual decline of estimated glomerular filtration rate (eGFR) among patients with T2DM is approximately 2.0-2.5 ml/min/year. Cyclooxygenase (COX)-dependent eicosanoids, such as 11-dehydro-thromboxane (Tx) B_2, are increased in T2DM patients, and are potentially involved in the regulation of renal blood flow. Animal models showed that COX inhibitors, such as aspirin, are associated with improvements in renal plasma flow and eGFR values.

Hypothesis

The primary endpoint of the LEDA trial is to evaluate the 1-year decline of eGFR in T2DM patients treated or not with low-dose aspirin (100 mg/day). Secondary endpoints will be the rapid decline in renal function, defined as a reduction of eGFR ≥5 ml/min, and change of renal function class after 1 year-follow-up. Furthermore, changes of urinary excretion 11-dehydro-TxB_2, before and after 1 year of treatment will be related to renal function modifications.

Study design

A phase 3 no-profit, multicentre, double-blind, randomized intervention trial of aspirin 100 mg/day vs. placebo (ClinicalTrials.gov Identifier: NCT02895113). All patients will be monitored at 6 and 12 months after randomization to assess drug adherence and eGFR changes.

Summary

The LEDA trial is the first double-blind, placebo-controlled, randomised clinical trial aimed at examining whether aspirin treatment may beneficially affect kidney function in patients with T2DM by reducing the annual eGFR decline. The trial will also examine whether the potential renoprotective effects of aspirin might be partly due to its inhibition of TxB_2 production.

Introduction

Several epidemiological data showed that the prevalence of type 2 diabetes mellitus (T2DM has reached epidemic proportions worldwide. The progressive increase in food intakes, the greater availability of refined grains and sedentary lifestyles exert an adverse impact on the risk of new-onset T2DM. It is expected that the number of people suffering from T2DM will double in the period 2000-2030[1].The most important increase in the incidence of T2DM is expected in developing countries, where the prevalence of obesity is also rapidly increasing. Unlike developing countries, in the United States and Europe the higher prevalence of T2DM is mainly related to the increased life expectancy of the general adult population and patients with T2DM. In Italy, the Casale Monferrato Study reported an increased rate of T2DM of approximately 45% (2.6% vs. 3.8%) in the period 1988-2000[2]. This was particularly related to aging, as the prevalence of T2DM increased mainly in the age group >65 years; of note, a doubling of the prevalence at the age ≥80 years was recorded. The pathophysiology of T2DM is multifactorial as, beyond genetic background, several acquired risk factors contribute to development of new-onset T2DM including pre-diabetic states (i.e., impaired fasting glycaemia and impaired glucose tolerance), overweight/obesity, dyslipidaemia and arterial hypertension[3].

T2DM is associated a high risk of long-term complications, that increase with the duration of hyperglycaemia. They can be divided into non-vascular and vascular complications, the latter including microvascular (i.e. nephropathy, retinopathy and neuropathy) and macrovascular (i.e. coronary, cerebrovascular and peripheral arterial diseases)[4] complications.

Renal dysfunction and T2DM. Worsening of kidney function is a peculiar feature of patients suffering from T2DM. It has been estimated that the annual decline of estimated glomerular filtration rate (eGFR) in patients with T2DM, is about 2.0-2.5 ml/min per year[5]. The Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicentre Study showed that non-albuminuric renal impairment is the predominant clinical phenotype in T2DM patients, particularly women, with reduced eGFR and that the non-albuminuric form is associated with a significant prevalence of CVD, especially at the level of the coronary vascular bed[6, 7]. A recent prospective, observational study[8] showed that, in a cohort of 1682 T2DM patients with preserved renal function (i.e. eGFR ≥60 ml/min/1.73 m^2) who were followed for 10 years, the decline of eGFR was -0.6±0.1 ml/min/year in patients with normal albuminuria rising to -2.7±0.4 ml/min/year in those with macroalbuminuria at baseline. The authors also found that together with abnormal albuminuria,

older age, hypertension, insulin treatment and lower baseline eGFR were the strongest predictors of annual eGFR decline in this population[8].

Chronic kidney disease has also a negative prognostic impact in patients with T2DM. The FIELD study, which included 9,795 T2DM patients showed that a reduced eGFR and the presence of albuminuria were independent predictors of renal and cardiovascular mortality[9].

Aspirin, eicosanoids and kidney (dys)function. Aspirin is an effective antithrombotic agent that inhibits the production of thromboxane (Tx) A_2 and other prostaglandins by blocking the enzyme cyclooxygenase 1 (COX-1). The antiplatelet action of ASA is via specific inhibition of COX-1 through an irreversible acetylation of serine-529 of COX-1. This enzyme exerts both cyclooxygenase [converting arachidonate into prostaglandin G2 (PGG2)] and peroxidase activities [converting PGG2 into PGH2, the biochemical precursor of many other prostaglandins and Tx]. Inhibition of COX-1 by aspirin results in complete inhibition of the pro-aggregating TxA_2. As platelets are a-nucleated, such aspirin-induced TxA_2 inhibition can be fully restored only through the synthesis of new platelets, i.e. after approximately 7 days from aspirin administration. Previous randomized clinical trials have shown that aspirin is effective as an antithrombotic agent at a dosage ranging from 50 to 1500 mg/day. **In patients treated with low-dose aspirin, serum TxB_2 levels are the most reliable marker of COX-1 inhibition.** Patrono et al.[10] have investigated the relationship between the aspirin dose and TxB_2 levels, showing that a single dose of 100 mg reduces by 98% the 1-hour concentration of serum TxB_2. Notably, serum TxB_2 returned to normal levels after a period compatible with the platelet half-life. **Urinary levels of TxB_2 metabolites such as 11-dehydro-TxB_2 and 2.3-dinor-TxB_2, reflect the production of TxB_2 in whole body[11].** As 11-dehydro-TxB_2 is excreted in higher amounts and has a longer half-life, its analysis in urine is largely used for clinical purpose[12-14]. Thromboxane binding to its receptor expressed by platelets, smooth muscle cells, endothelium and vessels may exert vasoconstriction and platelet aggregation[15].

It is noteworthy that urinary 11-dehydro-TxB_2 levels reflect platelet activation in T2DM patients, and is largely dependent on glycaemic control[16].

In the kidney, COX enzymes exert their physiologic regulatory functions in the macula densa, medulla and interstitium[17]. The products of COX enzymes, and in particular the balance between Tx and prostacyclin production, is crucial for kidney homeostasis[18]. Experimental studies showed that in the macula densa, COX enzymes favour renin production and are involved in the regulation of renal blood flow. In particular, in animal models, the administration of either aspirin or Tx receptor inhibitors was associated with improvements in renal plasma flow and eGFR values,

suggesting a pathogenic role for Tx in the progression of renal damage[19-22]. A previous study in kidneys from diabetic rats, has also demonstrated that an imbalance between TxA_2 and prostacyclin production could favour the onset of diabetic nephropathy[19]; furthermore, a higher urinary Tx/prostacyclin ratio was also found in patients with T2DM as compared to healthy volunteers[23].

Moreover, a recent cross-sectional study performed in 115 patients with stage 1–4 CKD, showed that urinary levels of 11-dehydro-TxB_2 [879 vs. 345 pg/mg creatinine] were significantly higher in stage 3–4 patients as compared to stage 1–2 ($p<0.0001$)[24].

To date, however, the data available in the literature on the long-term effects of low-dose aspirin (or other antiplatelet agents) on kidney function and progression of chronic kidney disease (CKD) in humans are scarce and inconclusive.

In prospective cohort study of 4,494 US male physicians, aspirin intake significantly reduced the risk for decline in kidney function compared with never use in the group of subject without cardiovascular risk factors[25].

In a study including 14 patients with severe congestive heart failure, the administration of picotamide, which is a TxA_2 synthase and TxA_2/prostaglandin H_2 receptor inhibitor, resulted in an improvement in effective renal plasma flow and eGFR[26].

In a retrospective cohort study of 3,585 patients with CKD undergoing cardiac surgery, it has been reported that pre-operative aspirin use was associated with a significant decrease in postoperative acute kidney injury[27].

In a recent observational cohort study, involving 800 patients with non-valvular atrial fibrillation[29], we found that the use of aspirin (100 mg/die) was significantly associated with a reduced risk of CKD progression over 2 years of follow-up. In particular, patients who were not receiving aspirin had a three-fold higher risk of progressing to an eGFR value <45 ml/min/1.73 m^2 at the end of follow-up compared to those treated with aspirin. Furthermore, levels of urinary **11-dehydro-TxB_2** excretion were inversely associated with the aspirin use, and strongly predicted the annual eGFR decline during the 2-years follow-up period.

Of note, in the First United Kingdom Heart and Renal Protection (UK-HARP-I) Study, which randomized 448 CKD patients to receive treatment with simvastatin 20 mg or aspirin 100 mg, allocation to treatment with 100 mg of aspirin daily was not associated with an excess of major bleeds, suggesting that the use of low-dose aspirin may be safe in CKD patients[28].

Aspirin and renal function in T2DM. The efficacy and safety of acetylsalicylic acid (aspirin, ASA) as an antithrombotic agent has been assessed both in apparently healthy people at low risk of

cardiovascular complications (primary prevention), and in patients at high-risk, such as those with a prior myocardial infarction or ischemic stroke (secondary prevention). Patients with T2DM represent an important group of patients in whom treatment with ASA should be carefully considered. The evidence that T2DM patients without previous cardiovascular events have similar cardiovascular risk compared to non-diabetic individuals with prior myocardial infarction, could make reasonable the use of aspirin as primary prevention strategy for cardiovascular diseases in this clinical setting. However, while there is consolidated evidence about the use of ASA for secondary prevention in patients with T2DM, there is no evidence of an effective use for primary cardiovascular prevention[30, 31] ; thus, currently, the use of ASA in this specific group of T2DM patients remains largely at physician discretion[4].

However, ASA may exert effects beyond cardiovascular prevention in T2DM[32]; for instance some authors investigated the relationship between ASA administration and renal function in T2DM providing conflicting evidence.

A randomized trial including 76 patients with diabetic nephropathy showed that short-term use of high-dose ASA (1000 mg) was able to reduce the rate of proteinuria by the 15.9% as compared to control group, with no side effects related to the use of ASA[33].

A Swedish cohort study[34] that investigated the decline of renal function at 5-7 years in 801 patients with CKD, showed that chronic administration of ASA was associated with a slower decline of renal function compared to untreated patients (mean difference of 0.8 ml/min/1.73 m^2). The Authors reported that this beneficial effect of ASA was maintained also in the group of patients with diabetic nephropathy.

Conversely, the JPAD2 cohort study showed no effect of ASA on kidney function in 2536 Japanese patients but the allocation of patients to ASA (81 mg or 100 mg daily) or not was at physician discretion[35].

Primary endpoint. The aim of the study is to evaluate the decline of renal function, as assessed by absolute change in eGFR, calculated as the difference between eGFR at 12 months vs. baseline eGFR, in T2DM patients receiving low-dose aspirin (100 mg/day) or placebo.

Secondary endpoints. It will be considered as secondary endpoints:

- The rapid decline in renal function, defined as a reduction of eGFR ≥5 ml/min at 1 year,
- Change of renal function class (from G1 to G2, from G2 to G3a and so on) at 12 months, **dialysis or transplantation,**
- Urinary **11-dehydro-TxB_2 levels at the end of the follow-up** will be correlated to renal function modifications.

STUDY DESIGN

A phase 3, no-profit, multicentre, double-blind, placebo-controlled, randomised, intervention trial of 1-year treatment with aspirin 100 mg/day vs. placebo in patients with T2DM (ClinicalTrials.gov Identifier: NCT02895113). The inclusion and exclusion criteria are specified in Table 1. Table 2 reports the timeline of events and assessments. In all participants, the following data will be collected:

- anthropometric/clinical data (at each visit): age, gender, height, weight, body mass index, waist circumference, systolic and diastolic blood pressure, heart rate;
- medical history (at baseline visit): diabetes duration, cardiovascular risk factors such as smoking, alcohol intake, dyslipidemia, arterial hypertension[36], heart failure[37], metabolic syndrome[38], chronic obstructive pulmonary disease[39];
- use of **all** concomitant medications (at each visit): name and daily dosage of all medications taken by patients**;**
- blood analysis (at each visit): serum creatinine (measured using a Jaffe´ rate-blanked and compensated assay), urea nitrogen, complete blood count, fasting glucose, insulin (only for patients not treated with insulin), hemoglobin A1c, serum liver enzymes (aminotransferases and gamma-glutamyltransferase), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, uric acid, C-reactive protein (high sensitivity-CRP), and albumin-to-creatinine ratio on a morning spot urine **sample (at each visit).**

Measurements of blood parameters and the urinary albumin-to-creatinine ratio will be made with standard laboratory methods in each single center.

Estimation of kidney function. To estimate kidney function, eGFR will be calculated by the CKD-Epidemiology Collaboration formula (CKD-EPI)[40]. Patients will be classified into four categories based on eGFR according to the KDIGO guidelines: normal eGFR (> 90 ml/min/1.73m^2, Stage G1), mild decrease in eGFR (89-60 ml/min/1.73m^2, Stage G2), mild-moderate decrease in eGFR (59-45 ml/min/1.73m^2, Stage G3a), moderate to severe decrease in eGFR (44-30 ml/min/1.73m^2, G3b Stage) and severe decrease of eGFR (<30 ml/min/1.73m^2, Stage G4).

Data collection. Each investigator will collect the data and fill them in a certified electronic platform. Each center will be provided with personal login procedures for direct entry of individual patient data. Investigators will be also asked to fill all data in a paper form, which must be held until the end of the trial. Each investigator will be provided with a sequential numerical code (01, 02,

etc.), which will be used to identify the participating center at the time of filling in the electronic platform. Patients enrolled will be enumerated according to the order of enrolment. For example, the first patient from the center 01 will be identified with code “01-01”, the second with the code “01-02” and so on. The Promoter center (Sapienza University of Rome) will require further clarification of the data entered (for example, in the case of incorrect insertion), but will not require any information enabling the identification of the patient enrolled.

Collection of biological samples and measurement of urinary 11-dehydro-thromboxane B_2. Urine samples (10 ml each visit for each patient) will be collected in all participants in the morning between 8:00 and 9:00 AM after an overnight fast. The urine samples will be stored at -80°C until use. The levels of urinary 11-dehydro-TxB_2 will be measured centrally (at the Promoter center) using a commercial ELISA kit (Cayman Chemical, Ann Arbor, MI)[41]. The data will be expressed as ng/mg urinary creatinine. Urinary creatinine will be also centrally measured using a commercial enzyme immunoassay kit.

Informative sheet and informed consent. At enrolment, an investigator will explain to each patient the modalities and the aim of the trial. Each patient will then be asked to provide written informed consent before the inclusion in the study. It will be also specified that each participant is allowed to withdraw at any time, and to be informed about results at the end of the study. The trial will be conducted in accordance with the Standards of Good Clinical Practice (ICH-GCP), as expected by current regulations concerning clinical trials and the protection of personal data (Administrative Order. June 30, 2003, No. 196), and according to what established by the Declaration of Helsinki (version of the 59nd WMA General Assembly, Seoul, October 2008). In addition, an informative letter for the general practitioner will be delivered to each patient. The study protocol will be approved by the coordinating center’s Ethic Committee and, thereafter, by the Ethics Committee of each center outside the Policlinico Umberto I “Sapienza” University Hospital.

Treatment delivery. All patients will be randomly assigned to either aspirin 100 mg/day or placebo (Figure 1) to be taken after lunch (in non-fasting conditions) according to the randomization list (see below). Each patient will receive at baseline (T0) an amount of tablets for 6 months (T6) of treatment, when the patient will be seen on the control visit by the Investigator, who will also verify the adherence to the treatment prescribed by the withdrawal of drug bottle/empty placebo. Any error in the assumption of the drug by the patient (for instance, a reported early depletion of tablets or the presence of residual tablets in the container), must be promptly notified by the Investigator at the

Promoter center, which will assess in each case whether such errors compromise the continuation of the study for the patient. At the 6-months visit (T6), the investigator will give the patient the vial containing pills necessary for the rest of the study, until the 12-months visit (T12 visit) .

Randomization list. The randomization list will be generated by software and will be managed by the Promoter center that will assign the codes for all patients at each participating center in order of enrollment. An operator, who will not be involved in patients' recruitment, or in data analysis, will be in charge of code assignment and maintenance of randomization list in a safe place not accessible to other investigators.

Management and monitoring of participating centers. Each participating center will receive all study materials, including the study protocol and synopsis, the case report forms (T0, T6, T12), the information sheet and informed consent for the patient, as well as the approval of the Ethics Committee of the Promoter center, a letter of intent to the local ethics committees, and a letter for the general practitioners. Two investigator meetings are being planned; the first meeting will be held before the start of enrolments to explain the scientific rationale and the modalities of the study and the second to assess the state of enrolment. We will also plan to carry out in each center, after its activation, a site visit to check 1) the ability to conduct the study (approval if it will be obtained a copy of the local Ethics Committee and viewed the informed consent of the patients enrolled); 2) proper data collection; 3) the adequate storage of the biological samples. It will then be filled-in a form of relating to each center the evaluation after the visit. The participating centers will be required to retain all documentation relating to the study for the entire duration of the study, and thereafter for at least 10 years, and send it to the promoter center if needed.

Adverse events. In the case of any adverse event occurring during the trial, is mandatory for the investigator to report it promptly to the Promoter center. The major adverse events that should be reported include:

A. Side effects associated with the drug treatment:
    a. any occurrence of allergic reactions after taking the tablet;
    b. any gastrointestinal symptoms (e.g. nausea, heartburn);
    c. any major or minor bleeding (clinically significant);
    d. others.
B. Intercurrent disease/conditions that may be a contraindication to the continuation of the trial by the patient:
    a. any ischemic heart disease event (angina pectoris, acute myocardial infarction, cardiac revascularization procedures);

b. any acute erebrovascular event (ischemic stroke, transient ischemic attack);
c. any need to take anti-inflammatory drugs or antiplatelet drugs for more than 1 week;
d. other (e.g. pregnancy, immobilization from accident traumas)

Each investigator is requested to contact the Promoter center in the case of any doubt about the evaluation of the adverse event. In addition, each investigator will be required to complete and sign a pre-specified form and to send it to the Promoter center, along with any available clinical documentation to certify the adverse event.

Patients will be advised to contact the investigator immediately after the occurrence of any adverse event, without waiting for the next scheduled visit and before stopping the assigned treatment.

Statistical analyses. Categorical variables will be reported as percentages, whereas continuous variables expressed as means ± standard deviation or medians and interquartile ranges according to their distribution, which will be tested with the Kolmogorov-Smirnov test. Differences between the percentages will be assessed by using the chi-square test or by Fisher's exact test. The student's t test and Pearson correlation analyses will be used for normally distributed continuous variables. Appropriate non-parametric tests will be used for all other variables. Multiple linear regression analysis will be used to assess independent predictors of the absolute annual changes in eGFR, while the multiple logistic regression analysis will be used to identify factors associated with the rapid decline in kidney function. To evaluate the changes in eGFR stages over the trial will be used ordinal logistic regression models. The outcomes will be assessed at two follow-up times (at 6 and 12 months) simultaneously via regression models for longitudinal data, using a random subject-dependent intercept to take account of the dependence of replicate measurements on the same subjects.

An interim-analysis will be performed on the primary endpoint when 33% of patients will have been randomised and completed the 12 months follow-up. The Haybittle–Peto approach will be used: the trial will be ended using symmetric stopping boundaries at if $p<0.001$ at interim.

Statistical significance will be determined at a value of $p<0.05$. The analyses will be performed with the SPSS software version 20 for Windows and R v. 3.0.2 for Linux.

Calculation of the sample size of the trial. The minimum sample size of the trial is estimated on data from previous studies that have estimated an annual reduction of GFR in patients with T2DM

of about 2.5 ml/min/1.73 m^2 year[42]. On the basis of this data, and assuming a protective effect of aspirin of about 20% on the progression of eGFR with a DS of 1.5, it is estimated to include a minimum of 380 patients (190 treated with aspirin and 190 placebo) to have a 90% chance with a probability of error of type I $\alpha = 0.05$ to identify a significant reduction in the primary endpoint. With an expected 10% drop-out rate at follow-up, the final sample to be included in the trial will be of 418 patients (209 for each arm of treatment).

The planned sample size will allow us to achieve an 80% power for a correlation (in absolute value) of 0.14 or more between urinary 11-dehydro-TxB_2 and renal function modifications.

Summary

The LEDA trial will be the first multicentre, double-blind, placebo-controlled trial to examine whether treatment with aspirin (100 mg daily) may beneficially affect kidney function in adult patients with T2DM by lowering the annual decline of eGFR. The trial will also examine whether the potential renoprotective effects of aspirin might be partly due to its inhibition of TxB_2 production.

Disclosures

The trial will be supported by an unrestricted grant from Bayer S.p.A. for the production of aspirin and placebo. Bayer S.p.A. will not be involved in any phase of patients' recruitment or data analysis and interpretation.

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Table 1. Inclusion and exclusion criteria of the LEDA trial

Inclusion criteria
Diagnosis of type 2 diabetes [43, 44]. - random blood glucose ≥ 200 mg / dl (≥11.1 mmol/l) - fasting blood glucose ≥ 126 mg/dl (≥7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.* - blood glucose 2 hours after oral glucose tolerance test (75 g OGTT) ≥200 mg/dl. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.* - hemoglobin A1c ≥ 6.5% (≥ 48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.* - treatment with any glucose-lowering agent. *In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.
Exclusion criteria
1) History of cardiovascular or cerebrovascular events (**defined on history and/or instrumental findings provided by the patient**); 2) Presence of inadequate glycemic control (i.e., hemoglobin A1c ≥8%) 3) **Presence of uncontrolled blood pressure despite anti-hypertensive treatment (≥140/≥85 mmHg)** **4) Previous major bleeding (i.e. intracranial)** **5) Previous gastro-intestinal ulcer** 6) Clinical diagnosis of type 1 diabetes (diagnosis of diabetes and insulin use before 35 years of age); 7) Patients with chronic kidney disease G4 or G5 stage (i.e., eGFR <30 ml/min/1.73 m^2 or dialysis); 8) Chronic active infections or 9) Evidence of malignancy in the last 5 years. **Patients with in situ neoplastic disease successful treated only with local excision can be included in the study (including in situ non-melanoma skin cancer).** 10) Autoimmune systemic diseases; 11) **Sustained cardiac arrhythmias requiring anticoagulant treatment (i.e. atrial fibrillation). In this category isolated ventricular/supraventricular extra-systoles are not included**; 12) Use of non-steroidal anti-inflammatory drugs, or other antiplatelet agents in the previous 30

days;

13) Cirrhosis of any etiology

14) Use of anticoagulants;

15) Life expectancy <1 year;

16) Known allergy to aspirin;

17) Known pregnancy;

18) Severe psychiatric illness.

Table 2. LEDA trial - Timeline of visits and events

Event/Assessment (s)	Baseline visit	6-months visit	12-months visit
Informed consent	X		
Inclusion/exclusion	X		
Anthropometric/clinical data	X	X	X
Medical history and concomitant treatments	X		
Randomization	X		
Laboratory analysis	X	X	X
Assessment of kidney function	X	X	X
Urine sample collection and Thromboxane B_2 assessment	X	X	X
Study drug administration	X	X	
Withdrawal of the medication package and compliance monitoring		X	X
Adverse event monitoring		X	X

Figure 1. The LEDA trial design