Aspirin in the prevention of cardiovascular disease in type 2 diabetes

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Summary and discussion

Diabetes mellitus type 2 is a prevalent metabolic disease, defined by the presence of hyperglycemia. Patients with type 2 diabetes have an increased risk for atherothrombotic cardiovascular disease (CVD), which clearly defines morbidity and mortality associated with diabetes. The 2 to 4 fold increased risk for cardiovascular disease is caused by multiple concurrent pathophysiological mechanism such as endothelial dysfunction, increased vessel wall inflammation and platelet hyperaggregability. This thesis focusses on the role inhibition of platelet activation could play in prevention of cardiovascular events in patients with diabetes.

In ***chapter 2***, we describe in a narrative review current knowledge and evidence on pharmacological interventions for reducing the abovementioned increased risk for CVD in patients with type 2 diabetes. Strict glycaemic control is not associated with a significant reduction in CVD risk, although new hypoglycaemic agents might offer additional benefits. In contrast, it has been demonstrated that treatment of hypertension and dyslipidemia significantly reduce cardiovascular risk. Meticulous control of blood pressure to a level ≤130/80 mmHg, preferably using renin-angiotensin-system (RAS)-modulating agents is of proven value. Use of statins as LDL-cholesterol-lowering therapy, initiated at a LDL-cholesterol level of ≥2.60 mmol/L is firmly established. Mainly based on risk analogy, international guidelines advocated the use of aspirin in primary prevention of CVD in type 2 diabetes. However, there is no support from randomized controlled trials for this statement. Recent meta-analysis failed to demonstrate a reduction in CV risk with aspirin in patients with type 2 diabetes in primary prevention. Nevertheless, an intensified approach in order to identify and treat cardiovascular risk factors in patients with type 2 diabetes, stratified to individual patients, is necessary to reduce the excess in cardiovascular disease these patients suffer from.

In the diabetic individual, platelets are more easily activated, resulting in a hyperaggregatory state. Inflammatory pathways are upregulated in both early and later stages of atherosclerosis. As activated platelets at the site of vascular injury mediate inflammatory pathways, the hypothesis arises whether inhibition of platelet activation could dampen the inflammatory state of the vessel wall. Subsequently,

antiplatelet therapy may reduce incidence of cardiovascular diease, not only by inhibiting platelet activation and aggregation but also by limiting local vascular inflammation fueling the process of atherosclerosis. We hypothesize that in a patient were both vessel wall inflammation and activated platelets coincide, i.e. in the patient with diabetes mellitus type 2, use of aspirin could reduce low-grade vascular inflammation.

In the first part of this thesis, we describe the results of a double blind, placebo controlled cross-over study focusing on this hypothesis. In ***chapter 3***, the primary results of this study are presented. We included 40 patients with well-controlled diabetes mellitus type 2 who were free of manifest cardiovascular disease at time of inclusion. Patients were selected from practices of general practitioners affiliated with the Leiden University Medical Center (LUMC) and had to have diabetes for more than 1 year, HbA1c < 10% and a CRP > 1.0 mg/L. Importantly, concurrent statin therapy was not allowed. Patients were treated with aspirin 100 mg/day or 300 mg/day versus placebo in a cross-over design. Six weeks on treatment were followed by a 4 week washout period. Hereafter, the second 6 week treatment period took place. Before and after each treatment period, we measured CRP and Il-6 levels. Primary endpoint was difference between CRP on treatment versus placebo. Use of aspirin resulted in a CRP reduction of 1.23 $\pm$ 1.02 mg/L (mean $\pm$ SEM), whereas use of placebo resulted in a mean increase of 0.04 $\pm$ 1.32 mg/L (P = 0.366). Aspirin reduced IL-6 with 0.7 $\pm$ 0.5 pg/mL, whereas use of placebo resulted in a mean increase of 0.2 $\pm$ 0.8 pg/mL (P = 0.302). There were no significant differences in effects on CRP and IL-6 between 100 and 300 mg aspirin. Our results indicate that aspirin did not result in reduction of low-grade inflammation in patients with type 2 diabetes without cardiovascular disease, although a modest effect could not be excluded. No significant differential effects between aspirin 100 and 300 mg were found.

Several randomized controlled trials (RCT) have tested the hypothesis that in patients with diabetes, aspirin may reduce the increased CV risk. However, recent studies using low-dose aspirin (≤100 mg/day) failed to show a significant reduction of CV events. One of the explanations of this apparently attenuated clinical benefit of aspirin in patients

with diabetes is failure of aspirin to produce the expected pharmacological effect in patients with diabetes. This concept, aspirin non-responsiveness or resistance, is defined as failure of aspirin to inhibit platelet thromboxane A2 production or inhibit tests of thromboxane-dependent platelet function. Aspirin resistance is reported to be more prevalent among patients with diabetes. In ***chapter 4***, we analyzed in our aforementioned crossover study on the effects of aspirin on vessel wall inflammation determinants of aspirin non-responsiveness. At the end of each treatment period, we tested platelet aggregation using light aggregometry with various agonists. Non-responsiveness was defined as arachidonic acid-induced aggregation of >20% after aspirin therapy. Seven (18%) patients did not respond adequately to aspirin, which was predicted by high levels of LDL-cholesterol and triglycerides and poor glycemic control (HbA1c>7%) at baseline in multivariate logistic regression. Five non-responders used 100 mg whereas two used 300 mg (odds ratio 3.0, 95% confidence interval 0.5-17.7). Use of 300 mg resulted in increased inhibition of collagen-induced aggregation compared to 100 mg (45% vs. 21%, *P*=0.016). Since a lower prevalence of non-responsiveness was suggested and collagen-induced aggregation was more inhibited in patients using aspirin 300 mg compared with 100 mg, we hypothesize that an increase in dosage may improve aspirin effectiveness in patients with type 2 diabetes.

Circulating cells promoting revascularization at sites of ischemia have been proposed to play an important role in maintenance and repair of the vascular endothelium. In patients with diabetes, both number and function of these vasculogenic cells are impaired. Several lines of evidence suggest that activated platelets at the site of endothelial injury are necessary to recruit circulating vasculogenic cells. In ***chapter 5*** we studied the effects of aspirin on the number of circulating vasculogenic cells. Again, we used the previously discussed randomized, double blind, placebo controlled crossover trial among 40 participants with type 2 diabetes mellitus. Participants were randomized to receive aspirin 100 mg/day or aspirin 300 mg/day and placebo during two treatment periods. After each period, we enumerated $CD34^+$ stem cells and endothelial progenitor cells (EPCs or $CD34^+/VEGFR\text{-}2^+$ cells) using flow cytometric analysis. The effect of aspirin on *in vivo* systemic platelet activation

was assessed by measuring levels of soluble P-selectin (sP-sel). The total number of EPCs was significantly reduced after aspirin treatment, on average 12 cells/mL (95%CI -23 to -2; P=0.023). The effect of aspirin 300 mg/day (-15 cells/mL, 95%CI -30 to 0, P=0.046) was more pronounced than with aspirin 100 mg/day (-8 cells/mL, 95%CI -26 to 8, P=0.27). Effects of 300 mg/day on EPC and sP-sel were significantly correlated (P<0.001, r=0.75). No significant differences were observed in the number of $CD34^+$ stem cells at the end of the aspirin treatment period compared with placebo. Therefore, we conclude that in patients with type 2 diabetes, the number of EPC is reduced by aspirin treatment, more profound at doses of 300 mg/day. The number of circulating $CD34^+$ stem cells was not affected by the use of aspirin. These results suggest that treatment with aspirin may impair the endogenous regenerative capacity of the vasculature.

The results from our study on aspirin and diabetes offers new insights on the reasons why use of aspirin does not appear to be associated with reduction in CV risk in patients with type 2 diabetes. First of all, although often acclaimed, we could not find a significant effect of aspirin on vessel wall inflammation. Second, even in a controlled setting such as a RCT, aspirin non-responsiveness is a rather frequent finding. The dose-dependent inhibition of collagen-induced aggregation supports other findings that aspirin non-responsiveness in part may be overcome by higher dosages of aspirin. However, at these higher dosages of aspirin, we found a 40% reduction of $CD34^+$/VEGFR-2^+ cells, which may be associated with a negative effect on endogenous vessel wall repair. Thus, in patients with diabetes, the theoretical protective effect of using higher dosages of aspirin with a lower incidence of aspirin non-responsiveness may be balanced by a reduction in vessel repair capacity. The net effect on clinical outcome could be negligible, which is compatible with the results from several meta-analysis on CV protection with aspirin in patients with type 2 diabetes. Hence, the results of our study stress the importance to use clinical outcomes instead of laboratory-defined endpoints in studies designed to test the hypothesis that use of higher doses of aspirin is an effective strategy to overcome aspirin resistance.

In the second part of this thesis, the results of several epidemiological studies on the prevalence and clinical consequences of aspirin non-responsiveness or resistance are reported. Based on the failure of aspirin to inhibit platelet thromboxane A2 production or to inhibit tests of platelet function, a variety of laboratory tests to define and quantify aspirin resistance have been proposed. Yet, a uniform definition is still lacking. Numerous studies on prevalence of aspirin resistance in different populations have been published in recent years. In ***chapter 6***, we describe the results of the first systematic review and meta-analysis on prevalence of aspirin non-responsiveness in a secondary prevention setting. Moreover, to explain potential heterogeneity, we examined whether definition used, dosage of aspirin, and clinical setting contributed to prevalence of resistance. Two reviewers independently searched electronic databases using a predefined search strategy. To be included in our analysis, articles had to contain a laboratory definition of aspirin resistance, use aspirin as secondary prevention, and report associated prevalence. We estimated pooled prevalence of aspirin resistance and stratified studies based on differences in definition of aspirin resistance, population characteristics, and aspirin dosage. Mixed model analysis was performed for prevalence of aspirin resistance both with and without fixed effects for the laboratory method used to define aspirin resistance, the characteristics of the population studied, and the dosage of aspirin, and with an identification number for each study as a random effect. We included 34 full-text articles and 8 meeting abstracts. The mean prevalence of aspirin resistance was 24% (95% CI 20%-28%). After adjustment for differences in definition, used dosage, and population, a statistically significant higher prevalence was found in studies with aspirin dosage ≤100 mg compared with ≥300 mg (36% [95% CI 28%-43%] vs 19% [95% CI 11%-26%], P ≤0001). Studies measuring platelet aggregation using light aggregometry with arachidonic acid as an agonist had a pooled unadjusted prevalence of 6% (95% CI 0%-12%). In studies using point-of-care platelet function–analyzing devices, the unadjusted prevalence was significantly higher, at 26% (95% CI 21%-31%). In conclusion, our systematic review on prevalence of aspirin resistance indicates that persistent platelet reactivity can be found in approximately 1 in 4 patients on aspirin therapy for secondary prevention of cardiovascular events. Biochemical method to define aspirin resistance and aspirin dosage significantly influence prevalence of aspirin resistance.

A crucial question to be answered is whether patients with laboratory defined aspirin non-responsiveness are at higher risk for cardiovascular events compared to their aspirin responsive counterparts. In other words, is the laboratory phenomenon of aspirin resistance of any clinical relevance? To address this issue, we conducted a systematic review and meta-analysis on all reports on all available evidence on the association between aspirin non-responsiveness and subsequent clinical cardiovascular events. The results are described in ***chapter 7.*** To be included in our analysis, studies had to report on patients who used aspirin for secondary cardiovascular prevention, had to contain a clear description of a method to establish the effects of aspirin on platelet reactivity, and had to report recurrence rates of cardiovascular events. Odds ratios of cardiovascular outcome of eligible studies were pooled in a random-effects model. We included 15 full-text articles and 1 meeting abstract. Fifteen of these studies revealed an adverse association between laboratory aspirin resistance and occurrence of cardiovascular events. The pooled odds ratio of all cardiovascular outcomes was 3.8 (95% CI, 2.3-6.1) for laboratory aspirin resistance which implies that patients biochemically identified as having laboratory aspirin resistance are more likely to also have "clinical resistance" to aspirin because they exhibit significantly higher risks of recurrent cardiovascular events compared with patients who are identified as (laboratory) aspirin sensitive.

Since publication of our two systematic reviews, multiple reports have corroborated our findings. Laboratory defined aspirin resistance appears to be a prevalent condition in several cohorts of patients at risk for cardiovascular disease. The discussion on the optimal laboratory method to define aspirin resistance is still not settled. Assays vary in accuracy, specificity for inhibition of cyclooxygenase-1 pathway and reproducibility. Moreover, prospective data on clinical events in patients who are reported to be aspirin non-responsive are still scarce. Not surprisingly, it is not known whether the physician taking care of the aspirin non-responsiveness patient should alter currently used aspirin to further protect his patient from cardiovascular events. We have to wait for a clear definition and reliable assay of aspirin resistance and the results of the tests should influence clinical decision making (i.e. by changing the dosage or type of antiaggregatory drug). Until then, we have to refrain from individualization of antiaggregatory medication in patients at high risk for cardiovascular events.

Although aspirin is a potent vasoprotective drug, it is generally believed to have no effects on blood pressure. In contrast, two recent RCT's have shown that 100 mg aspirin strongly decreases blood pressure in subjects with grade I essential hypertension when it was administered at bedtime, whereas – if anything – blood pressure might slightly increase when aspirin was taken upon awakening. An important yet unanswered question is by which mechanism aspirin could time-dependently lower blood pressure. As the main regulators of blood pressure behave according to circadian rhythms, these may be potential targets of time-dependent aspirin therapy. We investigated the effect of 100 mg aspirin administered at bedtime compared with upon awakening, on plasma renin activity and aldosterone levels over 24 hours and excretion of cortisol and catecholamines in 24h-urine. A randomized, placebo-controlled, double blind, crossover trial was performed in 16 grade I hypertensive subjects. Results of this trial are reported in ***chapter 8***. During two periods of 2 weeks separated by a 4-week washout period, participants used both aspirin at morning and at night, which was blinded with placebo. After both periods, subjects were admitted for 24 hours to measure the aforementioned parameters. Aspirin intake at bedtime compared with upon awakening reduced average (24h) plasma renin activity by 0.08 µg/L/h (95% confidence interval (95%CI) 0.03–0.13, *P*=0.003) without affecting aldosterone levels (95%CI -0.01–0.01 nmol/L, *P*=0.93). Cortisol excretion in 24h-urine was 52 nmol/24h (95%CI 5–99, *P*=0.05) lower and dopamine and norepinephrine excretion were 0.25 µmol/24h (95%CI 0.01–0.48, *P*=0.04) and 0.22 µmol/24h (95%CI -0.03–0.46, *P*=0.02) lower in patients treated with bedtime aspirin. In conclusion, aspirin taken at bedtime compared with upon awakening significantly diminished 24h plasma renin activity and excretion of cortisol, dopamine and norepinephrine in 24h-urine. Decreased activity of these pressor systems forms a biologically plausible explanation for the finding that aspirin at night may reduce blood pressure whereas aspirin at morning does not.

In summary, in this thesis we analyzed different aspects on the role aspirin could play in prevention of cardiovascular disease in patients with diabetes. Strong evidence suggests against its liberate use, as in primary prevention setting no clinical benefit was found. Aspirin did not reduce CRP as marker for vascular inflammation in patients with diabetes. Our systematic review showed that laboratory defined aspirin

non-responsiveness appears to be an entity which is both prevalent and of clinical relevance. In patients with diabetes, glycemic control and dyslipidemia are associated with aspirin non-responsiveness. Higher dosages of aspirin might overcome the non-responsiveness. However, it is possible that this impairs endothelial regenerative capacity.

Future studies should focus on how to select patients in whom platelet inhibition for cardiovascular prevention might be advantageous. Furthermore, a randomized controlled trial in selected patients should unequivocally demonstrate a significant and relevant clinical benefit. Ultimately, depending on patient characteristics such as risk for cardiovascular disease, responsiveness of antiaggregatory drugs and metabolic state, an individualized appraisal can be made whether to use platelet inhibition to prevent CV disease or not.