Aspirin in the prevention of cardiovascular disease in type 2 diabetes

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Effects of aspirin on serum C-reactive protein and interleukin-6 levels in patients with type 2 diabetes without cardiovascular disease: a randomized placebo-controlled crossover trial

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Abstract

Aim: Low-grade inflammation plays a pivotal role in atherogenesis in type 2 diabetes. Next to its antithrombotic effects, several lines of evidence demonstrate anti-inflammatory properties of aspirin. We determined the effects of aspirin on inflammation – represented by C-reactive protein (CRP) and interleukin-6 (Il-6) – in type 2 diabetic subjects without cardiovascular disease and assessed differential effects of aspirin 300 mg compared to 100 mg.

Methods: A randomized, placebo-controlled, double blind, crossover trial was performed in 40 type 2 diabetic patients. In two periods of 6 weeks, patients used 100 mg or 300 mg aspirin and placebo. Plasma CRP and Il-6 levels were measured before and after both periods.

Results: Use of aspirin resulted in a CRP reduction of 1.23±1.02 mg/L (mean±SEM), whereas use of placebo resulted in a mean increase of 0.04±1.32 mg/L (*P*=0.366). Aspirin reduced Il-6 with 0.7±0.5 pg/mL, whereas use of placebo resulted in a mean increase of 0.2±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.

Conclusions: Our results indicate that a six-week course of aspirin does not improve 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.

Introduction

Patients with type 2 diabetes have a 2-4 fold increased risk for cardiovascular events (1). The excess global mortality attributable to diabetes in the year 2000 was estimated to be 2.9 million deaths and is expected to increase given the growing prevalence worldwide (2,3). Several mechanisms related to metabolic disturbances in diabetes contribute to the increase in cardiovascular risk. Patients with type 2 diabetes exhibit chronic low-grade inflammation which promotes atherosclerotic processes and endothelial cell dysfunction (4,5). C-reactive protein (CRP) is an acute phase reactant, which is produced primarily by the liver in response to circulating inflammatory cytokines including interleukin-6 (Il-6). In several large population-based studies both in apparently healthy individuals (6) and diabetic patients (7,8), CRP levels predicted cardiovascular events, independent of conventional risk factors. Moreover, it is suggested that CRP is a causative factor in the development of atherosclerosis (9).

Use of acetylsalicylic acid or aspirin as inhibitor of platelet aggregation is an effective strategy to reduce cardiovascular events in patients at high risk. Recognizing the increased risk of cardiovascular disease, current guidelines, as issued by the American Diabetes Association (ADA), advocate use of 75-162 mg aspirin daily in patients with type 2 diabetes that have additional risk factors (10). In a large prospective study, Ridker *et al*. showed that risk reduction of cardiovascular events by aspirin was more profound among patients in the highest quartile of CRP compared to the lowest quartile (11). This observation suggests that next to the antiplatelet effects, aspirin may attenuate low-grade inflammation in atherosclerosis. Indeed, in LDL-receptor deficient mice, low-dose aspirin decreased markers of vascular inflammation and increased stability of aortic atherosclerotic plaques (12). Inhibition of activation of nuclear factor (NF)-κB, a family of cellular transcription factors involved in the inflammatory response, by salicylates is supposed to be responsible for anti-inflammatory action of aspirin (13,14). In addition, aspirin-induced acetylation of vascular cyclooxygenase-2, results in production of local endogenous anti-inflammatory eicosanoids (15-epi-lipoxin A_4) in the endothelium (15).

Several studies addressing the effects of aspirin on CRP have yielded conflicting results. Ikonomidis *et al*. studied the effects of 300 mg aspirin daily during three weeks on CRP in 40 men with stable coronary artery disease. CRP levels were significantly lower after aspirin therapy compared to placebo (16). Feng *et al*. administered 81 or 325 mg aspirin to 32 healthy men during seven days and found no significant effect of either dosage on CRP levels (17). Feldman *et al*. studied the effects of low-dose aspirin (81 mg every day or 325, 81 or 40 mg every-third-day for 31 days) on CRP in 57 healthy volunteers and found no significant changes in CRP levels from baseline (18). To date, no studies investigated the effect of aspirin on low-grade inflammation in type 2 diabetes. Additionally, there is no information regarding a potential dosage-related effect of aspirin on CRP.

We conducted a randomized controlled trial to test the hypothesis that use of aspirin results in a decrease of low-grade inflammation represented by CRP and Il-6 in subjects with type 2 diabetes without cardiovascular disease. Moreover, we assessed whether there was a differential effect on CRP and Il-6 reduction between 100 and 300 mg aspirin.

Methods

Subjects were recruited from general practitioners affiliated to the Leiden University Medical Center. Subjects were eligible if they had been diagnosed with type 2 diabetes for at least 1 year, had glycosylated haemoglobin A (HbA1c) levels <10% and CRP levels >1.0 mg/L, and were both aged above 18 years old and capable to give informed consent. Subjects were excluded if they had any history of cardiovascular disease (defined as myocardial infarction, acute coronary syndrome, percutaneous coronary intervention, coronary artery bypass grafting, heart failure, severe cardiac arrhythmia, cerebrovascular accident, transient ischemic attack or peripheral vascular disease) or known contraindications to use of aspirin (defined as history of asthma, any bleeding disorder, gastrointestinal tract bleeding or known allergy to acetylsalicylic acid). Other exclusion criteria were presence of uncontrolled hypertension, severe renal or hepatic dysfunction, pregnancy, concurrent participation in other research projects

or blood donation and use of non-steroidal anti-inflammatory drugs, anticoagulant medication, corticosteroids or statins. All subjects gave written informed consent and the study was approved by the Leiden University Medical Center medical ethics committee and performed in accordance with the Declaration of Helsinki.

The study had a prospective, randomized, placebo-controlled, double blind, crossover design. All subjects (n=40) received one period placebo and the other period aspirin. Subjects were randomly assigned to receive aspirin 100 mg (n=20) or 300 mg (n=20). The first treatment period with aspirin or placebo for six weeks was followed by a washout period of four weeks. Thereafter, those assigned to placebo in the first period received aspirin for six weeks and those assigned to aspirin received placebo for additional six weeks. Double blind study medication was prepared and stored at the department of clinical pharmacy of Leiden University Medical Center. A computer-generated randomization code was prepared by this department. Medication was pre-packaged based on a block size of four. Each consecutive subject was given the next consecutive randomization number and eligible subjects were assigned in a 1:1 ratio to receive the study drug or placebo.

Subjects visited the research site after an overnight fast at the beginning and at the end of each six-week treatment period. By a structured interview, we asked for compliance, possible adverse events and changes in medication. To further assess compliance, remaining pills were counted. Non-compliance was defined as remaining pill count ≥10%. At each visit, blood samples were drawn from antecubital veins.

Laboratory measurements

At baseline, routine haematological and chemical variables were determined according to standard procedures. CRP was measured with an ultra sensitive protein-enzyme linked immunosorbent assay (ELISA) from DSL (Webster, Texas, USA). The sensitivity was 0.002 mg/L and the interassay coefficients of variation ranged from 2.8 to 5.1% at different levels. Determination of Il-6 was performed with a standard ELISA technique according to the manufacturer's guidelines (Sanquin, Amsterdam, the Netherlands). The lowest detectable level was 1 pg/mL and for samples below that level 0.5 pg/mL was used in the calculations. All CRP and Il-6 assays were performed after completion of the study.

Statistical analysis

Based on results from former studies (16,19), we calculated a required sample size for this cross-over trial of 40 patients to have 90% power to detect a 40% CRP reduction by aspirin compared to placebo at the 5% level of significance using the paired samples *t* test.

Continuous variables are presented as mean values ± standard deviation and categorical variables as frequencies (percentages), unless otherwise stated. CRP and Il-6 levels were not normally distributed. Therefore, these variables were expressed as medians and interquartile ranges. Comparisons between continuous variables were performed with independent samples *t* tests or Mann-Whitney *U* tests if not normally distributed. Categorical variables were compared with Pearson χ^2 or Fisher's exact tests as appropriate. Correlations of baseline CRP levels with patient-related factors were analyzed with univariate correlation analysis and multivariate linear regression analysis.

Changes in levels of CRP and Il-6 before and after aspirin and placebo were analyzed using paired samples *t* tests or Wilcoxon signed ranks tests as appropriate. To assess which patient-related factors were associated with effects of aspirin on CRP and Il-6, we compared baseline characteristics between highest and lowest quartiles of CRP and Il-6 reduction with independent samples *t* tests.

Analyses were performed using SPSS version 12.01 (SPSS, Chicago, Illinois, USA). All analyses were two-sided, with a level of significance of α=0.05.

Results

Subject characteristics are summarized in Table 1. No statistical differences between the groups were observed. All subjects were compliant to study medication and no adverse events occurred. Median baseline CRP levels were 4.13 (2.65-10.58) mg/L. Baseline CRP was positively correlated with female sex (Pearson correlation coefficient 0.426, *P*=0.007), BMI (coefficient 0.324, *P*=0.044), HbA1c (coefficient 0.381, *P*=0.017) and triglycerides (coefficient 0.584, *P*<0.001) in univariate analysis. Multivariate analysis revealed that HbA1c (regression coefficient 2.459, *P*=0.043) and triglycerides (coefficient 4.308, *P*<0.001) were independently related to CRP.

Table 1 – Baseline characteristics

	Aspirin 100 mg (*n* = 20)	Aspirin 300 mg (*n* = 20)	*P*
Age (yrs)	59.0 ± 10.5	54.5 ± 9.6	0.161
Female sex	9 (45)	5 (25)	0.185
BMI (kg/m^2)	30.0 ± 6.3	31.5 ± 5.3	0.420
Waist circumference (cm)	103 ± 12	106 ± 11	0.420
Hip circumference (cm)	106 ± 13	107 ± 10	0.936
Systolic tension (mm Hg)	154 ± 15	150 ± 16	0.468
Diastolic tension (mm Hg)	90 ± 9	90 ± 9	0.939
Smoking	2 (10)	2 (10)	1.000
Laboratory data			
Glucose (mmol/L)	8.3 ± 3.0	7.7 ± 1.1	0.422
HbA1c (%)	6.2 ± 1.4	5.9 ± 0.8	0.426
CRP (mg/L)	4.34 (2.53-8.76)	4.02 (2.65-12.63)	0.768
Creatinine (µmol/L)	81.2 ± 15.2	85.1 ± 13.9	0.402
HDL-cholesterol (mmol/L)	1.5 ± 0.4	1.4 ± 0.3	0.253
LDL-cholesterol (mmol/L)	3.5 ± 1.0	3.8 ± 0.8	0.299
Triglycerides (mmol/L)	1.8 ± 1.3	2.0 ± 1.1	0.563
VWF:Ag (IU/mL)	1.4 ± 0.5	1.2 ± 0.5	0.260
Fibrinogen (g/L)	4.0 ± 0.7	4.2 ± 0.7	0.255
Medications			
ACEI/ARB	5 (25)	9 (45)	0.185
Diuretics	3 (15)	3 (15)	1.000
β-blockers	3 (15)	5 (25)	0.695
Oral hypoglycaemic drugs	14 (70)	13 (65)	0.736
Insulin	4 (20)	4 (20)	1.000

Data are *n* (%), means ± standard deviation (SD) or medians and interquartile ranges. ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BMI, body mass index; HDL, high-density lipoprotein; CRP, C-reactive protein; LDL, low-density lipoprotein; VWF:Ag: von Willebrand factor antigen.

Effects on CRP

CRP values are presented in Table 2a, both for all subjects and separated in those using 100 and 300 mg aspirin. Absolute changes in CRP during each treatment period are shown in Figure 1a.

Use of aspirin resulted in a CRP reduction of 1.23±1.02 mg/L (mean ± standard error of the mean (SEM)), whereas use of placebo resulted in a mean increase of 0.04±1.32 mg/L (difference: 1.27 mg/L, 95% confidence interval (CI): -1.54 to 4.09, *P*=0.366).

Among subjects randomized to 100 mg aspirin, a reduction of 1.08±1.44 mg/L was observed after aspirin and an increase of 0.19±0.52 mg/L after placebo (difference: 1.28 mg/L, 95%CI: -1.96 to 4.51, *P*=0.420). In the group of subjects using 300 mg aspirin, a reduction of 1.39±1.49 mg/L was observed after aspirin and a reduction of 0.11±2.63 mg/L after placebo (difference: 1.27 mg/L, 95%CI: -3.67 to 6.22, *P*=0.596). The changes in CRP by aspirin were similar for subjects using 100 and 300 mg aspirin (*P*=0.884).

Table 2a – Changes in CRP

	Aspirin			Placebo		
	Before	After	*P*	Before	After	*P*
Overall	4.85 (2.03-12.43)	4.99 (2.24-8.64)	0.788	4.38 (2.00-9.21)	4.94 (2.52-9.55)	0.361
Aspirin 100 mg	4.85 (2.36-9.55)	5.59 (2.24-8.55)	0.823	4.28 (1.96-7.50)	4.00 (1.57-8.94)	0.709
Aspirin 300 mg	5.09 (1.84-16.65)	4.63 (1.77-8.64)	0.940	4.60 (2.21-19.12)	6.84 (2.91-15.09)	0.411

CRP values are presented as medians and interquartile ranges (mg/L). *P*-values are for differences in CRP before and after treatment with aspirin and placebo.

We compared subjects in the highest and the lowest quartile of CRP reduction by aspirin with respect to all baseline variables. Subjects with the strongest CRP reduction (highest quartile) had significantly lower HbA1c levels than those with the lowest reduction (5.3±1.2 versus 6.3±0.7%, *P*=0.023). There were no differences in other patient-related factors.

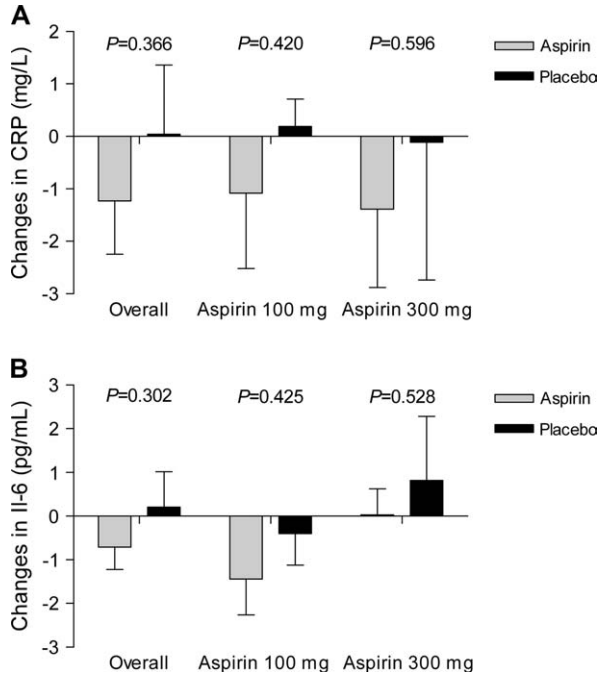

Figure 1 - Changes in (A) CRP and (B) Il-6 levels during treatment with aspirin and placebo. Changes are calculated as levels after minus levels before treatment and presented as means ± standard error of the mean. *P*-values are for differences in CRP and Il-6 changes by aspirin and placebo.

Effects on Il-6

Il-6 values are presented in Table 2b, both for all subjects and separated in those using 100 and 300 mg aspirin. Absolute changes in Il-6 during each treatment period are shown in Figure 1b.

Use of aspirin resulted in an Il-6 reduction of 0.7±0.5 pg/mL (mean ± SEM), whereas use of placebo resulted in a mean increase of 0.2±0.8 pg/mL (difference: 0.9 pg/mL, 95%CI: -0.9 to 2.7, *P*=0.302). Among subjects randomized to 100 mg aspirin, a reduction of 1.4±0.8 pg/mL was observed after aspirin and a reduction of 0.4±0.7 pg/mL after placebo (difference: 1.0 pg/mL, 95%CI: -1.6 to 3.7, *P*=0.425). In the group of subjects using 300 mg aspirin, an increase of 0.03±0.6 pg/mL was observed

after aspirin and an increase of 0.8±1.5 pg/mL after placebo (difference: 0.8 pg/mL, 95%CI: -1.8 to 3.4, *P*=0.528). The changes in Il-6 by aspirin were similar for subjects using 100 and 300 mg aspirin (*P*=0.158). The amount of Il-6 reduction was not related to any patient-related factor.

Table 2b – Changes in Il-6

	Aspirin			Placebo		
	Before	After	*P*	Before	After	*P*
Overall	2.7 (0.5-4.3)	1.9 (0.5-3.4)	0.225	2.2 (0.5-3.5)	2.5 (0.5-4.9)	0.557
Aspirin 100 mg	3.1 (1.3-5.1)	1.9 (0.5-4.1)	0.140	2.4 (0.5-3.6)	2.5 (0.7-4.8)	0.979
Aspirin 300 mg	2.0 (0.5-3.9)	1.7 (0.5-3.3)	0.777	1.7 (0.5-3.4)	2.6 (0.5-4.9)	0.495

Il-6 values are presented as medians and interquartile ranges (pg/mL). *P*-values are for differences in Il-6 before and after treatment with aspirin and placebo.

Discussion

We tested the hypothesis that use of aspirin results in a decrease of low-grade inflammation in subjects with type 2 diabetes without cardiovascular disease. In our study, aspirin did not significantly reduce levels of CRP and Il-6. Furthermore, there was no differential effect on CRP and Il-6 reduction between 100 and 300 mg aspirin. This is the first study that addressed the effect of aspirin on inflammation in subjects with type 2 diabetes. Our results are in line with two earlier studies evaluating the effects of aspirin on CRP in healthy volunteers, in which no effects were found (17,18). However, subjects in our study had CRP levels well above the levels in the former studies, probably due to the proinflammatory diabetic state. In contrast, a third study addressed the effects of 300 mg aspirin daily on CRP and Il-6 in 40 men with coronary artery disease and found significantly lower levels of CRP and Il-6 after treatment with aspirin compared to placebo (1.0 (0.5-3.1) versus 1.4 (0.5-4.1) mg/L, *P*<0.05 and 2.9 (2.5-3.4) versus 3.5 (3.2-4.6) pg/mL, *P*<0.05, respectively) (16). However, in this study, levels of CRP and Il-6 after aspirin were compared with levels

after placebo. The absolute changes of these variables after the two treatments were not assessed although this would have been the appropriate comparison given the design of the study. We evaluated changes in CRP and Il-6 levels during aspirin and placebo treatment, thereby taking into account potential differences in baseline CRP and Il-6 levels between both treatment periods due to high intra-individual variation (20). To illustrate this, if we would have used the approach of the aforementioned study (16), we also would have found a significantly lower CRP after aspirin 300 mg compared to placebo (*P*=0.037). In our view, the design we have used in our study better reflects the true nature of aspirin's effects on CRP.

Our study was powered to detect a 40% reduction in CRP by aspirin. However, due to much larger inter- and intra-individual variance of CRP values than assumed based on prior studies (16,19), we cannot exclude a small effect of aspirin on CRP. In addition, our data suggest a more profound effect on CRP reduction by aspirin 300 mg than by 100 mg, but our study was not powered to detect a difference between both dosages. Indeed, large prospective studies on primary prevention of cardiovascular events in patients with diabetes using an aspirin dosage higher than 100 mg daily showed a more beneficial outcome (21,22) than studies using 100 mg aspirin or less daily (23,24), though a direct comparative study has not yet been performed. Therefore, a large-scale study is warranted to assess the effects of aspirin 300 mg on CRP in diabetic patients. Noteworthy, inhibition by salicylates of NF-κB resulting in down-regulation of pro-inflammatory target genes may require even higher dosages of aspirin (25).

Baseline CRP values were independently related to high levels of triglycerides and HbA1c, which is confirmed by many other studies (8,26-28). A previous study revealed that patients with both high levels of CRP and HbA1c are at particularly high risk for poor cardiovascular outcome (29). In our study, subjects with the strongest CRP reduction by aspirin were found to have significantly lower HbA1c levels compared to those with the weakest effect of aspirin on CRP. Apparently, diabetic patients with a good glycaemic regulation are more susceptible to the potential anti-inflammatory effects of aspirin than poorly controlled subjects. On contrary, patients with high HbA1c levels, which are at the highest risk of cardiovascular events, do not benefit.

The following potential study limitations warrant comment. First, our sample size

was relatively small, given the observed larger variation in CRP levels than *a priori* assumed (16,19). Second, lack of reduction of CRP and Il-6 levels by aspirin could theoretically be explained by non-adherence to study medication. However, several features of our study argue against existence of non-compliance. Subjects were highly motivated to participate in this trial, medication was blinded, and intervention periods were short. All subjects were fully compliant as assessed by interview and pill count.

In conclusion, our results indicate that use of aspirin does not improve vascular endothelial inflammation represented by CRP and Il-6 in patients with type 2 diabetes without cardiovascular events. A more modest reduction in CRP by aspirin 300 mg could not be excluded.

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