Aspirin in the prevention of cardiovascular disease in type 2 diabetes

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Prevalence of persistent platelet reactivity despite use of aspirin: a systematic review

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Am Heart J 2007;153(2):175-181

Abstract

Background: The absolute risk of recurrences among patients using aspirin for prevention of cardiovascular events remains high. Persistent platelet reactivity despite aspirin therapy might explain this in part. Reported prevalences of this so-called aspirin resistance vary widely between 0% and 57%.

Objectives: To systematically review all available evidence on prevalence of aspirin resistance and to study determinants of reported prevalence.

Methods: Using a predefined search strategy, we searched electronic databases MEDLINE, EMBASE, CENTRAL and Web of Science. 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.

Results: 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%) versus 19% (95% CI 11-26%), p<0.0001). Studies measuring platelet aggregation using light aggregometry with arachidonic acid as 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, 26% (95% CI 21 – 31%).

Conclusions: There is large heterogeneity in studies reporting on aspirin resistance. Both aspirin dosage and method of defining aspirin resistance strongly influence estimated prevalence. On average, it appears that about one in four individuals may express biochemically defined aspirin resistance.

Introduction

Cardiovascular diseases are the most common cause of mortality and morbidity in western countries in the twenty-first century. In the United States, the mortality of cardiovascular diseases was nearly 40% of total mortality in 2003 (1). As aggregation of platelets highly contributes to the development of cardiovascular events, inhibition of this process could play an important role in prevention of cardiovascular disease (2).

Nowadays, aspirin (acetylsalicylic acid) forms the cornerstone in secondary prevention of cardiovascular events. The effect of low-dose aspirin is most likely based on the permanent inactivation of cyclooxygenase-1 (COX-1), which results in an irreversible inhibition of the production of thromboxane A2 by platelets (3,4). Thromboxane A2 is a potent platelet activator that also causes vasoconstriction and smooth muscle proliferation (5,6). A decrease in thromboxane A2 leads to reduced aggregation of platelets (7).

The clinical effectiveness of aspirin on the prevention of cardiovascular events has been well established. The Antithrombotic Trialists' Collaboration has documented a 22% reduction of death and serious ischemic vascular events by antiplatelet therapy compared with placebo, in their most recent meta-analysis of 287 randomized trials, incorporating more than 200,000 patients (8).

However, not all patients profit to the same extent, which could be explained by a variety of pharmacodynamic, pharmacokinetic and biochemical features (9). Addressed biochemically as persistent platelet reactivity in vitro despite use of aspirin, this phenomenon is called aspirin resistance, though a uniform definition is lacking (10-12). 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 has been proposed. Aspirin resistance has received much attention, in both medical journals (11,13,14) and lay media (15).

However, both prevalence and impact of aspirin resistance are still not well known. There are only few studies on the clinical consequences of being labeled aspirin resistance (16,17).

Collectively, they suggest a higher recurrence rate of cardiovascular events in aspirin resistant patients. Estimates of prevalence of aspirin resistance vary greatly, with a range from 0% (18) to 57% (19). It is still not known which factors might influence reported prevalences.
In our study, we systematically reviewed all available evidence on prevalence of aspirin resistance among patients using aspirin in secondary prevention setting. Moreover, we examined whether the reported prevalences relate to used definition, dosage of aspirin and clinical setting.

Methods

We used electronic databases to identify relevant reports. The following databases were searched: MEDLINE (from January 1966 to October 2005), EMBASE (from January 1974 to October 2005), the Cochrane Central Register of Controlled trials (CENTRAL) (from 1800 to 2005) and Web of Science, using predefined search terms (Appendix 1). We used both MeSH terms and free text words. We used no language restrictions. Furthermore, we tried to identify additional studies by searching the reference lists of relevant studies and reading reviews, editorials and letters on this topic. Authors of appropriate identified studies were contacted to obtain additional data not reported in the original report. Both full-text articles and meeting abstracts were included.
To be included in the analysis, selected studies had to meet all of the following inclusion criteria: 1) the study should be a cross-sectional survey or a cohort study that included consecutive patients; 2) the study should contain a clear definition of aspirin resistance; 3) the patients involved should use aspirin for secondary prevention cardiovascular events; 4) the study population should be well described; and 5) the study should report data on prevalence of aspirin resistance. Reviewers were not blinded to journal, author or institution of publication.
To assess quality of identified studies, we used a prespecified data collection form to abstract information for each report regarding year of publication, duration and setting of study, study design, measurement of exposure, completeness of follow-up,

blinding, case definition and matching of patients and total sample size. To answer our research question we collected data on study population, dosage of aspirin, definition of aspirin resistance, and prevalence of aspirin resistance.

Selection, quality assessment and data extraction of studies to be included in this review were all independently done by two reviewers (MMCH and JDS). Disagreements were resolved by consensus and discussion with a third party (MVH). Statistical analysis was based on a linear mixed model for prevalence of aspirin resistance, both with and without correcting for between-trial factors, i.e. the laboratory method used to define aspirin resistance, the characteristics of the population studied and the dosage of aspirin. The analysis was executed through the mixed model procedure as implemented in SPSS, version 12.01. We evaluated the significance of the correcting fixed effects through use of the f-test, by interpretation of calculated 95% confidence intervals. The analysis was carried out overall as well as across several subgroups. The reciprocal of the total number of participants in each study was calculated for weighting. Furthermore, to assess heterogeneity among studies, we analyzed prevalence data of different studies using generic inverse variance data entry and random effects model analysis in Review Manager 4.2.2. (Cochrane Collaboration). Quantification of the effect of heterogeneity was assessed by means of I^2, ranging from 0-100%. I^2 demonstrates the percentage of total variation across studies due to heterogeneity (20). Kappa statistics for agreement between reviewers were performed manually for each process in study selection. The overall kappa was calculated as a weighted mean of those different values.

For further analyses, we stratified studies based on differences in definition of aspirin resistance, population characteristics and aspirin dosage. We identified three groups based on laboratory techniques used: definitions based on measurements with the platelet function analyzer (PFA)-100, rapid platelet function assay (RPFA) and light transmission aggregometry (LTA). The PFA-100 device (Dade Behring Inc, Deerfield, Ill., USA) measures in-vitro shear-stress-induced platelet activation in terms of platelet occlusion of a membrane coated with platelet agonists. Using a collagen/epinephrine cartridge (CEPI), closure times between 136s and 199s formed the cut-off value to differentiate between aspirin resistance and sensitivity. The Ultegra RPFA

device (Accumetrics Inc., San Diego, CA, USA) measures changes in light transmission related to the rate of aggregation, using a disposable cartridge with fibrinogen-coated beads and a platelet activator. Results are expressed as aspirin reaction units (ARU), with an ARU ≥ 550 indicating aspirin resistance.
LTA measures the increase in light transmission through a suspension of platelet-rich plasma when agonists stimulate platelet aggregation.
We divided the described population into three groups. First, all trials with patients who used aspirin for secondary prophylaxis after myocardial infarction, with stable or unstable angina, and after or during revascularization were labeled as CAD group. Second, the stroke group consisted of studies with patients with previous stroke or transient ischemic attack. Last, we defined a rest group for other patient groups, mostly consisting of patients with peripheral arterial disease or a non-specific reason for aspirin use as secondary prevention. We categorized four dosage groups: ≤ 100 mg, 101 – 299 mg, ≥ 300 mg or no specified dosage.
The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

We included thirty-four full-text articles and eight meeting abstracts (21-28) in our systematic review (Figure 1). Overall, kappa statistics were 0.82, indicating good interobserver agreement. The overall prevalence, weighted for study size, was 24% (95% confidence interval (CI) 20% - 28%), with ranges from 0% (18) to 57% (19). Table 1 presents overall prevalences, both unadjusted and adjusted for differences in definition, population and aspirin dosage. There was a significant heterogeneity among studies (χ^2 682.87, p<0.00001, I^2 94%).

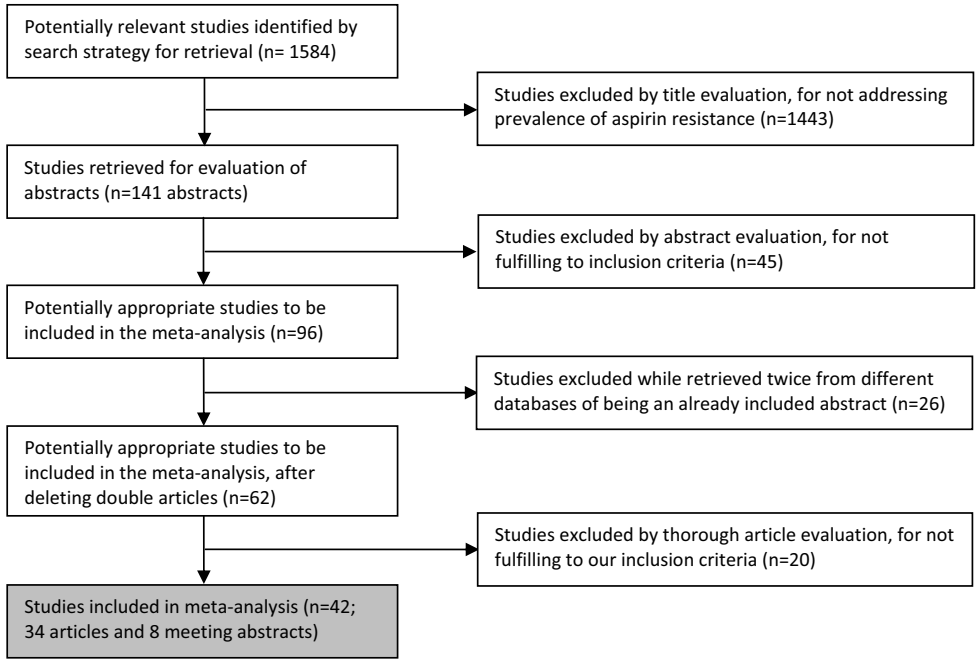

Figure 1 - Flow Chart Study Selection.

Heterogeneity was largely explained by the laboratory methods used to define aspirin resistance. Most included studies (n=22) used the PFA-100 as method to determine aspirin resistance (19,22-24,26,29-45). Six studies used the RPFA assay (36,46-50). LTA was used in several studies (n=15) (18,21,25,27,28,35,36,47,51-57). Within the studies using LTA, the choices of agonists and cut-off values to define aspirin resistance varied widely. A few studies (n=4) used other methods which are less used and validated to detect aspirin resistance than above-described techniques (19,58-60). The pooled mean unadjusted prevalence among the five studies included in our meta-analysis, which used arachidonic acid as agonist in LTA, was 6% (95% CI 1 – 12%). In studies using PFA-100 or RPFA, the pooled mean unadjusted prevalence was significantly higher, 26% (95% CI 21 – 31%, $p<0.0001$).

Table 1 - Prevalence of aspirin resistance specified by used definition of aspirin resistance, study population and aspirin dosage

	Mean prevalence	
	Not adjusted, % (95% CI)	Adjusted*, % (95% CI)
Overall	23.8 (19.5-28.0)	27.1 (21.5-32.6)
Definition		
PFA-100	28.1 (22.2-33.9)	29.0 (23.1-34.8)
RPFA	18.9 (12.1-25.8)	26.2 (18.6-33.9)
LTA	15.4 (7.8-23.0)	21.3 (15.1-27.5)
Rest	35.0 (6.0-64.0)	31.8 (21.6-42.0)
Population		
CAD	22.4 (17.5-27.4)	22.9 (17.0-28.7)
Stroke	26.0 (16.3-35.7)	32.1 (22.4-41.8)
Rest	27.3 (9.5-45.1)	26.3 (16.5-36.0)
Dosage		
≤100mg	27.1 (18.7-35.6)	35.6 (28.1-43.2)
101-299mg	29.6 (21.8-37.5)	28.2 (20.9-35.6)
≥300mg	21.7 (11.8-31.7)	18.6 (11.3-26.0)
Unknown	19.8 (10.7-28.9)	25.8 (16.2-35.4)

*Adjusted for definition of aspirin resistance, study population and used dosage PFA-100 indicates aspirin resistance determined using platelet function analyzer 100 (Dade-Behring Inc., Deerfield, USA) technique; RPFA indicates aspirin resistance determined using rapid platelet function analyzer (Ultegra, Accumetrics Inc, San Diego, USA); LTA indicates aspirin resistance determined using light transmission aggregometry; CAD indicates coronary artery disease

Most studies examined aspirin resistance in patients with coronary artery disease (CAD, n=28) (18,22-24,26-28,30,32,33,35,37,39,41-46,49-51,53-58). The stroke group consisted of eight studies (21,29,34,36,38,52,59,60). The rest group for other patient clusters, mostly consisting of patients with peripheral arterial disease or a non-specific reason for aspirin use as secondary prevention consisted of seven studies (19,25,31,34,40,47,48). There was no difference in mean adjusted prevalence between the three groups.

Reported daily aspirin dosages varied between 80 mg and 1500 mg. Ten studies used aspirin ≤100 mg (31,33,34,40,41,46,49,53,57,58), thirteen studies 101 – 299 mg (24,26,29-33,36-39,45,49) and thirteen studies ≥ 300 mg (19,21,29,33,35,42,47,49,51, 52,54,59,60). Twelve studies did not report a specific dosage (18,22,23,25,27,28, 43,44,48,50,55,56). Studies on aspirin resistance in users of aspirin in a dosage ≥300 mg daily show a significantly lower adjusted prevalence compared with studies with aspirin ≤100 mg (18.6% (95% CI 11.3 – 26.0%) versus 35.6% (95% CI 28.1 – 43.2%), p<0.0001).

Discussion

Among studies in patients using aspirin for secondary prophylaxis of arterial thromboembolism, our meta-analysis showed an overall prevalence of approximately 25% of laboratory defined persistent platelet reactivity. There is large heterogeneity in studies, reflecting large variance in used method to define aspirin resistance, population studied and used dosage of aspirin.

Analysis of unadjusted prevalence in studies using LTA shows prevalence significantly below the unadjusted prevalence for the point-of-care devices. Light aggregometry employing arachidonic acid as agonist is a well established, though labor intensive method, reflecting biochemical action of aspirin most directly, as the main effect of low-dose aspirin on platelet activation is inhibition of the COX-1 dependent conversion of arachidonic acid into thromboxane A2 (3). Several studies demonstrate poor concordance between PFA-100 and LTA method (35,36). This discrepancy suggests that PFA-100 or RPFA measures different aspects of platelet function. Gonzalez et al. studied both platelet aggregation using arachidonic acid, PFA-100 closure time and markers of thromboxane A2 production in healthy subjects on aspirin (61). Although intake of 100 mg aspirin resulted in a 75% reduction in 11-dehydrothromboxane B2 in healthy subjects and all individuals displayed more than 90% inhibition of platelet aggregation, regression analysis revealed no association between 11-dehydrothromboxane B2 levels and PFA-100 closure times. Furthermore, increased levels of VonWillebrand Factor (VWF) are associated with

failure to prolong PFA-100 closure time in aspirin-treated patients (31). In healthy individuals, VWF plasma levels were strongly associated with CEPI closure time, but no relationship was found between closure time and platelet aggregation, suggesting that PFA-100 results reflect VWF activity more than platelet function (62).

Studies on aspirin resistance in users of aspirin in a dosage of ≥300 mg daily show a significantly lower adjusted prevalence rate compared with studies with aspirin ≤100 mg (18.6% (95% CI 11.3 - 26.0%) versus 35.6% (95% CI 28.1 - 43.2%), p<0.0001).
Consequently, our data suggest that increasing the dosage of aspirin could reduce the prevalence of aspirin resistance. Results from other studies support this hypothesis. In a population of 102 patients with type 2 diabetes mellitus, Abaci et al. assessed PFA-100 closure time before and after the administration of 100 mg aspirin (63). Aspirin resistance, defined by a closure time below 300s, was found in 34 of 102 patients. Prolongation of closure time > 300s was obtained in 15 of these 34 patients after additional ingestion of 300 mg aspirin. The effect of PFA-100 guided dosage adaptation on aspirin resistance (defined by PFA-100 CEPI-closure time <170s) was studied in a population of 212 CAD patients (64). At an initial dosage of 100 mg aspirin, 18.4% had a closure time in the normal range. After reinforcement of compliance and increase in dosage to 300 mg, only 1.4% had a closure time within the normal range. Used dosage influenced prevalence of aspirin resistance measured by RPFA as well. An aspirin dosage ≤100 mg independently predicted presence of aspirin resistance (OR 2.2, 95% CI 1.1 - 4.4, compared with dosage > 100 mg) in a population of 468 CAD patients (49).
Presupposed there is an adverse relationship between aspirin resistance and risk of cardiovascular events, and aspirin dosage is a major determinant of resistance, one would expect aspirin dosage to influence event rate in aspirin prevention trials. However, in the Antiplatelet Trialists' Collaboration meta-analysis, in trials comparing different daily doses of aspirin (≤75 mg, 75-150 mg, 160-325 mg and 500 - 1500 mg) versus no aspirin, no particular range of aspirin dose was preferable for the prevention of serious vascular events. Possibly, the potentially beneficial effect of a higher aspirin dosage in reducing prevalence of aspirin resistance is offset by the more profound suppression of the vasculoprotective effects of prostacyclin occurring at higher dosages of aspirin (3,65,66).

The strength of our study lies in the systematic nature of the reviewing process. By prespecifying inclusion criteria and a sensitive search strategy, we were capable to review all retrievable studies with a minimum risk for bias. Thus, we were able to provide an extensive and to our knowledge complete overview on available data on prevalence of aspirin resistance. However, the following potential limitation to our study requires comment. As in all systematic reviews, our results may have been influenced by publication bias. This could result in inflated estimates of prevalence rates. Even though we tried to minimize publication bias by applying no formal language restriction and including meeting abstracts, a funnel plot on all included studies suggests an inverse relationship between size of population and aspirin resistance prevalence (data not shown). On contrary, there was no difference in mean prevalence derived from studies published in peer-reviewed journals and from those published as meeting abstract.

In conclusion, our systematic review on prevalence of aspirin resistance indicates that in approximately one in four patients on aspirin therapy for secondary prevention of cardiovascular events, persistent platelet reactivity can be found. Biochemical method to define aspirin resistance and aspirin dosage significantly influence prevalence of aspirin resistance. More studies are needed to determine which method is most predictive in identifying patients at high risk of cardiovascular events despite aspirin therapy. Moreover, prospective studies are needed to answer the question whether aspirin resistance could be overcome by an increase in dosage.

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Appendix 1

Predefined Search Terms for Electronic Databases

Medline

((“aspirin”[MeSH] OR aspirin OR aspirin* OR Acetylsalicylic Acid OR salicylate* OR salicylic*) AND (resistance OR resistant OR failure OR failing OR nonrespon* OR nonrespon*) AND (clinical consequences OR clinical consequence OR clinical implications OR clinical implication OR incidence OR prevalence OR “Treatment Outcome”[MeSH] OR “Outcome Assessment (Health Care)”[MeSH]) NOT (“insulin resistance*” OR insulin resistance)) OR ((“aspirin resistance” OR “aspirin failure” OR ((aspirin/administration and dosage OR aspirin/therapeutic use OR aspirin) AND drug resistance) OR ((resistance[title word] OR resistant[title word] OR failing[title word] OR failure[title word] OR “non responsiveness”[title word] OR non-responders[title word] OR non-response[title word] OR nonrespon*[title word]) AND aspirin[title word])) NOT “insulin resistance”)

Embase

(aspirin resistance OR ASA resistance OR aspirin failure OR resistance to aspirin).af OR ((*Acetylsalicylic Acid/ OR aspirin.ti) AND (drug resistance/ OR drug resistance.mp)) OR ((resistance.ti OR resistant.ti OR failing.ti OR failure.ti OR non responsiveness.ti OR nonrespon$.ti OR nonrespon$.ti) AND (aspirin.ti or Acetylsalicylic Acid.ti) NOT heart failure.mp)

Cochrane Central Register of Controlled Trials (Central)

(“aspirin resistance” OR “aspirin failure” OR “ASA resistance”) in All Fields OR (aspirin AND (resistance OR resistant OR failing OR failure OR “non responsiveness” OR nonresponders OR non-response OR nonrespon*)) in Record Title NOT (“insulin resistance” OR “heart failure”) in All Fields

Web of science

ti=((aspirin resistance OR aspirin failure OR resistance to aspirin OR (aspirin same resistance) OR (aspirin same failure))) NOT ts=(heart failure OR insulin resistance)