Time for aspirin : blood pressure and reactivity

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Chapter 1

General introduction

Despite major improvements in treatment and prevention over the last decades, cardiovascular disease is still one of the leading causes of morbidity and mortality worldwide.[1, 2] In Europe, cardiovascular disease is responsible for approximately four million deaths each year. Low-dose aspirin is a cornerstone in the prevention of cardiovascular disease, because it reduces the risk of recurrent cardiovascular events with about a quarter.[3] Historically, the natural form of aspirin (*salicin*) was extracted from willow tree bark (*salix*) which was already used in ancient Egypt and Greece to treat pain and fever.[4] Major drawbacks of this ancient drug were its side effects, especially irritation of the stomach. In 1897, a chemist named Felix Hoffman modified the compound into acetylsalicylic acid to reduce its side effects. The Bayer company registered the substance under the name "Aspirin®" as a pain killer in 1899.[5] A bleeding tendency in patients using aspirin was already reported in 1945[6], but the effect of aspirin on blood platelets was not known until 1968.[7] Even before this mechanistic discovery, a general practitioner named Lawrence Craven was one of the first to systematically investigate the possible role of aspirin to prevent myocardial infarctions in 1950 (Figure 1).[8] He recommended all his male patients between the age of 40 and 65 years to take aspirin, and observed no myocardial infarctions. Followed by large randomized placebo-controlled trials in the 80s, the role of low-dose aspirin in the prevention of cardiovascular disease was established.[9] In the current era new benefits of aspirin continue to be discovered,

A B C D

Figure 1 – **(A)** Aspirin, in the form of salicin extracted from willow bark or -leaves, was already used by the ancient Egyptians, as recorded in the Ebers Papyrus roll. The Greek medical doctors (e.g. Hippocrates) later adopted this treatment. **(B)** In 1897, a Bayer company chemist Felix Hoffman, purified acetylsalicylic acid. **(C)** Aspirin tablets in an early (pre-1921) Bayer bottle. **(D)** The general practitioner Lawrence Craven was one of the first to systematically describe the protective effect of aspirin on myocardial infarction. With permission from respectively the Leipzig University Library, Bayer Healthcare, Eric E. Johnson and the Minnesota University Library.

for example in the prevention of cancer.[10] In this thesis, we investigate a new strategy to improve the effectiveness of aspirin: the reduction of blood pressure and morning platelet reactivity by taking aspirin at bedtime instead of on awakening.

BEDTIME ASPIRIN AND BLOOD PRESSURE

High blood pressure (hypertension) is an important risk factor for cardiovascular disease. It is believed to cause 22% of all heart attacks in western Europe.[11] Even small reductions of blood pressure (3 to 5 mmHg) decrease the risk of myocardial infarction and stroke in population-based studies.[12, 13] Although there are many different antihypertensive agents, the blood pressure goal of $\leq$140/90 mmHg is reached by only half of all hypertensive patients.[14] Thus, simple and effective interventions to improve blood pressure control could have a large impact on the incidence of cardiovascular disease.

Aspirin, a non-steroidal anti-inflammatory drug (NSAID), was originally thought to increase blood pressure, because it inhibits blood pressure lowering prostaglandin synthesis. However, in contrast to other NSAIDs, aspirin was shown not to increase blood pressure in a meta-analysis.[15] In recent studies aspirin was even associated with considerable reductions of blood pressure (5 to 7 mmHg), but only when taken at bedtime instead of on awakening.[16-18] An insight into the mechanism behind this remarkable time-dependent effect of aspirin on blood pressure was given by the ASPirin In Reduction of TENSION I (ASPIRETENSION I) study.[19] In this study, aspirin intake at bedtime compared with intake on awakening reduced parameters known to influence blood pressure, such as plasma renin activity and 24 hour cortisol, dopamine and norephinephrine excretions.[19] However, these studies were conducted in healthy participants, patients with untreated hypertension or pregnant women, all without a clear clinical indication for aspirin use.[20] Use of low-dose aspirin is only indicated when benefits outweigh the harms (bleeding). Until now, this is only the case for patients who are at high risk for or have established cardiovascular disease.[9, 20, 21] Yet, the potential blood pressure lowering effect of bedtime aspirin was never assessed in patients with established cardiovascular disease, who may also use concomitant antihypertensive drugs and who have more advanced atherosclerosis than patients in previous studies. In the ASPirin In Reduction of TENSION II (ASPIRETENSION II) study, described in **chapter 3**, we assessed whether aspirin intake at bedtime compared with intake on awakening also reduces blood pressure of patients already using aspirin for prevention of cardiovascular disease.

BEDTIME ASPIRIN AND MORNING PLATELET REACTIVITY

Platelets are small anucleated cells, of which large numbers (150-450 x 10^9/L) are present in the human blood.[22] Platelets have a lifespan of 7-10 days and new platelets are constantly produced by bone marrow megakaryocytes. To maintain a stable physiological concentration, new platelets are released at a rate of 10% per day. In normal conditions platelets circulate in the bloodstream in a resting state. However, where blood vessels are damaged, platelets are exposed to stimuli and get activated, change their shape and aggregate to form a hemostatic plug.[23, 24] This is beneficial where hemostasis is needed, but harmful when a plug forms on a ruptured or eroded atherosclerotic plaque and causes myocardial infarction or stroke.

Already in the 1980s, a circadian rhythm was observed in the frequency of acute cardiovascular disease, with the highest incidence during the morning hours (6 to 12 AM).[25] In a meta-analysis the excess risk of acute cardiovascular events during morning hours was estimated to be around 40%.[26] Also in patients treated with cardiovascular drugs such as platelet inhibitors and beta-blockers, recurrent events occur in a circadian pattern.[27] After the discovery of the morning peak in cardiovascular events it was shown that platelet reactivity also follows a circadian rhythm, with a peak of platelet reactivity during the morning (6-12 AM).[28-32] Given the important role of platelets in the development of acute cardiovascular events, it is reasonable to assume that the morning peak of platelet reactivity contributes to the morning peak of acute cardiovascular events.[33] If true, reduction of morning platelet reactivity might prevent arterial thrombosis during morning hours and thereby prevent a proportion of morning cardiovascular events. This may be achieved by intake of aspirin at bedtime instead of on awakening.

After intake, aspirin is rapidly absorbed by the stomach and small intestine, reaching its maximal concentration in blood already after 20 minutes. Thereafter, aspirin is rapidly de-acetylated and cleared from the circulation.[34] Aspirin inhibits platelet reactivity by inactivating the platelet cyclo-oxygenase-1 enzyme, thereby preventing the production of thromboxane A_2, a potent amplifier of platelet aggregation. Because platelets lack the DNA to renew the enzyme, cyclo-oxygenase-1 is inhibited by aspirin for the whole lifespan of a platelet (7-10 days). Still, aspirin has to be taken each day, because new platelets are released at a rate of 10% per day.[35] These newly released platelets are uninhibited by aspirin and are capable to produce thromboxane A_2.[35] It has been suggested that the presence of 10% uninhibited platelets is enough to abolish the effect of aspirin on platelet reactivity.[7, 36] Importantly, previous studies showed that 95% of all platelets have to be inhibited by aspirin to achieve an effective reduction of platelet reactivity.[37]

With a daily platelet turnover of 10%, this implies that only 90% of all platelets are inhibited by aspirin at the end of its dosing interval (24 hours after intake), whereas 95% inhibition is needed to achieve effective inhibition of platelet reactivity.[37] This is also supported by a recent study, which showed that platelet aggregation was insufficiently inhibited 24 hours after morning aspirin intake in 25% of the patients with established cardiovascular disease.[38] The majority of patients take their aspirin on awakening, which in most cases is after the start of the morning peak of platelet reactivity at 6 AM. Consequently, after morning aspirin intake, 10% of platelets are uninhibited just before the next morning intake 24 hours later. So, with aspirin intake on awakening, 10% uninhibited platelets are present during the morning hours, when the risk of cardiovascular events is the highest (Figure 2). Because it is desirable to achieve optimal inhibition of platelet reactivity during the high risk morning hours, it might be beneficial to take aspirin at bedtime instead of on awakening. By taking aspirin at bedtime, the proportion of uninhibited platelets during morning hours would theoretically be reduced to 5%, and thereby inhibition of platelet reactivity is more effectively achieved during morning hours (Figure 2).This was already suggested by previous authors[39-41], but for the first time we evaluate this in clinical trials, described in **chapter 3** and **4**.

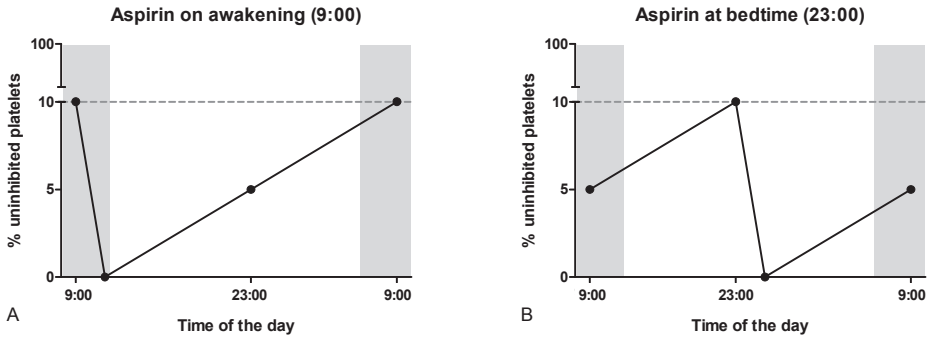

Figure 2 – Theoretical representation of the effect of aspirin intake on awakening (9:00h) or at bedtime (23:00h) on the proportion of uninhibited platelets during 24 hours. The grey shaded area represents the timing of the morning peak of cardiovascular events and platelet reactivity. Short after aspirin intake, all platelets are inhibited (0% uninhibited). Subsequently, new platelets are released at a rate of 10% per day. Due to aspirins short half-life, these newly released platelets are uninhibited. This implies that 10% uninhibited platelets are present 24 hours after aspirin intake, which is situated during the high risk morning hours when aspirin is taken on awakening (**panel A**). The proportion of uninhibited platelets during the morning hours is theoretically reduced to 5% by taking aspirin at bedtime (**panel B**). This might be beneficial, because it is known that 95% platelet inhibition is required to achieve a clinically effective reduction of platelet reactivity by aspirin.

AIMS AND OUTLINE OF THIS THESIS

The main aim of this thesis is to assess the effect aspirin intake on awakening compared with intake at bedtime on blood pressure and the morning peak of platelet reactivity. Both outcomes are particularly suitable to study with a cross-over study, which carries specific methodological advantages. **Chapter 2** gives an introduction to cross-over studies, which helps to understand the design and methodology of the studies in subsequent chapters. **Chapter 3** and **4** describe the results of two clinical trials, which were carried out to investigate the time-dependent effect of aspirin on blood pressure and platelet reactivity. To examine the effect of platelet reactivity on the risk of secondary cardiovascular events, we carried out a cohort study in men who survived a first myocardial infarction, which is described in **chapter 5**. Following international guidelines, patients with cardiovascular disease take several medications on a daily basis, of which beta-blockers are one of the most frequently prescribed. However, there is still debate about the effect of beta-blockers on platelet reactivity. By performing a systematic review and meta-analysis in **chapter 6** we synthesize all available evidence and estimate the magnitude of beta-blockers' effect on platelet reactivity. In **chapter 7** we summarize and discuss the results of the studies presented in this thesis.

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