Thromboxane Biosynthesis in Stroke and Post-stroke Dementia

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Druk: **Ridderprint offsetdrukkerij, Ridderkerk**

Thromboxane Biosynthesis in Stroke and Post-stroke Dementia

Thromboxaan biosynthese na een beroerte
en bij dementie na een beroerte

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector Magnificus

Prof.dr.ir. J.H. van Bemmel

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

woensdag 15 mei 2002 om 13.45 uur

door

Fop van Kooten

Geboren te Rotterdam

Promotiecommissie:

Promotor:	Prof.dr. P.J. Koudstaal
Overige leden:	Prof.dr. J. van Gijn Prof.dr. A. Hofman Prof.dr. P.R. Saxena

Financial support of the Stichting Neurovasculair Onderzoek Rotterdam is gratefully acknowledged.
The studies on thromboxane biosynthesis were supported by a grant from the European Union (BMH1-CT93-1533).
The studies on dementia were financially supported by a grant from the Netherlands Program for Research on Aging (NESTOR), funded by the Ministry of Education, Culture, and Sciences and the Ministry of Health, Welfare, and Sports.
Additional financial support was provided by Janssen-Cilag B.V.

Aan mijn ouders,
Inge, Jens & Sten

This thesis is based on the following manuscripts

Chapter 1 Partly based on: van Kooten F, Koudstaal PJ. Epidemiology of post-stroke dementia. *Haemostasis* 1998;28:124-133.

Chapter 2 van Kooten F, Ciabattoni G, Patrono C, Schmitz PIM, van Gijn J, Koudstaal PJ. Evidence for episodic platelet activation in acute ischemic stroke. *Stroke* 1994;25:278-281.

Chapter 3 van Kooten F, Ciabattoni G, Patrono C, Dippel DWJ, Koudstaal PJ. Platelet activation and lipid peroxidation in patients with acute ischemic stroke. *Stroke* 1997;28:1557-1563.

Chapter 4 van Kooten F, Li SC, Dippel DWJ, Ciabattoni G, Koudstaal PJ. Atrial fibrillation is associated with increased thromboxane biosynthesis in patients with acute cerebral ischemia. *Submitted.*

Chapter 5 van Kooten F, Ciabattoni G, Koudstaal PJ, Dippel DWJ, Patrono C. Increased platelet activation in the chronic phase after cerebral ischemia and intracerebral hemorrhage. *Stroke* 1999;30:546-549.

Chapter 6 van Kooten F, Bots ML, Breteler MMB, Haverkate F, van Swieten JC, Grobbee DE, Koudstaal PJ, Kluft C. The Dutch Vascular Factors in Dementia Study: rationale and design. *J Neurol* 1998;245:32-39.

Chapter 7 van Kooten F, Ciabattoni G, Koudstaal PJ, Grobbee DE, Kluft C, Patrono C. Increased thromboxane biosynthesis is associated with poststroke dementia. *Stroke* 1999;30:1542-1547.

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GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

Burden of stroke

With 25 to 30 thousand new patients per year and an incidence of 170/100.000, stroke is a major health problem in the Netherlands, as it is in other western countries. It accounts for almost 10% of the annual death in the Netherlands. Approximately 80% of stroke is of ischemic origin, and 20% cerebral hemorrhage. For the individual patient, the consequences of stroke are often devastating. Approximately 20% of the patients do not survive the first weeks and case fatality at 1 year is approximately 35%.[1] However, mortality strongly depends on type of stroke, and is higher in hemorrhagic than in ischemic cases. Of the survivors, only 65% is discharged home directly. Approximately 15% is discharged to a nursery home, and 20% to a stroke recovery or a rehabilitation center.[2] Even patients who are discharged to their own home are often not able to live fully independently, partly due to their physical handicap, but often also due to cognitive impairment. In approximately 25% of the cases, cognitive dysfunction is severe enough to the extent that patients fulfill all criteria of dementia.[3,4,5] Although the highest incidence of ischemic stroke is between the 6th and 7th decade of life, it is a disease of all ages. In subjects between 15 and 45 years of age, the incidence of ischemic stroke is between 6 and 15/100.000/year.[6,7,8] The mortality in this group of relatively young adults, is about 20% in the first 6 years after stroke and only less than half of the survivors is eventually able to perform a job.[9] Although the consequences of stroke are often dramatic for the individual patient, these figures also indicate the large burden of stroke on the community and the health care system. As a result of the high incidence of stroke, a relatively small reduction of a few percent of stroke or stroke recurrence would mean a large reduction in number of patients appealing to the health care system in absolute terms. Obviously, this makes every effort reducing the incidence or recurrence of stroke, even with only a few percent, more than worthwhile.

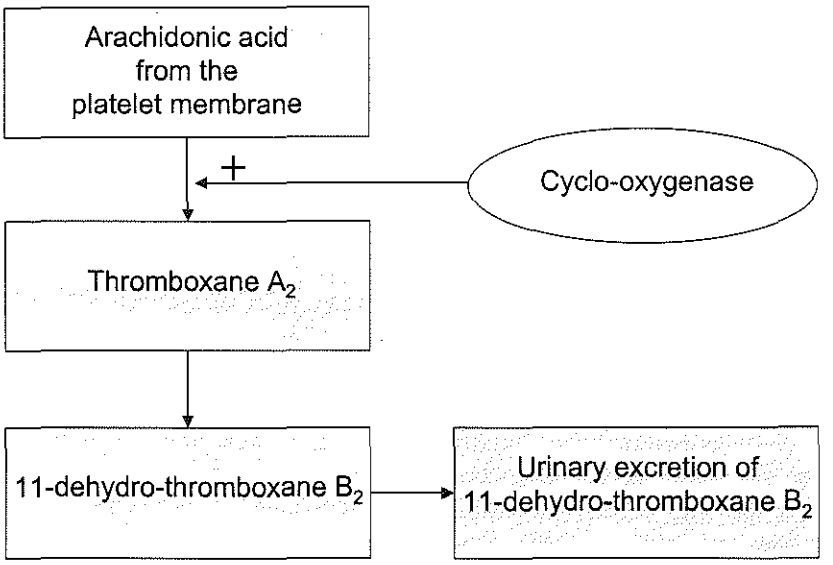

Figure 1.1 Schematic representation of thromboxane biosynthesis in activated platelets

Measures of platelet activation in cerebral ischemia

Since thromboembolism from extracranial arteries is widely regarded as one of the most common causes of transient ischemic attacks and ischemic strokes, platelet function in these conditions has received increasing attention in the past decades. In older studies, platelet hyperaggregability was reported after stroke,[10,11,12,13,14] in particular in young stroke patients.[10] Others attempted to study in vivo platelet activation by measuring blood levels of β-thromboglobulin.[15,16,17,18,19,20,21] β-thromboglobulin is a platelet-specific protein which is secreted from α-granules of activated platelets. However, the validity of these in vitro studies has been challenged.[22] A major disadvantage of this method is the possibility of ex vivo platelet activation that may occur during and after blood sampling.[23] This artifact may, at least partly, explain the inconsistent results that were found in the studies using β-thromboglobulin as a measure of platelet activation. The blood levels of β-thromboglobulin varied widely among

these studies, and they showed a considerable overlap with the levels of control patients. Another drawback of these studies was that they were not confined to the acute phase after the ischemic event.

Urinary 11-dehydro-TXB_2 as a measure of platelet function and other possible sources of thromboxane biosynthesis

Theoretically, the artifact of blood sampling can be avoided by measuring potential products of platelet activation that are excreted in the urine. A validated method measuring urinary metabolites of thromboxane A_2, was introduced in 1987 by Ciabattoni et al.[24] Thromboxane A_2 represents an amplification mechanism of platelet activation and platelet aggregation by virtue of its being synthesized and released in response to a variety of agonists (e.g., collagen, ADP, and thrombin) and in turn inducing platelet aggregation and further release of thromboxane A_2. Figure 1.1 represents a simplification of the thromboxane pathway in platelets. Catalyzed by the enzyme cyclooxygenase, thromboxane A_2 is formed from arachidonic acid of the membrane of activated platelets. Thromboxane A_2 is further metabolized, and one of its stable metabolites, 11-dehydro-TXB_2, is excreted in the urine. Although it is an elaborate and time-consuming procedure, 11-dehydro-TXB_2 can be measured accurately with a validated radioimmunoassay.[24]

Clearly, urinary 11-dehydro-TXB_2 excretion reflects in vivo thromboxane biosynthesis. Whether thromboxane biosynthesis reflects platelet activation depends on other potential sources of thromboxane production. In this respect, monocytes and macrophages are known to be potential sources of thromboxane synthesis by being able to express the enzyme cyclooxygenase-2 in response to inflammatory cytokines and growth factors.[25] In addition, transcellular biosynthesis of thromboxane A_2 may occur through the biochemical cooperation of cells expressing cyclooxygenase-2 (e.g., vascular endothelial cells) with aspirinated platelets.[26] However, cyclooxygenase-2 is less sensitive to suppression by aspirin than cyclooxygenase-1,[27] and low dose aspirin is probably able to suppress cyclooxygenase-1, but not cyclooxygenase-2 activity. Because platelets have no DNA, they are not able to produce new proteins, and are thus not able to replace the cyclooxygenase-1 enzyme blocked by aspirin. DNA containing cells are able to replace blocked cyclooxygenase enzymes and it is assumed that thromboxane production in DNA containing cells is less susceptible to suppression with low doses of aspirin. Therefore, we

consider that thromboxane synthesis that can be suppressed with low dose aspirin is of platelet origin, and cyclooxygenase dependent and thus reflects platelet activation.

Thromboxane biosynthesis in stroke

At the time we started our studies, enhanced thromboxane biosynthesis, as reflected by increased urinary excretion of thromboxane metabolites, had been reported in patients with unstable angina and acute myocardial infarction.[28,29,30] Koudstaal et al. reported increased thromboxane biosynthesis in patients with acute ischemic stroke.[31] This enhanced thromboxane biosynthesis could be largely suppressed by low dose of aspirin, which confirmed its platelet origin.[31] This study showed that it was feasible to measure enhanced thromboxane biosynthesis in stroke patients. The number of patients, however, was limited, and therefore, associations with cardiovascular risk factors and stroke characteristics could not be studied satisfactorily. In addition, no data were available concerning the dynamics of platelet activation in the first days after onset of symptoms. The presence of increased platelet activation in the acute phase of ischemic stroke would present a rationale for antiplatelet therapy in this setting.

Diagnosis of post-stroke dementia

As it is suggested that daily intake of aspirin is associated with a reduction of cognitive decline, both in normal and in demented subjects, we wanted to study the relationship between thromboxane A_2 biosynthesis and cognitive function and dementia. One of the major tasks was to establish the diagnosis of dementia in the study patients.

Diagnosis of dementia

The diagnosis of post-stroke dementia encompasses the diagnosis of dementia and the diagnosis of stroke. Whether a stroke patient is demented or a demented patient has had a stroke, are completely different problems, each emphasizing different aspects of the diagnosis. In demented patients, it may be difficult to find evidence for stroke, as stroke may have been clinically asymptomatic or simply forgotten by the patients and their families. It may be difficult to diagnose dementia in stroke patients due to somatic disorders caused by stroke which may interfere with cognitive evaluation. The most difficult task is to establish a direct relationship between dementia and stroke.

The French psychiatrist Pinel introduced the term dementia to refer to patients with intellectual deterioration and idiocy.[32] The concept has been changing ever since and only after the introduction of the DSM-III criteria,[33] was dementia operationally defined. For the diagnosis of dementia, memory impairment was an essential feature. In addition, impairment of one or more other cognitive domains was required, and the impairment had to be severe enough to interfere with social or occupational functioning. Bedside tasks, but not neuropsychological examination, were suggested to assess the cognitive abilities. These features are also required for the dementia diagnosis in the DSM-IV criteria,[34] however, neuropsychological assessment is also recommended to evaluate cognitive functions. Furthermore, the impairment in memory, cognitive, social, and occupational functioning should represent a decline from a previously higher level. The NINDS-AIREN criteria for dementia,[35] which were adopted from the ICD-10,[36] are different from the DSM-IV criteria in that two or more cognitive domains have to be impaired in addition to memory impairment, instead of one or more. The impairment of an additional cognitive domain is necessary to compensate for the impairment in a cognitive domain due to stroke. The definitions of dementia in the DSM-IV, ICD-10, and thus the NINDS-AIREN criteria have in common that they were mainly developed bearing Alzheimer's disease in mind. Memory impairment is an essential feature for the dementia diagnosis in these criteria. Therefore, it is still controversial whether they can be applied in patients with vascular dementia, because memory disturbance is not a universal finding in cognitive impairment due to vascular causes.[37] On the other hand, it has been shown that dementia diagnosis based on criteria which included memory disturbance could better identify patients at increased risk of adverse outcome following ischemic stroke.[38] In studies which compared different diagnostic criteria in patients admitted with suspected dementia, distinct groups of patients were identified using the various criteria,[39,40] even though the frequency of demented patients was similar in one study.[40] The ICD-10 and the DSM-IV criteria appeared more sensitive to diagnose vascular dementia than the NINDS-AIREN, which was more specific.[40] This makes the latter more suitable for research purposes and the former for clinical practice. The choice of a particular set of criteria appeared more useful demonstrating a vascular etiology for the diagnosis of vascular dementia than in excluding a vascular cause in suspected Alzheimer's disease.[39] In patients after ischemic stroke, the frequency of dementia was found to depend on the diagnostic criteria used. [4,38] The diagnosis based on the MMSE (cut off <24) and less restrictive neuropsychological paradigms, i.e. impairment in more than 2 cognitive domains (memory impairment not required), and functional impairment

seemed to over-diagnose dementia. Based on clinical judgement, dementia is probably underdiagnosed.[38]

Problems encountered when implementing dementia criteria in stroke patients

In the Dutch Vascular Factors in Dementia Study,[5] part of the studied population consisted of a consecutive series of patients from the Rotterdam Stroke Databank, a hospital-based stroke registry. In this study, pre-stroke cognitive function was assessed by a structured interview and the score on the Blessed Dementia Scale.[41] Between 3 and 9 months after stroke, cognitive function was assessed by neurological examination and a series of neuropsychological screening instruments, the MMSE,[42] the Geriatric Mental Status-organic scale (GMS),[43] and the Dutch version of the cognitive and self-contained part of the Cambridge Examination for Mental Disorders of the Elderly, the CAMCOG.[44] In patients with clinical suspicion of a dementia syndrome, extensive neuropsychological examination was carried out. The diagnosis of dementia was assessed by a diagnostic panel that consisted of a neuropsychologist, two neurologists, and a physician of the Rotterdam Stroke Databank, based on information of a close informant, the results of the neuropsychological evaluation, and the clinical impression on examination. For the diagnosis, the DSM-III-R criteria for dementia[45] were applied. One of the major problems we encountered was that only 300 (59%) of the 505 stroke patients over 54 years of age and alive 3 months after stroke could be included in the study. This is in accordance with previous large studies in which between 40 and 60% of ischemic stroke patients, alive at 3 months after stroke and fulfilling the age criteria, could be or were included.[3,4] Patients who were unfit for neuropsychological testing were excluded in these studies, for example patients with impaired consciousness or severe aphasia, the latter since valid nonverbal cognitive tests were lacking. By excluding patients with aphasia, however, the reported prevalence of dementia might be an underestimation. On the other hand, inclusion of mild-to-moderate aphasic patients may lead to an overestimation of dementia, because aphasia may interfere negatively with neuropsychological testing. The same applies for other neurological deficits such as hemianopia and neglect, disorders, which, in our experience, made it more difficult to perform neuropsychological testing. In patients with cognitive deterioration and severe somatic complaints leading to dependency, it is difficult to accurately discriminate between the cognitive and the somatic complaints as the most probable cause of deterioration in occupational and social functioning. This dilemma may have led to differences in the reported prevalence of dementia after stroke. The timing of

evaluation, 3-9 months after stroke, may have been too early for some of our patients, because they could still have been improving during this phase. Because the major recovery from stroke takes place during the first 3-6 months, and only small numbers of patients improve thereafter,[46,47] some authors state that only after 6 months an accurate judgement concerning cognitive function can be made.[48] This is supported by a study, which reported improvement in memory of patients tested 3 and 6 months after stroke.[49] Finally, despite a structured interview with a close informant and the use of the Blessed Dementia Scale to assess pre-stroke functioning, it is often difficult to assess whether cognitive deterioration has been present before onset of stroke, and if so, to assess its severity. Operational criteria concerning this part of the diagnosis have not been defined. However, pre-stroke dementia is not rare and has been reported in 8-16% of the patients in prospective stroke cohort studies,[3,50] where it has been systematically evaluated only in the latter study. In this study, pre-stroke dementia was defined as an IQCODE score[51] greater than 103, which was shown to have a diagnostic accuracy for dementia of 90.4%.[52] Pre-stroke dementia was present in 33 (16.3%) of 202 patients, of whom 1 had already been diagnosed as demented. Whether or not the diagnosis of pre-stroke dementia can be made accurately, it is clear that a substantial proportion of the patients with post-stroke dementia already had cognitive disturbances before stroke onset. Pre-stroke cognitive decline was reported in nearly 40% of another stroke cohort.[4] It is obvious that pre-stroke cognitive function should be evaluated as soon as possible after stroke, to reduce loss of information and eliminate the influence of post-stroke functioning on the scores.

Scope of this thesis

In this thesis, we addressed the following questions:

- What is the time course of platelet activation in the acute phase of stroke?
- Can platelet activation in the acute phase of stroke be adequately suppressed by aspirin?
- Which risk factors for stroke, which patient characteristics, and which stroke characteristics are associated with increased platelet activation?

- What is the relationship between platelet activation, atrial fibrillation and severity of symptoms in patients with acute ischemic stroke?

- Does increased platelet activation occur in the chronic phase after stroke?

- Is increased platelet activation in the chronic phase after stroke a risk factor for cognitive decline and dementia after stroke?

In our studies of urinary 11-dehydro-TXB_2 as a measure of platelet activation, we examined the time course of platelet activation in acute ischemic stroke (chapters 2 and 3), to provide a rationale for choice of treatment in the acute phase. In chapter 3, we also studied the relationship between platelet activation and lipid peroxidation, and the effect of aspirin on the production of both eicosanoids in acute ischemic stroke in order to clarify the pathway of isoprostane biosynthesis in this condition. The combined data of the acute phase studies were used to investigate the relationship between platelet activation, atrial fibrillation and the use of aspirin in patients with cerebral ischemia (chapter 4). In chapter 5, we describe platelet activation in the chronic phase after TIA, ischemic stroke, and primary intracranial hemorrhage in relation with vascular risk factors and stroke characteristics. In chapter 6, we describe the rationale and design of a large study on vascular factors in dementia in which the cohort described in chapter 7 was embedded. Finally, we studied thromboxane biosynthesis in relation to post-stroke dementia, since it was suggested that daily intake of aspirin is associated with a reduction of cognitive decline (chapter 7).

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2

PLATELET ACTIVATION IN ACUTE ISCHEMIC STROKE

CHAPTER 2

EVIDENCE FOR EPISODIC PLATELET ACTIVATION IN ACUTE ISCHEMIC STROKE

Summary

Background and purpose. Enhanced thromboxane biosynthesis has previously been reported in patients with acute ischemic stroke. In this study we examined the time course of thromboxane biosynthesis after the onset of symptoms in 13 patients with acute cerebral infarction.

Methods. We obtained 5 to 8 consecutive 6-hour urine samples from each of these 13 patients within the first 48 hours after onset of symptoms to study the dynamics of platelet activation in this setting. The urinary excretion of the major enzymatic metabolite of thromboxane B_2, 11-dehydro-thromboxane B_2, was measured by a previously validated radioimmunoassay. The excretion rate was compared with that of 20 control patients with nonvascular neurological diseases.

Results. Eleven (85%) patients had at least one value exceeding 2 SD of the control mean (251 pmol/mmol creatinine). The proportion of samples with an elevated 11-dehydro-thromboxane B_2 level was markedly similar in each of the eight 6-hour collection periods (mean, 52±8%; range, 40 to 67%). In 4 patients (31%) the excretion rate was elevated in all measurements obtained. In the 11 patients with enhanced thromboxane biosynthesis, no uniform pattern of changes over time in metabolite excretion emerged, with 3 patients having peak values at 0-12 hour, 3 at 12-24 hour, 3 at 24-36 hour and 2 at 36-48 hour. The level and dynamics of 11-dehydro-thromboxane B_2 excretion were related neither to the neurological symptoms nor to the type or site of the cerebral ischemia.

Conclusions. We conclude that episodes of platelet activation occur repeatedly during the first 48 hours after the onset of symptoms of an acute ischemic stroke. Given its apparent dynamic nature, this ongoing process may be amenable to pharmacologic modulation.

Introduction

Clinical and experimental studies suggest that platelets have a major role in the pathogenesis of cerebral ischemia.[1,2,3] In a previous study we reported enhanced thromboxane biosynthesis, reflected by the urinary excretion of a major thromboxane metabolite, 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) in half of the patients with ischemic stroke, and in one-third of patients with a transient ischemic attack.[4] Metabolite excretion could be largely suppressed by low-dose aspirin, suggesting its origin from platelets. However, the temporal relationship between increased thromboxane biosynthesis and onset of symptoms could not be assessed since in the majority of patients only single measurements of 11-dehydro-TXB_2 were obtained.

To study more precisely the dynamics of platelet activation after acute ischemic stroke, we have performed repeated measurements of urinary 11-dehydro-TXB_2 during the first 48 hours after onset of symptoms.

Patients and methods

Study patients. We prospectively studied 13 consecutive patients (8 men and 5 women; mean age, 67.8 ± 19.4 years) with acute ischemic stroke who were admitted to the Dijkzigt University Hospital of Rotterdam and were included in the Rotterdam Stroke Databank between February and July 1992. The University Hospital of Rotterdam is an area hospital with an urban population. This center has no selection criteria for the admission of stroke patients, but young stroke patients are referred more frequently than to the nonacademic centers in the region. All patients were screened according to a strict protocol that consists of a full neurological examination, standardized blood tests, chest X-ray, at least one and usually two CT-scans of the brain, duplex scanning of the carotid arteries, and cardiac workup including standard 12-lead electrocardiography, and depending on other findings, 24-hour ECG monitoring and echocardiography. The patients were examined within 24 hours after the onset of neurological symptoms. Nature and time course of the symptoms were recorded by means of a detailed check-list.[5] In patients with stroke in the carotid territory, the symptoms were further subdivided according to the presence of cortical signs (aphasia, dysgraphia, dyslexia, or hemianopia), or one of the following lacunar syndromes: pure motor hemiplegia, pure sensory stroke, or sensorimotor stroke.[6] Apart from the neurological history, the following vascular risk factors were recorded:

smoking habits, hypercholesterolemia (history of hypercholesterolemia and/or fasting total cholesterol level more than 8.0 mmol/l), hypertension (history of hypertension and/or systolic blood pressure more than 160 mm Hg and/or diastolic pressure more than 90 mm Hg, treated or not), diabetes mellitus (type I or II, treated or not), and a history of intermittent claudication, stable angina pectoris, prior myocardial infarction, or previous vascular surgery (carotid, coronary, or peripheral vascular surgery). Stroke severity was assessed by means of the score on the modified Rankin scale (the Oxford Handicap Scale)[7,8] on admission, and functional outcome by means of the score on this scale at 3-month follow-up.

The routine laboratory investigations included hemoglobin, hematocrit, leukocyte, erytrocyte and platelet counts, erythrocyte sedimentation rate, blood urea, creatinine, fasting cholesterol and glucose, liver enzymes, and syphilis serology. The CT-scans were reviewed by two neurologists, without knowledge of the clinical features or the results of the biochemical studies.

Control patients. We used the data from our previous study,[4] in which 11-dehydro-TXB_2 excretion was measured in 20 control patients (11 men and 9 women; mean age, 64.2 years; range, 41-85 years) with nonvascular neurological disorders, such as cerebral trauma, Parkinson's disease, epilepsy, or cervical spondylotic myelopathy, who were admitted to the same hospital. Urine was collected during the night as soon as possible after admission to the hospital.

Exclusions. Patients were excluded if they had been taking aspirin or other nonsteroidal anti-inflammatory drugs in the preceding 10 days. All other drugs were continued during the study period. Also excluded were patients requiring invasive investigations, in particular angiography, within the subsequent 48 hours, patients with possible vasculitis (erythrocyte sedimentation rate >40 mm in the first hour), renal disease (creatinine greater than 150 μmol/L), unstable angina pectoris (recent onset of class III-IV chest pain according to the Canadian Heart Association, in the absence of an increase in the MB fraction of plasma creatinine kinase), or hematuria.

Urine measurements. Six-hour samples of urine were collected during the first 48 hours after the onset of symptoms, starting as soon as possible after admission to the hospital. In each patient at least 5 six-hour samples were obtained. The volume of each urine sample was recorded, and the creatinine concentration was measured. The samples were frozen immediately after voiding and stored at -20°C until extraction. Immunoreac-

tive 11-dehydro-thromboxane B_2 was extracted from 10-ml aliquots of each coded urine sample (the pH was adjusted to 4.0 to 4.5 with formic acid) on SEP-PAK C18 cartridges (Waters Associates, Milford, Mass.) and eluted with ethyl acetate. The eluate was subjected to silicic acid column chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol). The overall recovery, as determined by the addition of 11-dehydro-[^{3}H]thromboxane B_2, averaged 80 ± 6%. Immunoreactive 11-dehydro-TXB_2 eluted from silicic acid columns was assayed at a final dilution of 1:30 to 1:1000, as described elsewhere.[9] The urinary excretion rate of 11-dehydro-thromboxane B_2 was expressed as picomoles/millimole of creatinine.

Table 2.1 Baseline Characteristics and Functional Outcome of Study Patients

Patient	Sex	Age (Y)	Modified Rankin		Stroke Subtype	Cardiovascular Risk Factors
			entry	3-month		
1	F	81	3	6	Cortical	HT
2	F	19	2	1	Cortical	Smoking, Stroke
3	M	69	5	2	Cortical	...
4	M	77	5	4	Lacunar	Smoking, HT, DM
5	F	64	2	4	Cortical	HT
6	M	85	5	5	Cortical	HT, AF, SE
7	F	94	4	4	Cortical	...
8	M	76	4	2	Cortical	AF, MI
9	M	58	5	4	Cortical	Smoking
10	M	61	5	6	Cortical	DM
11	F	79	2	1	Cortical	HT, AF, MI
12	M	73	5	4	Cortical	...
13	M	45	2	0	Lacunar	Smoking, HT

HT, hypertension; DM, diabetes mellitus; AF, atrial fibrillation; SE, systemic emboli; MI, myocardial infarction.

Statistical analysis. Percentages in independent groups were compared by the chi-square test (without correction for continuity). Fisher's exact test was used where necessary. Mean values in independent groups

were compared by the Mann-Whitney *U* test. All analyses were performed with the personal computer program STATA, version 3.0.

Results

The baseline characteristics of the study patients are detailed in table 2.1. No significant difference in age and gender existed between patients with cerebral ischemia and control patients. All patients had symptoms and signs related to the carotid artery territory, 2 had a lacunar syndrome. Only 3 patients had no identifiable vascular risk factors.

Table 2.2 Modified Rankin scale

Rankin	Description of Handicap
0	No symptoms
1	Minor symptoms, not interfering with lifestyle
2	Minor handicap: symptoms that lead to some restriction in lifestyle but do not interfere with the patient's capacity to look after himself
3	Moderate handicap: symptoms that significantly restrict lifestyle and prevent totally independent existence
4	Moderately severe handicap: symptoms that clearly prevent independent existence although not needing constant attention
5	Severe handicap: totally dependent patient, requiring constant attention, night and day
6	Death

Table 2.3 shows that 11 patients (85%) with ischemic stroke had at least one urinary 11-dehydro-TXB_2 value exceeding 2 SD of the control mean (251 pmol/mmol creatinine). In 45 of all 86 urine samples (52%) an elevated 11-dehydro-TXB_2 level was found.

Table 2.3 Individual urinary 11-dehydro-thromboxane B_2 excretion rates within the first 48 hours after onset of symptoms in patients with cerebral infarction

Patient	11-dehydro-thromboxane B_2, pmol/mmol creatinine, during consecutive collection periods in hours after onset of symptoms								No. of samples per patient	% of samples with increased metabolite excretion per patient
	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48		
1	...	300*	...	260*	329*	1151*	580*	282*	6	100
2	...	...	146	127	188	195	141	307*	6	17
3	162	148	186	236	248	106	226	167	8	0
4	174	184	134	170	...	207	127	108	7	0
5	272*	...	254*	346*	162	...	122	193	6	50
6	149	144	322*	518*	472*	...	591*	332*	7	71
7	277*	242	293*	489*	390*	345*	...	50	7	71
8	...	491*	309*	...	2002*	1906*	268*	343*	6	100
9	180	177	284*	212	180	185	150	115	8	13
10	624*	1094*	810*	614*	592*	618*	838*	680*	8	100
11	437*	347*	274*	292*	380*	...	...	...	5	100
12	150	...	361*	242	331*	452*	...	308*	6	67
13	...	261*	173	144	121	...	241	51	6	17
	9	10	12	12	12	9	10	12	← No. of samples per collection period	
	44	50	67	50	58	56	40	50	← % of samples with increased metabolite excretion per collection period	

*Values of urinary 11-dehydro-thromboxane B_2 excretion > 2 SD of controls. (> 251 pmol/mmol creatinine)

In 4 patients (31%) all 11-dehydro-TXB_2 measurements obtained were elevated, although with different time-related patterns (Figure 2.1). No relation was found between the level and dynamics of 11-dehydro-TXB_2 excretion and stroke severity on admission. Patients who showed repeated (at least two) episodes of enhanced thromboxane biosynthesis had a worse functional outcome (median Rankin scale score, 4) compared with patients who had no or single increase in urinary 11-dehydro-TXB_2 excretion (median Rankin scale score, 2). However, this difference was not statistically significant (P=0.11).

As detailed in table 2.3, the proportion of samples with an elevated 11-dehydro-TXB_2 excretion level was strikingly similar in each of the eight 6-hour collection periods and averaged 52±8% (range, 40% to 67%).

The peak 11-dehydro-TXB_2 excretion rate averaged 605±518 for the whole group and 674±537 pmol/mmol creatinine for the 11 patients with enhanced thromboxane biosynthesis. Of the 11 patients with enhanced thromboxane biosynthesis, 3 (27%) had their peak 11-dehydro-TXB_2 values at 0 to 12 hours, 3 (27%) at 12 to 24 hours, 3 (27%) at 24 to 36 hours, and 2 (19%) at 36 to 48 hours after the beginning of symptoms. In summary, there was no consistent pattern of changes over time in metabolite excretion.

Small differences in baseline characteristics, including laboratory findings, between patients with (n=8) or without (n=5) repeated elevation of 11-dehydro-TXB_2 levels included lower levels of creatinine (P=0.04), and fibrinogen (P=0.07) in patients with at least two elevated 11-dehydro-TXB_2 measurements. In addition, significantly fewer patients smoked in this group (P=0.004). The number of patients was too small for further exploration in a multivariate analysis.

Discussion

In this study we found that the vast majority (85%) of patients with acute ischemic stroke have at least one episode of enhanced thromboxane biosynthesis, as reflected by the urinary excretion of 11-dehydro-TXB_2, during the first 48 hours after the onset of symptoms. This prevalence is considerably higher than the 51% figure we found in a previous study.[4] This difference can be explained by the repeated instead of single urine sampling performed in the present study.

No uniform pattern of time-related changes in 11-dehydro-TXB_2 was observed. We found no correlation between the level of 11-dehydro-TXB_2 and stroke severity on admission. However, we did find that patients with prolonged or repeated episodes of enhanced thromboxane biosynthesis had a worse functional outcome than patients with no or a single elevation in 11-dehydro-TXB_2 excretion. The difference was not significant, probably because the groups were too small. If this finding is confirmed in larger studies, it suggests that the number and duration of episodes with enhanced thromboxane biosynthesis are more important than the level of 11-dehydro-TXB_2 with regard to stroke outcome.

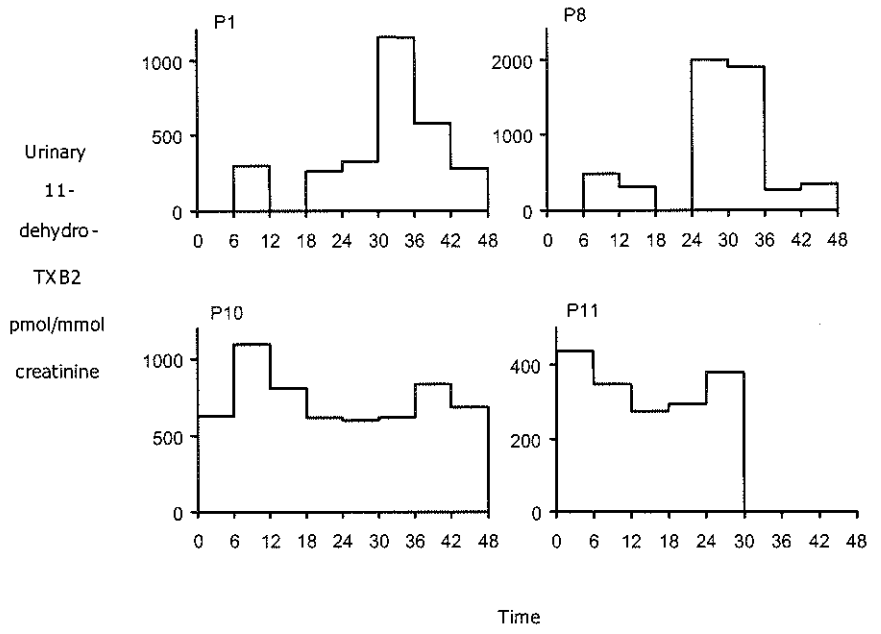

Figure 2.1 Line graphs show urinary 11-dehydro-thromboxane B_2 excretion levels in relation to time after the onset of symptoms in patients showing enhanced thromboxane biosynthesis in all measurements obtained.

Overall, these different individual patterns of enhanced thromboxane biosynthesis are consistent with our suggestion[4] that platelet activation occurs repeatedly during the first 48 hours after the onset of symptoms of an acute ischemic stroke. This suggestion is reinforced by the finding of elevated excretion of 11-dehydro-TXB_2 beyond 24 hours after the onset of symptoms in approximately half of the patients; given the 45 minutes half-life of this metabolite,[10] this would not be expected if thromboxane-dependent platelet activation occurred as a single episode, at the time of the event.

In a previous study[4] we have demonstrated that aspirin can profoundly suppress increased thromboxane biosynthesis. Together with our current finding that the majority of patients with acute cerebral ischemia show biochemical evidence of episodic platelet activation within the first 48 hours after the event, this may provide a rationale for testing the efficacy and safety of aspirin in this setting. A large multicenter trial (the International Stroke Trial) is currently under way to assess the value of antithrombotic

therapy in approximately 20,000 patients with acute ischemic stroke randomized within 48 hours after the onset of symptoms to receive aspirin, heparin, both or neither.[11]

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3

PLATELET ACTIVATION & LIPID PEROXIDATION IN ACUTE ISCHEMIC STROKE

CHAPTER 3

PLATELET ACTIVATION AND LIPID PEROXIDATION IN PATIENTS WITH ACUTE ISCHEMIC STROKE

Summary

Background and purpose. Both platelet activation and lipid peroxidation are potential sources of vasoactive eicosanoids that can be produced via the cyclooxygenase pathway, i.e. thromboxane (TX) A_2, or by free radical-catalyzed peroxidation of arachidonic acid, i.e. isoprostanes. We investigated the biosynthesis of TXA_2 and F_2-isoprostanes, as reflected by the urinary excretion of 11-dehydro-TXB_2 and 8-epi-prostaglandin (PG) $F_{2\alpha}$, respectively, in 62 consecutive patients (30 men, 32 women; mean age 67±14 years) with acute ischemic stroke.

Methods. At least two consecutive 6-hour urine samples were obtained during the first 72 hours after onset of symptoms. Urinary eicosanoids were measured by previously described radioimmunoassays.

Results. Repeated periods of enhanced thromboxane biosynthesis were found in 52% of patients. Urinary 11-dehydro-TXB_2 averaged 221±207 (mean±SD; n=197; range, 13 to 967) pmol/mmol creatinine in 30 patients treated with cyclooxygenase inhibitors (mostly aspirin) at the time of study, versus 392±392 (n=186; range, 26 to 2533) in 32 untreated patients (P<0.001). The corresponding values for 8-epi-$PGF_{2\alpha}$ excretion were 74±42 (range, 14 to 206) and 83±65 (range, 24 to 570) pmol/mmol creatinine (P>0.05). The correlation between the two metabolites was moderate both in untreated patients (r=0.41, P<0.001) and in patients with cyclooxygenase inhibitors (r=0.31, P<0.001). In a multiple regression analysis, increased thromboxane production was independently associated with severity of stroke on admission, atrial fibrillation, and treatment with cyclooxygenase-inhibiting drugs.

Conclusions. We conclude that during the first few days after an acute ischemic stroke (1) platelet activation occurs repeatedly in a cyclooxygenase-dependent fashion; (2) platelet activation is not associated with concurrent changes in isoprostane biosynthesis; (3) platelet activation is independently associated with stroke severity and atrial fibrillation; and (4) isoprostane biosynthesis is largely independent of platelet cyclooxygenase activity.

Introduction

Both platelet activation and lipid peroxidation are potential sources of vasoactive eicosanoids that can be produced via the cyclooxygenase pathway, i.e. thromboxane (TX) A_2, or by free radical-catalyzed peroxidation of arachidonic acid, i.e. isoprostanes. In previous studies, we reported enhanced thromboxane biosynthesis, reflected by the urinary excretion of a major thromboxane metabolite, 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) in patients with acute stroke.[1,2] Thromboxane production could be largely suppressed by low-dose aspirin, suggesting its cyclooxygenase dependent formation in platelets. The F_2-isoprostanes, a family of prostaglandin isomers, are free radical catalyzed products of arachidonic acid metabolism of which in vivo formation in human has first been reported by Morrow et al.[3] They are initially formed in situ on phospholipids, from which they are subsequently released, preformed presumably by phospholipases.[4] They circulate in plasma and are excreted in urine.[5,6] Among the F_2-isoprostanes, 8-epi prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$), is one of the most abundantly formed under physiological conditions.[5] It was shown to be a potent vasoconstrictive agent in animal models, both in renal[3,7] and in pulmonal vessels.[8,9] Furthermore, 8-epi-$PGF_{2\alpha}$ was found to cause platelet shape change but not aggregation.[10,11] Increased plasma levels of F_2-isoprostanes were found in smokers,[12] in patients with the hepatorenal syndrome,[13] and in patients with non-insulin dependent diabetes mellitus.[14] Increased urinary 8-epi-$PGF_{2\alpha}$ levels were found in healthy smokers,[15] after paracetamol overdose,[16] in patients treated with thrombolytic therapy for acute myocardial infarction,[16] and in hypercholesterolemia.[17] As F_2-isoprostanes are formed via free radical catalyzed peroxidation of arachidonic acid, it is suggested that an increased production may be a reflection of oxidative stress in these different clinical conditions. However, recent studies suggest that relatively small amounts of 8-epi-$PGF_{2\alpha}$ can also be formed in a cyclooxygenase-dependent manner.[16,18,19,20] Moreover, the metabolism in humans has been only incompletely characterized.[21]

In patients with ischemic stroke, both activation of platelet and monocyte cyclooxygenases,[20] and the free radical catalyzed pathway are potential sources of 8-epi-$PGF_{2\alpha}$ formation. Moreover, by its potential effect on vessel walls and platelets, this eicosanoid may have a negative influence on stroke outcome.

To investigate the actual rate of 8-epi-$PGF_{2\alpha}$ biosynthesis in vivo, we have studied the urinary excretion of this prostaglandin in patients with acute ischemic stroke. In addition, we related the production of 8-epi-$PGF_{2\alpha}$

to thromboxane biosynthesis, as reflected by urinary 11-dehydro-TXB_2 production. Finally, we evaluated the effect of cyclooxygenase inhibition on the production of both eicosanoids.

Subjects and methods

Study patients

We prospectively studied 62 consecutive patients (30 men and 32 women; mean age, 67±14 years) with acute ischemic stroke who were admitted to the Dijkzigt University Hospital Rotterdam and were included in the Rotterdam Stroke Databank between March and December 1994. The University Hospital Rotterdam is an area hospital serving an urban population. This center has no specific selection criteria for the admission of stroke patients; however, young stroke patients are referred more frequently to this center compared with the nonacademic centers in the region. All patients were screened according to a strict protocol consisting of a full neurological examination, standardized blood tests, at least one and usually two computed tomographic scans of the brain, duplex scanning of the carotid arteries, and a cardiac analysis that included standard 12-lead electrocardiography, and, if indicated, 24-hour electrocardiographic monitoring and echocardiography.

All patients were examined within 72 hours, and 42 of them within 24 hours after onset of neurological symptoms. In patients with stroke in the carotid territory, the symptoms were further subdivided according to the presence of cortical signs (aphasia, dysgraphia, dyslexia, or hemianopia) or one of the following lacunar syndromes: pure motor hemiplegia, pure sensory stroke, or sensorimotor stroke.[22] The computed tomographic scans were reviewed by at least two neurologists, without knowledge of the clinical features or the results of the biochemical studies. Cerebral infarctions were classified according to location and vascular territory.[23] Subcortical infarctions were further classified as small (≤15 mm) or large (>15 mm).

Apart from the neurological history, the following risk factors were recorded: smoking habits, hypercholesterolemia (history of hypercholesterolemia and/or fasting total cholesterol level over 6.5 mmol/L),[24] hypertension (history of hypertension and/or systolic blood pressure over 160 mm Hg and/or diastolic blood pressure over 90 mm Hg, treated or not), diabetes mellitus, (history of diabetes mellitus type I or II and/or a random blood glucose of 8.0 mmol/L or greater together with a HbA_{1c} level of 6.3% or more, treated or not),[25] atrial fibrillation (history of

atrial fibrillation and/or atrial fibrillation on ECG), and a history of intermittent claudication, angina pectoris, prior myocardial infarction, or prior vascular surgery (carotid, coronary, aorta bifurcation, or peripheral vascular surgery). We carefully recorded the medication that was used in the days prior to the stroke, and during the study period, distinguishing patients without antithrombotic or anticoagulant therapy, and those using cyclooxygenase inhibitors, heparin, oral anticoagulant therapy, or a combination of these. Patients with atrial fibrillation were heparinized and received oral anticoagulant treatment. In patients with aspirin for secondary prevention after stroke or myocardial infarction, treatment was continued during the study period. The other patients were included in the International Stroke Trial and were randomized for treatment with aspirin, heparin, both, or neither.[26] Stroke severity was assessed by means of the modified Rankin scale[27] on admission, and functional outcome by means of this scale at 3-months follow-up.

The routine laboratory investigations included hemoglobin, hematocrit, leukocyte, erythrocyte and platelet counts, erythrocyte sedimentation rate, blood urea, creatinine, fasting cholesterol and glucose, and liver enzymes.

Exclusions

Patients were excluded if they required invasive investigations, in particular angiography, during the study period. Also excluded were patients with vasculitis, renal disease (creatinine greater than 200 μmol/l), unstable angina pectoris (recent onset of class III to IV chest pain according to the Canadian Heart Association, in the absence of an increase in the MB fraction of plasma creatinine kinase), or macroscopic hematuria.

Urine measurements

Two to 8 six hour samples of urine were collected during the first 72 hours after the onset of symptoms, starting as soon as possible after admission to the hospital. The average delay between onset of symptoms and the start of urinary sampling was 14 ± 12 hours. The volume of each urine sample was recorded, and the creatinine concentration was measured. Samples of 50 mL, containing 1mM 4-hydroxy-TEMPO as an antioxidant, were stored in the refrigerator until they were frozen every six hours, and stored at -70°C until extraction. Analytical measurements related to eicosanoid biosynthesis were performed blindly to the pharmacologic treatments.

Immunoreactive 11-dehydro-TXB_2 and 8-epi-$PGF_{2\alpha}$ was extracted from 10-mL aliquots of each coded urine sample (the pH was adjusted to 4.0

with formic acid) on SEP-PAK C18 cartridges (Waters Associates, Milford, MA) and eluted with ethyl acetate. The eluates were subjected to silicic acid column chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol). The overall recovery averaged 74.5 ± 5.1 %. The eluates were dried, recovered with 5ml of buffer and assayed in the radioimmunoassay system at a final dilution ranging from 1:30 to 1:1000 for 11-dehydro-TXB_2 and 1:30 to 1:1000 for 8-epi-$PGF_{2\alpha}$.[28,29] The urinary excretion rate of 11-dehydro-TXB_2 and 8-epi-$PGF_{2\alpha}$ was expressed as picomoles per millimole of creatinine.

Validation of the 8-epi-$PGF_{2\alpha}$ assay in urine was provided by comparison of values obtained by TLC/EIA (using the same antibody used in the present study) with an independent analytical approach, NICI-GC/MS. An excellent correlation between the two methods was obtained: $r=0.99$, $n=9$, $p<0.001$. Moreover, 12 urine samples were extracted and measured by RIA using two different antisera with different cross-reactivities towards 8-epi-PGE_2 and similar values were obtained ($r=0.99$, $n=12$, $p<0.001$).[29]

Statistical analysis

Mean values between groups were compared using the Student's t-test. P-values <0.05 were considered statistically significant. For timeseries analysis, all potential prognostic variables were dichotomized by their medians, or trichotomized at the P33 and P67, when deemed appropriate. In an exploratory analysis, the relation between the levels of 8-epi-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 in consecutive urine samples and these variables was analyzed with analysis of variance for repeated measures, using the BMDP program 9d.[30] The difference between mean levels of 8-epi-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 for a certain variable was considered clinically meaningful, when there was a statistically significant difference ($p<0.05$; ANOVA) for at least 2 consecutive samples, or for a total of 4 samples in the series in the same direction. In a logistic regression analysis with stepwise forward selection of variables, we identified those factors that were independently related with repeatedly increased (i.e. more than once) 11-dehydro-TXB_2 levels.

Results

In the majority of patients (42) sampling started within 24 hours after stroke onset, and in 28 even within 12 hours. Two to 8 consecutive 6-hour urinary samples were obtained during the first 48 hours after admission. Thirty patients were treated with cyclooxygenase inhibitors; 26 used aspirin,

1 aspirin and the NSAID indomethacine, and 3 NSAIDs only, of whom 1 used ibuprofen, 1 diclofenac, and 1 naproxen. Three patients with NSAIDs were using this drug before onset of stroke and during the study it was continued. The patient on diclofenac started with this medication on admission. Of the 27 patients on aspirin, 13 were using it as secondary prevention before the onset of the current stroke, and it was continued during the study. Twelve patients started with aspirin at the beginning of the study, of whom 11 received it in accordance with the IST, and 1 after denying consent for randomization in the IST. In 2 patients who had received aspirin from the general practitioner and who denied consent for randomization in the IST, the medication was discontinued during the sampling period. Thus, a total of 17 patients were using antiplatelet medication before the start of the study, which was continued in 15, and stopped in 2, and 13 patients started with antiplatelet medication at the beginning of the study. No difference was found in excretion levels of both eicosanoids between patients who were on antiplatelet medication before the beginning of the study, and patients who started on admission nor such difference was found between patients on aspirin and patients on NSAIDs. Therefore all patients using cyclooxygenase inhibitors before and/or during the sampling period were compared to patients without cyclooxygenase inhibitors.

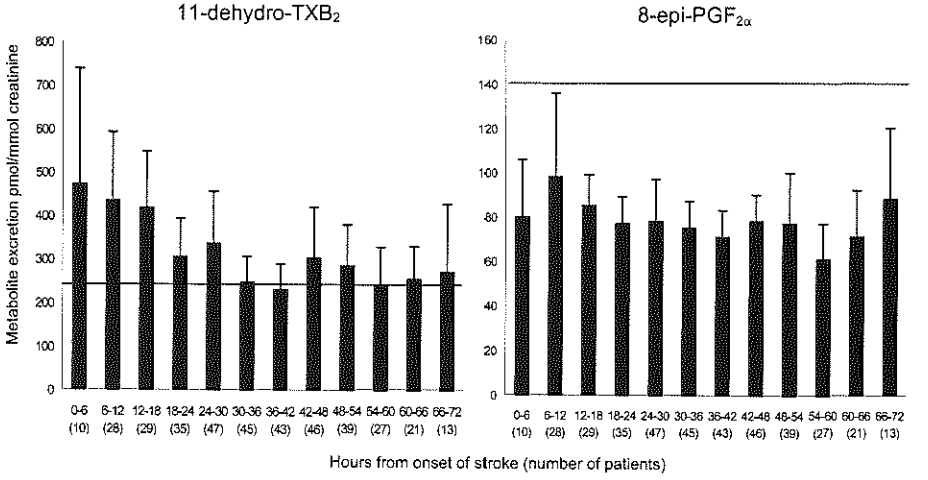

Figure 3.1 Mean values and 95% confidence intervals of urinary excretion of 11-dehydro-TXB_2 and 8-epi-$PGF_{2\alpha}$ for each time period in hours after onset of stroke. Line indicates the mean+2 SD of control subjects.

Compared with thromboxane excretion levels of controls from our previous study, mean+2SD=251 pmol/mmol creatinine,[1] 40 patients (65%)

had at least one sample with an increased excretion rate of 11-dehydro-TXB_2, while in 32 (52%) increased metabolite excretion was found repeatedly. For patients treated with cyclooxygenase inhibitors at the time of study, the corresponding percentages were 53% and 40% respectively. In untreated patients these percentages were higher (P=0.1), 75% and 63%, respectively.

In 30 patients treated with cyclooxygenase inhibitors, mostly 300 mg of aspirin daily, 197 samples were obtained. Urinary 11-dehydro-TXB_2 averaged 221±207 pmol/mmol creatinine (mean±SD, range:13-967) in these samples. In 186 samples of 32 untreated patients 11-dehydro-TXB_2 averaged 392±392 (range:26-2533). The difference was statistically significant ($p<0.001$). No statistically significant difference was found in the levels of 8-epi-$PGF_{2\alpha}$, with mean values of 74±42 (range:14-206) in patients on cyclooxygenase inhibitors, and 83±65 (range:24-570) in untreated patients.

Values of 8-epi-$PGF_{2\alpha}$ were only modestly correlated to 11-dehydro-TXB_2 excretion with a coefficient of 0.40 ($P<0.001$). The correlation coefficient was 0.35 in patients treated with cyclooxygenase inhibitors, and 0.41 ($P<0.001$), in untreated patients.

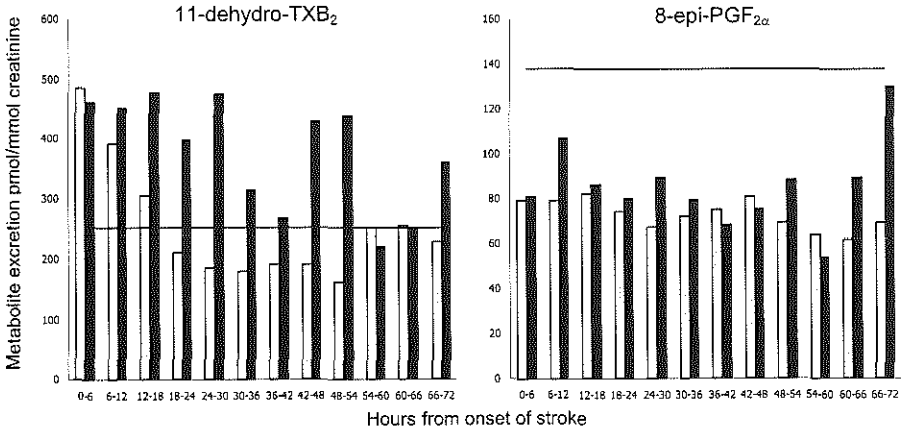

Figure 3.2 Mean values of urinary excretion of 11-dehydro-TXB_2 and 8-epi-$PGF_{2\alpha}$ for patients with and patients without cyclooxygenase inhibitors for each time period in hours after onset of stroke. Line indicates the mean +2 SD of control subjects. Gray bars indicate with cyclooxygenase inhibition and black bars without.

Figure 3.1 shows mean values and 95% confidence intervals of both eicosanoids of all patients for each time period. The level of 8-epi-$PGF_{2\alpha}$ fluctuated around 80 pmol/mmol creatinine. No consistent pattern was found

in its excretion over time. In contrast, excretion of 11-dehydro-TXB_2 was increased in the first period after stroke onset, with a subsequent decline thereafter. Taking medication into account, most of this decline could be attributed to cyclooxygenase inhibition. As shown in Figure 3.2, patients treated with cyclooxygenase inhibitors had a swift and constant decrease in 11-dehydro-TXB_2 excretion to normal values in the first 24 hours after stroke onset. Metabolite excretion remained constant in the subsequent periods. In contrast, patients without cyclooxygenase inhibition had repeatedly increased values of 11-dehydro-TXB_2 excretion. No difference was found in 8-epi-$PGF_{2\alpha}$ excretion between patients with and without cyclooxygenase inhibitors.

Table 3.1 Maximum 8-epi-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 production for demographic characteristics and cardiovascular risk factors

Characteristic	N	8-epi-$PGF_{2\alpha}$	11-dehydro-TXB_2
Age, years			
< 71	32	112±96	466±516
≥ 71	30	104±44	604±487
Sex			
Male	30	101±41	466±531
Female	32	116±97	596±475
Cardiovascular risk factors			
Smoking	23	122±105	553±577
Hypertension	51	104±46	515±453
Diabetes mellitus	11	118±40	501±355
Hypercholesterolemia	16	98±31	433±432
Intermittent claudication	3	74±32	1087±1205
Angina pectoris	8	126±49	662±521
Atrial fibrillation	13	152±132	1001±735‡
Congestive heart failure	6	133±55	1098±748†
History of cardiovascular disease			
Myocardial infarction	6	106±42	251±276
Stroke	12	102±50	509±338

Values are mean±SD; N is number of patients
† .1>P>.05, and ‡ .05≥P>.01, Student's *t* test.

In a first approach to evaluate univariately the relation between baseline characteristics, risk factors, stroke characteristics, and medication

on the one hand, and level of eicosanoids on the other, we explored differences in peak eicosanoid excretion.

Table 3.1 shows the mean value ± SD of peak eicosanoid excretion for demographic characteristics and cardiovascular risk factors. Higher values of 11-dehydro-TXB were found in patients with atrial fibrillation and in patients with congestive heart failure. For 8-epi-$PGF_{2\alpha}$ no differences in excretion rates were found. Table 3.2 shows the mean ± SD of peak eicosanoid excretion for stroke characteristics and medication. Patients with a cortical syndrome had higher peak values of 11-dehydro-TXB_2 than patients with a lacunar stroke or a stroke confined to the vertebro-basilar territory. Higher peak values of 11-dehydro-TXB_2 were also found in patients with severe strokes. Patients using cyclooxygenase inhibiting drugs had significantly lower peak values of 11-dehydro-TXB_2. Again, for 8-epi-$PGF_{2\alpha}$ no differences in excretion rate were found.

As a second step, we performed the timeseries analysis described in the methods section. In this analysis, 8-epi-$PGF_{2\alpha}$ levels were found to be elevated in patients with atrial fibrillation. Higher levels of 11-dehydro-TXB_2 were found in patients with a history of intermittent claudication, atrial fibrillation, cortical infarctions, severe strokes, poor stroke outcome, and in patients with a urinary catheter. Patients with a history of myocardial infarction and patients using cyclooxygenase inhibitors had lower values of 11-dehydro-TXB_2. Because patients with a urinary catheter had increased thromboxane metabolite levels, we examined whether this could be explained by urinary tract damage with subsequent hemorrhage and platelet activation. None of the patients had macroscopic hematuria. Furthermore, no relation was found between level of microscopic hematuria and level of 11-dehydro-TXB_2.

In a multiple logistic regression analysis, repeatedly increased 11-dehydro-TXB_2 levels were related to severity of stroke on admission, presence of atrial fibrillation, and antiplatelet treatment. Coumarin and heparin treatment was not associated with repeatedly increased 11-dehydro-TXB_2 levels.

Finally, repeatedly increased thromboxane metabolite excretion was not a statistically significant independent prognostic factor for outcome when added to stroke syndrome or stroke severity in a multiple logistic regression model, probably because of the rather small size of this study.

Table 3.2 Maximum 8-epi-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 production for stroke characteristics and medication

Characteristic	N	8-epi-$PGF_{2\alpha}$	11-dehydro-TXB_2
CT scan findings			
No infarction	22	92±47	592±562
Large cortical or subcortical infarction	27	125±101	566±534
Border zone infarction	3	142±28	605±340
Lacunar infarction	10	90±34	292±229
Large or borderzone infarction	30	127±96	570±514
No leukoaraiosis	53	111±79	539±526
Leukoaraiosis	9	93±49	510±369
Clinical stroke syndrome			
Cortical syndrome	42	120±86	633±563†
Lacunar syndrome	17	87±34	350±256†
Vertebrobasilar ischemia	3	68±39	161±58
Stroke severity			
Rankin < 4 at baseline	34	109±92	400±458‡
Rankin ≥ 4 at baseline	28	107±49	693±517‡
Stroke outcome			
Rankin < 4 at 3 months	41	105±84	491±570
Rankin ≥ 4 at 3 months	21	115±54	613±224
Medication			
No medication	20	108±53	547±448
CCO inhibitors only	20	96±48	376±306
Heparin only	9	151±159	922±907
OAC only	2	90±23	708±124
CCO inhibitors and heparin	9	106±49	356±271
CCO inhibitors and OAC	1	65	435
OAC and heparin	1	84	1214
Any CCO inhibitors	30	98±47	372±286‡
Any heparin	19	126±114	669±704
Any OAC	4	82±18	766±333

CCO indicates cyclooxygenase; OAC, oral anticoagulant. Values are mean±SD.

† .1>P>.05, and ‡ .05≥P>.01, Student's *t* test.

Discussion

In this study, 65% of patients with acute ischemic stroke had at least one period of enhanced thromboxane biosynthesis, while in half of the patients this occurred repeatedly. This is slightly less than the 85% and 61%

respectively we found in our previous study.[2] However, in the current study almost half of the patients received cyclooxygenase inhibitors. For untreated patients the results were in accordance with our previous findings.

In contrast, levels of 8-epi-$PGF_{2\alpha}$ seemed rather low with an average of approximately 80 pmol/mmol creatinine. Although no controls were used in this study, normal values of 8-epi-$PGF_{2\alpha}$ of 50.7±5.7 and 70.5±33.9 pmol/mmol creatinine were reported in other studies.[16,19] We found only a weak correlation between levels of 11-dehydro-TXB_2 and 8-epi-$PGF_{2\alpha}$. Whereas levels of 11-dehydro-TXB_2 decreased in time in patients with cyclooxygenase inhibiting drugs, no changes in urinary 8-epi-$PGF_{2\alpha}$ excretion were found in these patients. Although some studies suggests that 8-epi-$PGF_{2\alpha}$ can be formed in a cyclooxygenase dependent fashion from human platelets,[16,18] our results indicate that in a clinical condition where platelets are clearly activated, there are no concurrent changes in 8-epi-$PGF_{2\alpha}$ formation and excretion. Moreover, no significant differences in 8-epi-$PGF_{2\alpha}$ were found between patients with and without cyclooxygenase inhibiting drugs, again indicating that levels of 8-epi-$PGF_{2\alpha}$ found in patients with acute ischemic stroke are probably not produced in a cyclooxygenase dependent fashion. In univariate analysis, no factors that were found to be associated with 11-dehydro-TXB_2 production, were also related with 8-epi-$PGF_{2\alpha}$ excretion. Only in patients with atrial fibrillation was 8-epi-$PGF_{2\alpha}$ found increased on 2 consecutive periods in the timeseries analysis.

Increased levels of 8-epi-$PGF_{2\alpha}$ excretion were found after thrombolytic therapy in patients with acute myocardial infarction.[16] These findings suggest that the source of 8-epi-$PGF_{2\alpha}$ under these conditions is free radical catalyzed lipid peroxidation, associated with occlusion-reperfusion. Enhanced formation of 8-epi-$PGF_{2\alpha}$ might be expected in patients with acute stroke, a condition in which formation of free radicals occurs. However, in our patients, no evident peaks of 8-epi-$PGF_{2\alpha}$ excretion could be detected. In stroke patients it is difficult to predict if, and when reperfusion occurs. Still, we should have detected significant changes in 8-epi-$PGF_{2\alpha}$ excretion, if they occurred, since we collected 6 hour samples of urine during the time in which reperfusion might be expected in the majority of patients.[31] Alternatively, increased oxidant stress might occur as an early transient event, largely missed by the timing of our urine sampling or else, the signal-to-noise ratio might be to small to be detected at a distance from its source.

Increased 11-dehydro-TXB_2 excretion was univariately associated with a history of intermittent claudication or atrial fibrillation, the presence of a urinary catheter, cortical infarctions, severe strokes and worse outcome. A urinary catheter may induce urinary tract damage with subsequent

hemorrhage and possibly platelet activation. In our study, a urinary catheter was indeed associated with enhanced 11-dehydro-TXB_2 level, but also with stroke severity and outcome. In our ward, patients with severe strokes are always given a urinary catheter, which explains the relation with severity and outcome. None of the patients had macroscopic hematuria and no association was found between the level of microscopic hematuria and the level of urinary 11-dehydro-TXB_2. Therefore, it is unlikely that urinary 11-dehydro-TXB_2 levels were the result of urinary tract damage.

The other factors associated with increased thromboxane production, that is, atrial fibrillation, large subcortical and cortical infarctions, stroke severity and outcome, and the absence of cyclooxygenase inhibitors, may reflect the fact atrial fibrillation is more likely to cause large cortical infarctions which are mostly severe. Moreover, patients with atrial fibrillation usually receive heparin and coumarin treatment, and not cyclooxygenase inhibitors. However, in a multiple logistic regression analysis, atrial fibrillation, stroke severity, and cyclooxygenase inhibition were independently related to the level of urinary 11-dehydro-TXB_2.

An important remaining question is whether there exists a causal relationship between the extent and duration of platelet activation, as reflected by the level of 11-dehydro-TXB_2 excretion, and stroke severity and outcome. In this study, repeatedly increased thromboxane production was not a statistically significant independent prognostic factor for outcome when added to stroke syndrome or stroke severity in a multiple logistic regression model, probably because of the rather small size of our study. However, the results of trials that evaluate the value of antiplatelet therapy in acute ischemic stroke, of which the International Stroke Trial[26] and the Chinese Acute Stroke Trial[32] are by far the largest, may contribute to define the role of platelet activation in this setting. Preliminary results of these studies indicate that aspirin has a beneficial effect, albeit small, in acute ischemic stroke.[32,33]

We conclude that during the first few days after an acute ischemic stroke: 1) platelet activation occurs repeatedly in a cyclooxygenase-dependent fashion; 2) platelet activation is not associated with concurrent changes in F_2-isoprostane biosynthesis; 3) platelet activation is independently associated with stroke severity and atrial fibrillation; 4) F_2-isoprostane biosynthesis is largely independent of platelet cyclooxygenase activity.

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4

THROMBOXANE BIOSYNTHESIS AND ATRIAL FIBRILLATION IN ACUTE ISCHEMIC STROKE

CHAPTER 4

ATRIAL FIBRILLATION IS ASSOCIATED WITH INCREASED THROMBOXANE BIOSYNTHESIS IN PATIENTS WITH ACUTE CEREBRAL ISCHEMIA

Summary

Background and purpose. Patients with atrial fibrillation (AF) have a worse prognosis after acute ischemic stroke than patients without AF. In patients with cerebral ischemia and AF, aspirin seems to have a stronger protective effect against recurrent stroke than in patients without AF. We have studied the relationship between AF, aspirin treatment and thromboxane biosynthesis in the acute phase after cerebral ischemia.

Methods. Data from 3 of our previous studies on in vivo thromboxane biosynthesis after cerebral ischemia were combined. In these studies, we had obtained urinary samples from 131 patients in the acute phase after a transient ischemic attack (TIA) or ischemic stroke. The urinary excretion of the major enzymatic metabolite of TXA_2, 11-dehydro-TXB_2, was measured by means of a previously validated radioimmunoassay.

Results. Atrial fibrillation was present in 17 (13%) of the patients. The median (range) 11-dehydro-TXB_2 excretion in urine was 645 (37-2533) pmol/mmol creatinine in patients with AF, against 282 (32-5916) in patients with sinus rhythm ($P<0.001$). In a multiple linear regression analysis, AF and stroke severity were associated with increased urinary 11-dehydro-TXB_2 excretion, whereas use of aspirin was associated with decreased metabolite excretion. Use of aspirin was associated with nearly normal values in patients without AF, but in patients with AF metabolite excretion remained increased.

Conclusions. Patients with TIA or ischemic stroke and AF have significantly higher urinary excretion rates of 11-dehydro-TXB_2, as a measure of increased platelet activation, than those without AF. Aspirin significantly suppresses thromboxane biosynthesis both in patients with or without AF, but suboptimally in the former.

Introduction

Increased urinary excretion of 11-dehydro-TXB_2, which reflects in vivo platelet activation, was found in the acute phase,[1,2,3] and in the chronic phase[4] after ischemic stroke. Antiplatelet medication was, as expected, associated with a lower excretion level. However, the number of patients in each of these studies was small and did not allow firm conclusions as to the role of atrial fibrillation and its interaction with antiplatelet medication.

Patients with AF have a case fatality that is approximately twice as high as the case fatality in patients without AF.[5] Although in the International Stroke Trial (IST) the most common cause of early death in patients with AF was neurological damage from the initial stroke, in this study, slightly more patients with AF died from a fatal recurrent stroke of ischemic or unknown type. In previous studies in patients with AF the relative risk reduction of stroke and other major vascular events by aspirin was 22%,[6] whereas in patients with TIA or minor stroke of presumed vascular origin, the relative risk reduction by aspirin in any dosage above 30 mg was only 13%.[7] These findings suggest that platelet activation may play an even more important role in stroke patients with AF than in those without.

We combined data from 3 of our previous studies on in vivo thromboxane biosynthesis to investigate the relationship between AF, aspirin treatment and thromboxane biosynthesis in the acute phase of ischemic stroke.

Patients and methods

Study patients

We combined data of 3 studies, of which the methods are described in detail elsewhere.[1,2,3] The studies were performed in patients with acute ischemic stroke, admitted within 48 hours after onset. They were performed on the same neurological ward of the same hospital by the same investigators, and the methods used were nearly identical. Patients with TIA or ischemic stroke were recruited from the Rotterdam Stroke Databank, which is a register of stroke patients from the University Hospital Rotterdam. In all patients, the CT-scan findings were compatible with the diagnosis of cerebral ischemia. Patients with a cerebral infarction were subdivided according to a clinical classification: Total anterior circulation stroke, partial anterior circulation stroke, posterior circulation stroke and lacunar stroke.[8] Stroke severity was recorded by means of the modified

Rankin scale.[9] Apart from the neurological history, the following risk factors were recorded: smoking habits, hypercholesterolemia (history of hypercholesterolemia and/or fasting total cholesterol level >6.5 mmol/L),[10] hypertension (history of hypertension and/or systolic blood pressure >160 mm Hg and/or diastolic blood pressure >90 mm Hg, treated or not), diabetes mellitus (history of diabetes mellitus type I or II and/or a random blood glucose of ≥8.0 mmol/L together with an HbA1c level of ≥6.3%, treated or not),[11] AF (history of AF and/or AF on electrocardiogram), and history of intermittent claudication, angina pectoris, prior myocardial infarction, congestive heart failure and prior ischemic stroke. Use of antiplatelet medication at the time of urinary sampling was recorded in all studies.

Exclusions

Patients were excluded from the studies when invasive investigations were performed during, or in the days before the collection of urinary samples. In addition, patients with a possible vasculitis (ESR >40 mm in the first hour), renal disease (serum creatinine exceeding 150 mmol/l), unstable angina pectoris, and/or hematuria were excluded.

Control patients

11-dehydro-TXB_2 was measured in 20 control patients (11 men and 9 women; mean age, 64.2 years; range, 41-85 years) with non-vascular neurological disorders, such as minor cerebral trauma, Parkinson's disease, epilepsy, or cervical spondylotic myelopathy, who were admitted to the same hospital. Urine was collected during the night as soon as possible after the patient's admission to the hospital.[1]

Measurement of urinary 11-dehydro-TXB_2 excretion

All urine samples were immediately frozen after voiding and stored at -20 C until extraction. Immunoreactive 11-dehydro-TXB_2 was extracted from 10-mL aliquots of each coded urine sample (the pH was adjusted to 4.0 with formic acid) on SEP-PAK C18 cartridges (Waters Associates) and eluted with ethyl acetate. The eluates were subjected to silicic acid column chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol). Immunoreactive 11-dehydro-TXB_2 eluted from silicic acid columns was assayed at a final dilution of 1:30 to 1:1000, as described previously.[12] The urinary excretion rate of 11-dehydro-TXB_2 was expressed as picomoles per millimole of creatinine.

Baseline urine samples were taken as soon as possible after admission. In our first study,[1] a second sample was taken 24 hours hereafter

in the first 25 patients with cerebral ischemia. In 11 patients using aspirin, additional overnight urine samples were collected on the third, fifth and seventh day of treatment with 50 mg of aspirin. In the other studies[2,3] additional samples were taken repeatedly every six hours after baseline sampling.

Statistical analysis

Data were analyzed by means of STATA statistical software. The Student's *t* test was used to compare the urinary 11-dehydro-TXB_2 excretion between subgroups. P-values <0.05 were considered statistically significant. We considered the maximum 11-dehydro-TXB_2 excretion as most representative. The difference in the mean of 11-dehydro-TXB_2 excretion between groups was estimated with a 95% confidence interval. Multiple linear regression analysis was used to access the relationship between AF and urinary 11-dehydro-TXB_2 excretion after adjustment for confounding factors.

Results

AF was present in 17 (13%) of the 131 stroke patients. Table 4.1 shows the baseline characteristics for all patients in relation to the presence of AF. Patients with AF were older and more often had a history of congestive heart failure. They also had more often had a severe stroke syndrome.

Figure 4.1 depicts the individual measurements of urinary 11-dehydro-TXB_2 for patients with or without AF as a function of antiplatelet therapy. Patients with AF had significantly higher urinary 11-dehydro-TXB_2 excretion (median 645, range 37-2533) than those without (median 282, range 32-5916, $p<0.001$). Mean metabolite excretion values were lower in patients who were treated with aspirin. In patients without AF, these values were nearly in normal range, whereas in patients with AF the values remained far above normal values.

Table 4.1 Baseline characteristics of the study population, for patients with and without AF. (Number of patients, percentage between brackets)

Characteristic	With AF 17 (13)	Without AF 114 (87)	All Patients 131
Demographics			
Age (mean±SD) (years)	75±12	64±15‡	65±15
Male sex	7 (54)	66 (58)	73 (56)
11-dehydro-TXB_2 (median, range)	645 (37-2533)	282 (32-5916)‡	307 (32-5916)
Stroke characteristics			
TIA	0	19 (17)	19 (15)
Severe stroke (Rankin score >3)	12 (71)	32 (28)†	44(34)
Cortical infarction	11 (65)	60 (53)	71 (54)
Lacunar infarction	2 (11)	32 (28)	34 (26)
Risk factors			
Hypertension	9 (53)	46 (40)	55 (42)
Hyperlipidemia	0	6 (5)	6 (5)
Diabetes mellitus	1 (6)	7 (6)	8 (6)
Smoking	4 (24)	45 (39)	49 (38)
History			
Previous stroke	4 (24)	14 (12)	18 (14)
Angina pectoris	3 (18)	11 (10)	14 (11)
Congestive heart failure	4 (24)	4 (4)†	8 (6)
Myocardial infarction	2 (12)	11(10)	13 (9)
Intermittent claudication	2 (12)	4 (4)	6 (5)
Antiplatelet medication	5 (29)	25 (22)	30 (23)

† $P \leq 0.001$; ‡ $0.05 > P \geq 0.01$

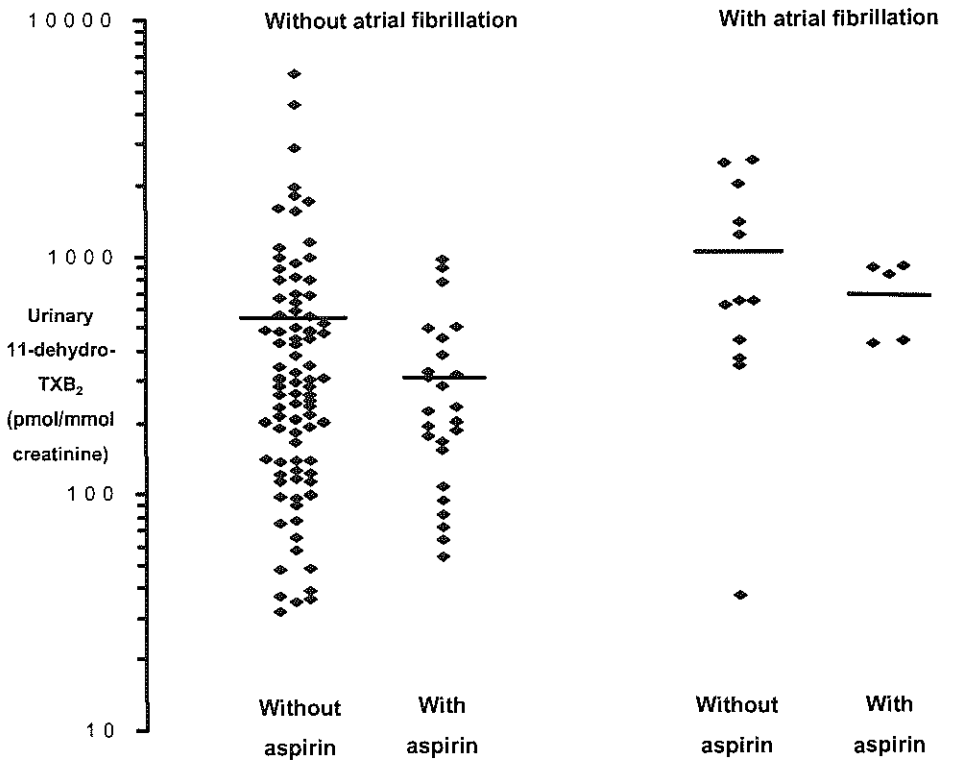

Figure 4.1 Individual urinary 11-dehydro-TXB_2 excretion rates depicted on a logarithmic scale for patients with sinus rhythm and AF, as a function of aspirin therapy. The horizontal bars represent mean values for each subgroup of patients.

In a multiple linear regression analysis, in which all factors from table 4.1 were taken into account, AF and stroke severity were associated with increased urinary 11-dehydro-TXB_2 excretion, as shown in table 4.2. AF and severe stroke increased the urinary metabolite excretion with approximately 350 pmol/mmol creatinine. No interaction could be demonstrated between antiplatelet effect and AF.

Table 4.2 Association of urinary 11-dehydro-TXB_2 levels with AF, adjusted for confounding factors by multiple linear regression analysis

	Δ urinary 11-dehydro-TXB_2 excretion (pmol/mmol creatinine)	95% CI
Atrial fibrillation	341	-45 to 727
Antiplatelet medication	-258	-542 to 25
Stroke severity (Rankin)	382	108 to 656

Discussion

The main finding of this study is that patients with TIA or ischemic stroke and AF had significantly higher urinary excretion rates of 11-dehydro-TXB_2, as a measure of increased platelet activation, than those without AF. Severity of stroke symptoms was another factor associated with increased platelet activation. Since AF most often leads to cortical or large subcortical infarctions, symptoms are expected to be more severe in patients with AF than in those without. Even after adjustment for stroke severity, AF remained an important determinant of 11-dehydro-TXB_2 excretion.

Our results are consistent with the clinical findings that antiplatelet medication reduces the risk of vascular events in AF patients with, and without stroke.[6] The results also suggest that aspirin treatment may be beneficial in patients with AF in the acute phase of stroke. The finding that the relative risk reduction of stroke and other vascular events by aspirin in patients with AF was 22%,[6] versus only 13% in patients with TIA or minor stroke of presumed vascular origin,[7] suggests that platelet activation may play an even more important role in stroke patients with AF than in those without. Indeed, we found higher urinary excretion levels of 11-dehydro-TXB_2 in patients with AF compared to those without. Excretion levels however, could not be suppressed significantly better by aspirin in AF patients. This finding suggests that platelet inhibition with 300 mg of aspirin may be insufficient in stroke patients with AF. In acute stroke patients with AF, no difference in occurrence of ischemic events or outcome could be demonstrated between patients treated with aspirin and those treated with unfractionated or high-dose low molecular weight heparin.[5,13] The combined data of the IST and Chinese Acute Stroke Trial (CAST)-trials,[14] in which

40,000 patients with acute ischemic stroke of whom approximately 20% with AF were included, did not show a different effect of aspirin in patients with or without AF. Patients with acute ischemic stroke with AF have a higher risk of early death, which seems mainly explained by older age and larger infarcts.[5] However, in the IST, more patients with AF died from a fatal recurrent stroke of ischemic or unknown type. Patients with AF had slightly more recurrent ischemic strokes in the first 14 days than patients without, 3.9% versus 3.3%, but the difference was just not statistical significant. In other studies the risk of early recurrence varies from 1.5% to 18% during the first 14 days after the initial event.[15,16,17,18] The low recurrence rates reported in the IST may therefore be an underestimation of the true rates.[5] Because the risk of stroke recurrence in aspirin treated patients with AF appears to be at least as high, and probably higher than in patients without AF and our finding that platelet activation is strongly associated with the presence of AF in the acute phase of stroke, one may suspect a causal relationship. Together with our other finding that aspirin alone does not adequately suppress platelet activation in stroke patients with AF this provides a rational for testing new antiplatelet regimens in this setting.

A potential weakness of our study is that it combined data from three previous studies. However, except for differences in the number of urinary samples taken in the acute phase of stroke, no differences in methodology of patient recruitment and workup, and in assessment of urinary 11-dehydro-TXB_2. Even the hospital ward, the laboratory, and the collaborators of these previous studies were identical. Another drawback of this study is the small number of patients in the subgroups of patients with AF, particular those treated with aspirin. Despite the small numbers, however, differences in metabolite excretion between patients with and those without AF were statistically significant.

We conclude that platelet activation in the acute phase of ischemic stroke is strongly associated with AF and that in these patients platelet activation is insufficiently suppressed by aspirin treatment alone.

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5

PLATELET ACTIVATION IN THE CHRONIC PHASE AFTER STROKE

CHAPTER 5

INCREASED PLATELET ACTIVATION IN THE CHRONIC PHASE AFTER CEREBRAL ISCHEMIA AND INTRACEREBRAL HEMORRHAGE

Summary

Background and purpose. Enhanced thromboxane (TX) biosynthesis has previously been reported in the acute phase after ischemic stroke. We investigated whether enhanced urinary 11-dehydro-TXB_2 excretion, a non-invasive index of platelet activation, was present in the chronic phase after a transient ischemic attack (TIA) or stroke, including intracerebral hemorrhage.

Methods. We obtained a single urinary sample from 92 patients between 3 and 9 months after onset of stroke or TIA. The urinary excretion of the major enzymatic metabolite of TXA_2, 11-dehydro-TXB_2, was measured by a previously validated radioimmunoassay. The excretion rate was compared with that of 20 control patients with nonvascular neurological diseases.

Results. Urinary 11-dehydro-TXB_2 averaged 294±139, 413±419, and 557±432 pmol/mmol creatinine, for patients with TIA, ischemic stroke and intracerebral hemorrhage, respectively, and was higher in all subgroups ($P<0.01$) than the value of 119±66 of controls. Increased 11-dehydro-TXB_2 excretion was present in 59% of all patients, in 60% ($P<0.001$) of patients with TIA, in 56% ($P<0.001$) of patients with ischemic stroke, and in 73% ($P<0.001$) of patients with intracerebral hemorrhage. Atrial fibrillation, no aspirin use, and severity of symptoms at follow-up contributed independently to the level of 11-dehydro-TXB_2 excretion, in a multiple linear regression analysis.

Conclusions. Platelet activation is often present in the chronic phase after stroke, including patients with intracerebral hemorrhage. Persistent platelet activation is associated with atrial fibrillation and poor stroke outcome, and can be substantially suppressed by aspirin treatment.

Introduction

In previous studies we have reported enhanced thromboxane biosynthesis, as reflected by the urinary excretion of a major TXA_2 metabolite, 11-dehydro-TXB_2, in patients with acute ischemic stroke.[1,2,3] Increased thromboxane production was found to occur episodically during the first 2 to 3 days after the onset of ischemic stroke,[1,2,3] and could be largely suppressed by low-dose aspirin,[1,3] thus suggesting its cyclooxygenase-dependent formation in platelets. Repeatedly increased 11-dehydro-TXB_2 excretion was independently related to stroke severity and atrial fibrillation. However, metabolite excretion was not a statistically significant independent prognostic factor for outcome when added to stroke syndrome or stroke severity in a multiple logistic regression model, probably due to the rather small size of that study.[3] Whether increased 11-dehydro-TXB_2 excretion is detectable in the chronic phase after stroke, and whether this is related to cardiovascular risk factors and stroke outcome is unknown.

In the present study we performed measurements of urinary 11-dehydro-TXB_2 excretion between 3 and 9 months after stroke onset in patients with TIA, ischemic stroke, or intracerebral hemorrhage, in order to investigate whether enhanced platelet activation is present in the chronic phase after stroke, and, if so, whether such persistent platelet activation is associated with stroke type, cardiovascular risk factors, and outcome.

Subjects and Methods

Study patients

Patients were recruited from the hospital based stroke cohort of the Dutch Vascular Factors in Dementia Study, of which the inclusion and exclusion criteria are detailed elsewhere.[4] The most important inclusion criteria for this study were that patients: 1/ had had ischemic stroke, intracerebral hemorrhage, or TIA with neurological deficit on admission to the hospital; 2/ had a reasonable life expectancy and were alive at follow-up; 3/ were 55 years of age or over at time of stroke onset; 4/ were natively Dutch speaking; and 5/ were not or only mildly aphasic (<3 on the Aphasia Severity Rating Scale from the Boston Diagnostic Aphasia Examination). Of the 300 patients who met these criteria, data were used of all non-demented patients of whom urinary samples were available. In order to achieve the demented/not demented ratio of 1:4 that was found in the whole cohort,[4] a random sample of demented stroke patients was added to the study group. Detailed information about cardiovascular risk factors and stroke

characteristics was obtained during hospital admission. In addition to a full neurological examination, ancillary investigations consisted of standardized blood tests, a chest radiograph, computed tomography and/or magnetic resonance imaging of the brain, duplex scanning of the carotid arteries, a cardiac analysis including 12-lead electrocardiography and, if indicated, 24 hour electrocardiographic monitoring and echocardiography. At follow-up, between 3 and 9 months after onset of stroke, blood pressure measurements were performed, urinary samples were collected, and details on medication used at the time of follow-up were obtained. Stroke severity was assessed by means of the modified Rankin scale.[5]

Control patients

We used the data from our previous study,[1] in which 11-dehydro-TXB_2 excretion was measured in 20 control patients (11 men and 9 women; mean age, 64.2 years; range 41 to 85 years) with nonvascular neurological disorders, such as minor cerebral trauma, Parkinson's disease, epilepsy, or cervical spondylotic myelopathy, who were admitted to the same hospital. Urine was collected during the night as soon as possible after admission to the hospital.

Urine measurements

Urine samples were collected 3 to 9 months after stroke. The creatinine concentration was measured and samples of 50 mL were immediately frozen and stored at –20°C until extraction. Analytical measurements of 11-dehydro-TXB_2 excretion were performed blinded to clinical characteristics. Immunoreactive 11-dehydro-TXB_2 was extracted from 10 mL aliquots of each coded urine sample (the pH was adjusted to 4.0 with formic acid) on SEP-PAK C18 cartridges (Waters Associates) and eluted with ethyl acetate. The eluates were subjected to silicic acid column chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol). Immunoreactive 11-dehydro-TXB_2 eluted from silicic acid columns was assayed at a final dilution of 1:30 to 1:1000, as described previously.[6] The urinary excretion rate of 11-dehydro-TXB_2 was expressed as picomoles per millimole of creatinine.

Statistical analysis

Data were analyzed by means of STATA statistical software.[7] The Student's *t* test was used to compare urinary 11-dehydro-TXB_2 excretion between groups. Multiple linear regression was used to assess the relation between the level of 11-dehydro-TXB_2 excretion and other clinical characteristics. Values of $P<0.05$ were considered statistically significant.

Results

The present study group consists of 92 patients, mean age, 73.8±8.2 years, of whom 53 were men and 39 women. Ten (11%) had had a TIA, 71 (77%) ischemic stroke, and 11 (12%) intracerebral hemorrhage.

The individual values of 11-dehydro-TXB_2 in all patients and controls are depicted in Figure 5.1. These values ranged between 105 and 496 (median, 287) pmol/mmol creatinine in patients with TIA, 80 and 2105 (median, 290) in patients with ischemic stroke, and between 96 and 1467 (median, 466) in patients with intracerebral hemorrhage. Compared to controls, 11-dehydro-TXB_2 excretion was significantly higher in patients with TIA ($P<0.001$), patients with ischemic stroke ($P=0.003$) and in patients with intracerebral hemorrhage ($P<0.001$). In 60% of the patients with TIA ($P=<0.001$), 56% of patients with ischemic stroke ($P<0.001$), and 73% of the patients with intracerebral hemorrhage ($P<0.001$), the excretion rate exceeded 2 SD of the mean value of control patients with nonvascular disorders (119±66 pmol/mmol creatinine). Overall, persistently increased urinary 11-dehydro-TXB_2 excretion was present in 59% of the patients.

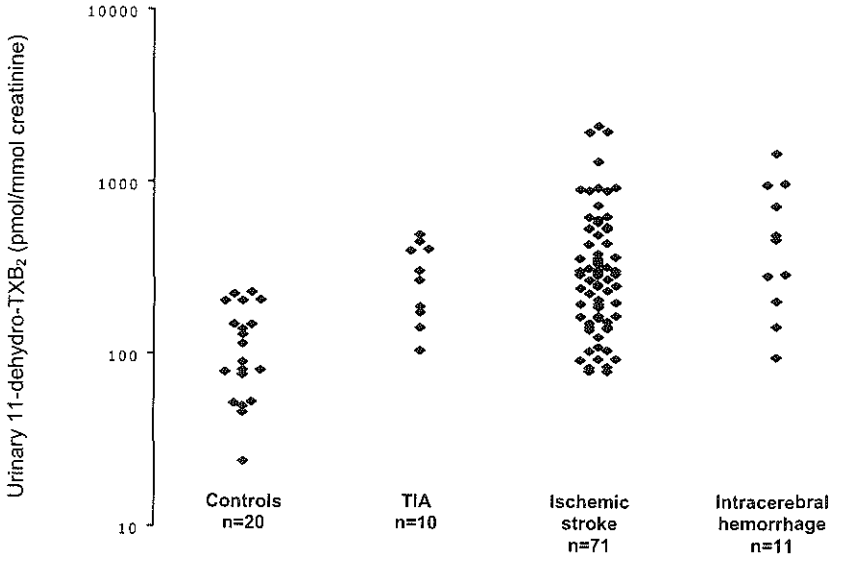

Figure 5.1 Individual measurements of urinary 11-dehydro-TXB_2, plotted on a log scale, for control patients (mean±SD, 119±66 pmol/mmol creatinine) and for patients with TIA (294±139), ischemic stroke (413±419), and intracerebral hemorrhage (557±432).

Table 5.1 11-dehydro-TXB_2 excretion values by demographic factors, cardiovascular risk factors, medication, and stroke characteristics

Characteristic	N	11-dehydro-TXB_2	*P*
Age, year			
< 75	44	385±368	0.46
≥ 75	48	447±433	
Gender			
Male	53	320±262	0.006
Female	39	549±512	
Cardiovascular risk factors			
Smoking	26	408±336	0.89
Hypertension	56	371±328	0.17
Diabetes mellitus	18	513±578	0.26
Hypercholesterolemia	30	326±188	0.13
Intermittent claudication	7	262±150	0.29
Angina pectoris	13	247±126	0.10
Atrial fibrillation	19	759±637	<0.001
Congestive heart failure	7	890±611	<0.001
History of cardiovascular disease			
Myocardial infarction	9	309±154	0.40
Stroke	22	365±243	0.49
Retinal infarction	2	517±584	0.72
Medication			
No oral anticoagulant and no aspirin	10	616±412	0.13†
Aspirin	56	294±294	0.004‡
Oral anticoagulant	26	606±500	0.95‡
Type of stroke			
TIA	10	294±139	0.04
Cerebral infarction	71	413±419	0.83
Intracerebral hemorrhage	11	557±432	0.27
Clinical subtype of stroke			
TACS	7	462±332	0.70
PACS	32	374±364	0.48
LACS	25	434±520	0.78
POCS	7	465±395	0.72
Severity			
Rankin score at follow-up ≤ 3	76	320±229	<0.001
Rankin score at follow-up > 3	16	877±667	

N is number of patients; values are mean±SD; TACS indicates total anterior circulation stroke; PACS, partial anterior circulation stroke; LACS, lacunar stroke; POCS, posterior circulation stroke.

† vs aspirin or oral anticoagulant; ‡ vs no aspirin and no oral anticoagulant.

Table 5.1 shows the urinary 11-dehydro-TXB_2 excretion of the 92 patients based on demographic characteristics, cardiovascular risk factors, use of antiplatelet and anticoagulant medication, and stroke characteristics. In the univariate analysis, urinary 11-dehydro-TXB_2 excretion was significantly higher in women (P=0.006), and in patients with atrial fibrillation (P<0.001) or congestive heart failure (P<0.001). Patients who used aspirin (N=56) had significantly lower excretion rates of 11-dehydro-TXB_2 than patients on oral anticoagulant treatment or patients without antiplatelet or anticoagulant treatment (P=0.004). Mean 11-dehydro-TXB_2 excretion in patients with TIA was significantly lower than that of patients with a cerebral infarction or a intracerebral hemorrhage (P=0.04). No association was found between the level of 11-dehydro-TXB_2 excretion and subtype of cerebral infarction. Poor stroke outcome, as measured by a Rankin score of >3 at follow-up, was associated with increased 11-dehydro-TXB_2 excretion (P<0.001).

Five patients had a recurrent vascular event between their qualifying event and the time of urinary sampling during follow-up. Three of them had an ischemic stroke, 1 a TIA, and 1 an intracerebral hemorrhage. Urinary 11-dehydro-TXB_2 excretion averaged 716±693 pmol/mmol creatinine, and was numerically higher, (P=0.09), than in patients without early recurrence. The 3 patients with ischemic stroke recurrence had significantly enhanced metabolite excretion: 976±840 pmol/mmol creatinine (P=0.01).

In a multiple linear regression analysis presence of atrial fibrillation and severe strokes (Rankin >3 at follow-up) were independently associated with increased 11-dehydro-TXB_2 levels, whereas treatment with aspirin was associated with reduced metabolite excretion (Table 5.2).

Table 5.2 Coefficients with 95% confidence intervals of factors that remained independently related to 11-dehydro-TXB_2 excretion in a multiple linear regression analysis

Factor	Coefficient	95% CI	*P*
Aspirin	-224	-355 to -94	0.001
Atrial fibrillation	291	133 to 449	< 0.001
Severe stroke	507	346 to 668	< 0.001

Discussion

The main finding of the present study is that biochemical evidence of in vivo platelet activation is detectable in the chronic phase after stroke in approximately 60% of patients. In the present study, also patients with intracerebral hemorrhage and TIA were included. Of the ischemic stroke patients, 56% had increased urinary 11-dehydro-TXB_2 excretion in the chronic phase. Perhaps unexpectedly, in the vast majority (73%) of patients with intracerebral hemorrhage, we also found enhanced thromboxane biosynthesis. This finding may suggest that increased platelet activation is a reflection of vascular risk factors, diffuse atherosclerotic lesions, or the extent of vascular damage due to the stroke. However, it is unlikely that platelet activation merely reflects a generalized vascular disease. A recent study in patients with peripheral arterial disease, has clearly demonstrated that hypertension, diabetes mellitus and hypercholesterolemia, but not peripheral vascular disease per sé, are associated with enhanced thromboxane biosynthesis.[8] In our study, we found no relationship between hypertension, diabetes mellitus and hypercholesterolemia on the one hand, and elevated levels of 11-dehydro-TXB_2 excretion on the other. However, two thirds of our patients were using aspirin at the time of sampling. Davì et al. reported that a daily regimen of low dose aspirin could largely suppress enhanced thromboxane biosynthesis in patients with hypercholesterolemia[9] and diabetes mellitus.[10] The relatively small number of patients with one or more vascular risk factors in our study probably explains why adjustment for aspirin intake did not eliminate its confounding effect in the multivariate analysis.

As in our previous study,[3] presence of atrial fibrillation and absence of aspirin therapy were associated with increased thromboxane production in the univariate analysis. We previously reported that poor stroke outcome tended to be associated with increased thromboxane production in the acute phase. In the present study, we found a statistically significant higher rate of thromboxane metabolite excretion in patients with a Rankin score > 3 at follow-up. The association between increased thromboxane production and atrial fibrillation may reflect, at least in part, the fact that atrial fibrillation is more likely to cause severe strokes. Moreover, patients with atrial fibrillation usually receive oral anticoagulant treatment, rather than aspirin. However, in the multiple regression analysis, both atrial fibrillation and stroke outcome were independently related to the rate of urinary 11-dehydro-TXB_2 excretion.

The study of Davì et al.[8] suggests that persistently increased platelet activation is a predictor of ischemic events in the setting of peripheral

arterial disease, since patients who experienced vascular events (myocardial infarction, cardiac death, ischemic stroke) during 48 months of follow-up, had significantly higher 11-dehydro-TXB_2 excretion at baseline than patients who remained event free. Five of our patients (5.4%), all of whom had had an ischemic stroke, had a vascular event in the time between their qualifying stroke and follow-up at 3 to 9 months later. In the 3 patients with a recurrent ischemic stroke, thromboxane metabolite excretion was significantly higher than in patients with no recurrences. This could not be explained by acute episodes of platelet activation due to the vascular event, because all patients were tested at least 3 months after their last event. This procedure was part of the protocol.[4] Although the number of patients is small, and the samples were taken after the recurrent event, the findings are in line with those of Davì et al. in a different clinical setting.[8] Whether persistent platelet activation is a risk factor for recurrent ischemic events in patients with ischemic and hemorrhagic stroke remains to be investigated in larger studies with longer follow-up.

We conclude that platelet activation is often present in patients in the chronic phase after stroke, including those with intracerebral hemorrhage. Persistent platelet activation, which is associated with atrial fibrillation and poor stroke outcome, can be substantially suppressed by aspirin treatment.

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6

THE DUTCH VASCULAR FACTORS IN DEMENTIA STUDY: RATIONALE AND DESIGN

CHAPTER 6

THE DUTCH VASCULAR FACTORS IN DEMENTIA STUDY: RATIONALE AND DESIGN

Summary

Dementia is a rapidly increasing health problem in the industrialized countries. With the aging of the population the number of demented persons increases both in relative and absolute terms. Obviously, there is a need for prevention and intervention strategies. We describe the methods and baseline findings of a large study aimed at identifying potentially modifiable vascular, thrombogenic, and metabolic determinants of dementia. The study population consists of subjects 55 years of age or older. Since the vascular wall of the cerebral vessels is different from that of the coronary or peripheral vessels, we formed three subgroups in which vascular risk factors for dementia are studied. Subjects with stroke were distinguished from subjects with coronary or peripheral artery disease, and from subjects without stroke or coronary or peripheral artery disease. To obtain a large enough number of subjects with stroke, cases and controls from a stroke registry were combined with cases and controls of a population based study from the same region. For the diagnosis of dementia the DSM-III-R criteria were used. Extensive information on cardiovascular risk factors was collected, including indicators of atherosclerosis. Blood and urine were sampled to study platelet function and thrombogenic and metabolic factors. The study population consists of 7466 subjects, of whom 300 were recruited from a hospital based stroke registry. Coronary or peripheral artery disease was present in 956 subjects, and stroke in 617. Dementia was present in 434 (5.8%) of all subjects. The prevalence of dementia was 3.0%, 24.0%, and 4.4% in subjects with a history of coronary or peripheral artery disease, a history of stroke, and subjects without a history of coronary or peripheral artery disease or stroke, respectively. The study will allow us to investigate the role of vascular factors in dementia, irrespective of its cause.

Introduction

Dementia is one of the most important causes of disability in the elderly. In the industrialized world, the prevalence of dementia is approximately 5%, increasing with age from 0.1% in subjects between 30 and 60 years of age, to approximately 30% in subjects over 90.[1] With the aging of the population, the proportion of persons over 75 years of age almost doubled from 3 to 6% in the Netherlands since 1960 (data from Statistics Netherlands, Voorburg 1996), the number of demented patients is increasing both in relative and absolute terms. In older age categories, severe dementia with the need for institutionalization has the highest prevalence.[2,3,4] Obviously, there is a need for strategies to reduce both the prevalence and the severity of dementia, in order to lighten the burden on society in terms of health care, disability, hospital and institutional care. The most obvious pathologic process to consider in this respect seems to be the vascular pathology, since vascular factors provide the best opportunities for prevention and intervention strategies. That dementia appeared to be more severe in patients with a presumed vascular cause,[3,5] should only encourage our interest in vascular mechanisms of dementia. Since 1990, we have embarked on a study of the role of vascular, thrombogenic and metabolic risk factors in relation to the presence and occurrence of dementia, the Dutch Vascular Factors in Dementia Study. We describe the methods and baseline findings of this study.

Rationale

It has been estimated that in 10% to 40% of all demented patients, vascular lesions or thromboembolic processes are the cause of the disease.[6] In stroke patients dementia was present in approximately 25%.[7,8] The disorder had been coined 'multi infarct dementia' by Hachinski in 1975.[9] However, after the introduction of brain imaging techniques, other vascular disorders than multiple infarctions were found to be related with dementia as well. White matter lesions are associated with cerebral infarctions,[10,11] but also with cognitive dysfunction, vascular risk factors, and atherosclerosis.[12,13] Multiple lacunar infarctions,[14] but also strategic single lacunar infarctions,[15] were associated with dementia. Various types of intracranial hemorrhages and their sequelae may produce dementia. The vascular mechanisms that may lead to dementia were described in detail by the NINDS-AIREN international work group, introducing criteria for

vascular dementia.[16] However, vascular factors may play an important role in development and the course of other types of dementia as well.[17,18] In this study, we aim to evaluate vascular risk factors in relation to dementia, irrespective of its cause. Since there is some evidence that the endothelium of cerebral vessels may be different from that of peripheral vessels, i.e. in expression of proteins like thrombomodulin that can alter the coagulant properties of the local vasculature,[19] the relative importance of various risk factors may differ between patients with peripheral artery disease and patients with cerebral artery disease. This is supported by the results of the CAPRIE study that has shown an effect of clopidogrel versus aspirin in patients with peripheral artery disease, but not in patients with coronary artery disease or stroke.[20] In the past alterations in levels of hemostatic proteins were thought to be the cause of atherosclerotic and atherothrombotic complications. Now it is recognized that abnormalities in coagulation may, at least partly, be the result of atherosclerotic changes, since the anticoagulant properties of atherosclerotic vessels is decreased.[21]

Therefore, we a priori distinguished between subjects with symptomatic coronary or peripheral artery disease, cerebral artery disease, and subjects free from vascular disease, to investigate the relationship of alterations in platelet function and hemostatic factors and dementia.

Objectives

Etiology of dementia. The study aims to identify which vascular, thrombogenic and metabolic risk factors are related to the risk of occurrence of dementia. Emphasis will be on: 1/ indicators of atherosclerosis: carotid intima media thickness, plaques, ankle-brachial systolic blood pressure index, ECG; 2/ indicators of platelet activation: platelet count, urinary 11-dehydro-thromboxaneB_2, serum plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor; 3/ indicators of endothelial deviations: von Willebrand factor, thrombomodulin, tissue-type plasminogen activator activity (t-PA activity), serum PAI-1; and 4/ haemostatic variables: fibrinogen, thrombin-antithrombin complex (TAT), plasmin-antiplasmin complex (PAP), fragment1+2, von Willebrand factor, activated proteinC-ratio (APC-ratio), D-dimer, and tPA-activity.

Diagnostic classification of dementia. We will evaluate how well the cardiovascular risk profile, location and degree of atherosclerosis, and disturbances in hemostatic and metabolic processes, relate to the current diagnostic criteria for vascular dementia.

Methods

Population

The population of the Dutch Vascular Factors in Dementia study consists of participants of the Rotterdam Study and of the Rotterdam Stroke Databank. The Rotterdam Study is a single center prospective follow-up study of disease and disability in the elderly, described in detail elsewhere.[22] Eligible participants included all residents 55 years and older living in the Rotterdam suburb of Ommoord, in the Netherlands. Baseline measurements were made from March 1990 to July 1993. Of the 10,275 eligible participants, 7,983 participated (78%). The study has been approved by the Medical Ethics Committee of the Erasmus University and written informed consent was obtained from all participants.

The Rotterdam Stroke Databank is a registry of patients with transient ischemic attack, ischemic stroke or a primary intracerebral hemorrhage, admitted to the department of Neurology of the University Hospital Rotterdam in the Netherlands. From March 1, 1993 until January 15, 1996, a total of 825 consecutive patients entered this registry of whom 300 met the criteria to enroll in the Dutch Vascular Factors in Dementia Study. Informed consent was obtained from all patients or in some cases from close relatives. The reasons for exclusion are detailed in Table 6.1.

Assessment of cognitive function and dementia

In the Rotterdam Study and in the Rotterdam Stroke Databank we used largely the same methodology to obtain information on cognitive function and to diagnose dementia, though small differences did occur, due to the specific problems of acute stroke patients.

In patients of the Rotterdam Stroke Databank, premorbid cognitive function was assessed by an interview with a close informant and the score on the Blessed Dementia Scale.[23] Cognitive function was assessed by neurological examination and by a series of neuropsychological screening instruments. Between 3 and 9 months after onset of stroke we performed the Mini-Mental State Examination (MMSE),[24] Geriatric Mental Status-organic scale (GMS),[25] and the Dutch version of the cognitive and self contained part of the Cambridge Examination for Mental Disorders of the Elderly, the CAMCOG.[26] In patients with a clinical suspicion of a dementia syndrome, extensive neuropsychological evaluation was carried out. Based on information of a close informant, the results of extensive neuropsychological evaluation, and the clinical impression at examination, the diagnosis of dementia was assessed in a diagnostic panel that consisted of a neuropsychologist, two neurologists, and a physician of the Rotterdam

Stroke Databank. For the diagnosis, the DSM-III-R criteria for dementia [27] were applied.

Table 6.1 Patient recruitment from the Rotterdam Stroke Databank for the Dutch Vascular Factors in Dementia Study

	Number of patients	Percentage
RSD-patients	825	100
- Inclusion in Dementia study	300	36.4
- Exclusion from Dementia study	525	63.6
< 55 years of age	198	24.0
Deceased within 3 months	122	14.8
Did not give consent	46	5.6
TIA and no signs on examination	42	5.1
Severe aphasia	41	5.0
Moved out of the region	29	3.5
No native Dutch speaking	17	2.0
Short life expectancy	13	1.6
Other intracranial pathology	12	1.4
Not testable other	3	0.4
Severe psychiatric disorder	2	0.2

The assessment of cognitive function and dementia in the Rotterdam Study is described in detail elsewhere.[28,29] In short, the screening for dementia included the MMSE and the GMS-organic scale. Subjects with a MMSE <26 and/or a GMS-organic score >0, were subjected to a more extensive screening procedure, consisting of the structured psychiatric diagnostic interview of the CAMDEX, the CAMCOG, and an interview with a close informant by a physician. All subjects with a CAMCOG <80 and/or a suspicion of dementia were referred for further examination which included examination by a neurologist and more extensive neuropsychological testing. Neuro-imaging was obtained if possible. For subjects with incomplete work-up, additional information was obtained from medical records. Based on all available information, the final diagnosis was made by a diagnostic panel

consisting of a neurologist, a neuropsychologist, and two physicians of the Rotterdam Study, using the DSM-III-R criteria.

Cardiovascular risk factors

In the Rotterdam Study information on health status, medical history, current drug use, and smoking behavior was obtained using a computerized questionnaire, which included a Dutch version of the Rose questionnaire for assessment of prevalent angina pectoris and intermittent claudication.[30] A previous history of myocardial infarction was based on the questions 'Did you ever have a myocardial infarction for which you were hospitalized?'. Information on a stroke history was obtained by the question 'Did you ever suffer from a stroke, diagnosed by a physician?'. For stroke, supplementary medical information including a detailed history, information on neuroimaging, and copies of hospital discharge records was obtained from general practitioner or hospital discharge records. Stroke diagnosis was based on all available medical information as detailed elsewhere.[31] Diabetes mellitus was considered present when subjects were currently using oral blood sugar lowering drugs or receiving insulin treatment. With respect to smoking behavior, subjects were categorized in groups of current smokers, former smokers and those who had never smoked. Height and weight were measured and body mass index (kg/m^2) was calculated. Sitting blood pressure was measured at the right upper arm using a random-zero sphygmomanometer. The average of two measurements obtained at one occasion, separated by a count of the pulse rate, was used in the present analysis. Hypertension was defined as a systolic blood pressure of 160 mmHg or over or a diastolic blood pressure of 95 mmHg or over or current use of antihypertensive drugs for the indication hypertension. Blood sampling procedures and storage have been described elsewhere.[32]

In the Rotterdam Stroke Databank detailed information about cardiovascular risk factors, stroke characteristics, and premorbid mental and physical status was obtained during hospital admission, using the same methodology as in the Rotterdam Study. In addition to a full neurological examination, ancillary investigations consisted of standardized blood tests, chest x-ray, computed tomographic scanning and/or magnetic resonance imaging of the brain, duplex scanning of the carotid arteries, a cardiac analysis including standard 12-lead electrocardiography, and, if indicated, 24-hour electrocardiographic monitoring and echocardiography. Blood pressure measurements, anthropometry, and blood and urine sampling were performed between 3 and 9 months after the initial stroke. As indicator of platelet activation we measured the urinary excretion of thromboxane A_2, 11-dehydro-thromboxaneB_2, as described elsewhere.[33,34]

Non-invasive assessment of atherosclerosis

In both the Rotterdam study and in the Rotterdam Stroke Databank carotid atherosclerosis was assessed through ultrasonography of both carotid arteries. To measure carotid intima-media thickness, ultrasonography was performed with a 7.5 MHz linear array transducer. This procedure has been described in detail previously.[35,36,37] Plaques were defined as a focal widening relative to adjacent segments, with protrusion into the lumen either composed of only calcified deposits, only non-calcified material or a combination of both. No attempt was made to quantify the size or extent of the lesions.[38]

The presence of atherosclerosis in the arteries of the lower extremities was evaluated by measuring the systolic blood pressure level of the posterior tibial artery on both the left and right side using an 8 Mhz continuous wave Doppler probe (Huntleigh 500 D, Huntleigh Technology, Bedfordshire, UK) and a random-zero sphygmomanometer.[12,35] A single blood pressure reading was taken on each side with the subject in a supine position. The ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm (ankle-arm index) was calculated for each leg, and the lowest used in the analysis.[39]

Follow-up

The cohort will be followed over time to detect new cases of dementia. This part of the study may further clarify the causal and prognostic significance of risk factors for dementia. In addition, patients with prevalent dementia will be monitored for progression.

In the Rotterdam Stroke Databank, subjects are followed each year, until 3 years after onset of stroke. At three years, assessment of cognitive function and dementia will be repeated. In patients in whom mental deterioration is suspected during follow-up, cognitive function will be assessed immediately. In patients with recurrent stroke assessment of cognitive function and dementia will be repeated 3 months after the recurrence. In the Rotterdam Study, assessment of cognitive function will be performed every two years.

The estimate number of incident cases during 3 year follow-up in the population part of the study is 200. This number is based on data of previous studies,[40,41,42] in the absence of an increased risk in patients with cardiovascular disease or stroke. The actual number therefore might be higher. For patients of the Rotterdam Stroke Databank with a recent stroke and free from dementia at baseline examination, the number of incident

cases of dementia in 3 years is estimated to be 55, based on findings in a large stroke databank in North America.[43]

Data-analysis

A priori three groups of subjects were constructed: subjects with coronary or peripheral artery disease, subjects with stroke and subjects free from coronary or peripheral artery disease and stroke. Coronary or peripheral artery disease was defined as a history of either myocardial infarction, angina pectoris, intermittent claudication, percutaneous transluminal coronary angioplasty (PTCA) or coronary bypass surgery. Subjects of the stroke group consisted of participants of the Rotterdam Stroke Databank and of Rotterdam Study participants with a medically confirmed history of stroke, either with or without coronary or peripheral artery disease. The approach taken to address the research questions is schematically shown in Figure 6.1.

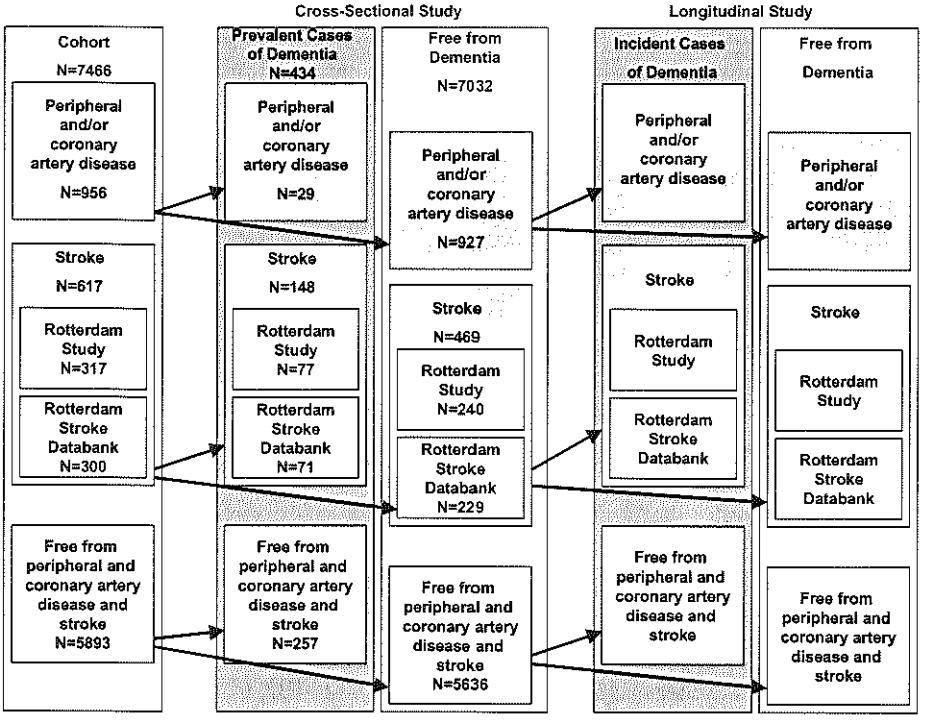

Figure 6.1 Schematic overview of the cohort groups (N, number of patients)

For the cross-sectional data analysis subjects with dementia are selected and an equal number of controls, matched for gender, and frequency matched in age strata of 5 years, schematically shown in Figure 6.2.

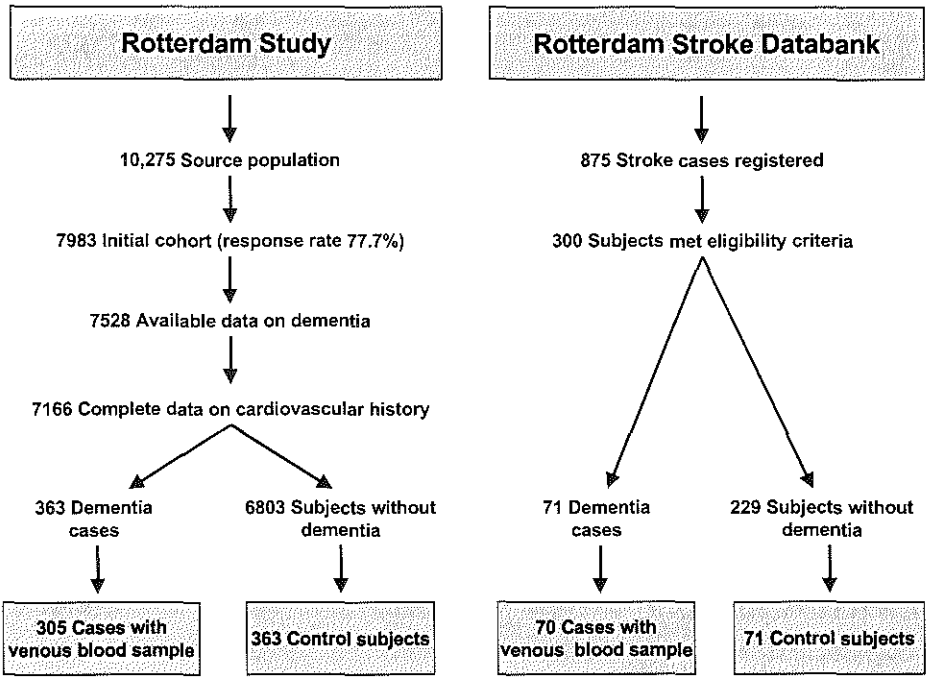

Figure 6.2 Schematic approach for selection of cases and controls in the Dutch Vascular Factors in Dementia Study.

Description of the cohort

Of the participants of the Rotterdam Study complete information on cognitive screening was available in 7528 subjects. Of those subjects data on medical history, i.e., myocardial infarction, angina pectoris, intermittent claudication, stroke, PTCA was present for 7166 subjects. From the 825 patients that entered the Rotterdam Stroke Databank, 300 subjects were included over the predefined time period. Of the patients aged 55 or over, 122 (14.8%) died within 3 months after stroke onset, 46 (5.6%) did not give informed consent, 42 (5.1%) had had a transient ischemic attack without any

sign on neurological examination, 41 (5.0%) had a severe aphasia, < 3 on the Aphasia Severity Rating Scale from the Boston Diagnostic Aphasia Examination (BDAE),[44] and another 76 (9%) were excluded for various other reasons. This resulted in a study population of 7466 subjects.

Table 6.2 General characteristics of the study population†

	Coronary or peripheral artery disease	Stroke	Free from coronary and peripheral artery disease and stroke	All subjects
Number of subjects	956	617	5893	7466
Age (years)	72.2(8.7)	72.9(9.5)	69.3(9.4)	69.9(9.4)
Female (%)	48.3	48.4	62.7	59.6
Systolic bloodpressure (mmHg)	139.4(22.8)	150.9(24.3)	139.1(22.1)	140.1(22.7)
Diastolic bloodpressure (mmHg)	71.5(11.5)	80.4(12.9)	74.0(11.5)	74.2(11.8)
Pulse rate (beats/min)	71.3(12.1)	77.0(16.7)	74.4(12.7)	74.2(13.0)
Weight (kg)	74.7(12.1)	74.1(12.6)	72.8(12.0)	73.2(12.2)
Height (cm)	167.4(9.2)	167.7(10.4)	166.6(9.4)	166.8(9.5)
Body mass index (kg/m^2)	26.6(3.8)	26.4(4.1)	26.2(3.9)	26.3(4.0)
Current smoking (%)	18.4	29.0	22.9	22.8
Diabetes mellitus (%)	7.0	13.7	3.6	4.9
Angina pectoris (%)	49.0	11.3	0.0‡	7.2
Intermittent claudication (%)	11.3	5.7	0.0‡	1.9
Myocardial infarction (%)	49.3	15.0	0.0‡	7.4
Stroke (%)	0.0‡	100.0‡	0.0‡	8.2
Dementia (%)	3.0	24.0	4.4	5.8

† Values are proportions or means with standard deviations in parentheses. Values are unadjusted.
‡ Selection criterium for the subgroups.

Results

General characteristics are given in Table 6.2. Among the 7166 participants of the Rotterdam Study, 363 (5.1%) were diagnosed as being

demented. The highest prevalence was found among subjects with stroke, 24.3%, and the lowest among subjects with or without peripheral or coronary artery disease, 3.0% and 4.4%, respectively. In the Rotterdam Stroke Databank, 71 (23.7%) patients were diagnosed as being demented. Subjects with vascular disease were older, more frequently men, and were more likely to have diabetes mellitus. Subjects with stroke had a higher systolic and diastolic blood pressure.

Table 6.3 Differences in general characteristics between subjects with and without dementia across study groups.† D + subjects with dementia, D – subjects without dementia

	Coronary or peripheral artery disease		Stroke		Free from coronary and peripheral artery disease and stroke	
	D +	D -	D +	D -	D +	D -
Number of subjects	29	927	148	469	257	5636
Age (years)	84.1	71.8‡	77.7	71.5‡	84.5	68.5‡
Female (%)	58.6	48.0	63.4	43.8‡	76.0	62.0‡
Systolic bloodpressure (mmHg)	140	139	144	153‡	142	139‡
Diastolic bloodpressure (mmHg)	70	72	78	81	73	74
Pulse rate (beats/min)	72	71	77	77	74	74
Weight (kg)	63.4	75.0‡	69.0	75.4‡	64.9	73.1‡
Height (cm)	160	168‡	163	169‡	160	166‡
Body mass index (kg/m^2)	24.8	26.7‡	25.7	26.5	25.4	26.2‡
Current smoking (%)	22.2	18.3	18.9	31.9	13.8	23.3
Diabetes mellitus (%)	11.5	6.9	15.1	13.3	5.2	3.6

† Values are unadjusted proportions or means.
‡ *P* value < 0.05

Table 6.3 shows differences in general characteristics between subjects with or without dementia across the study groups. In all study groups, higher age was associated with dementia. Demented stroke subjects were approximately 6 years younger than demented subjects in the other groups. In all groups, dementia was associated with both reduced body

weight and height, and with reduced body mass index. Demented stroke subjects had significantly lower systolic blood pressure levels than non-demented stroke subjects. In subjects free from vascular disease the reverse association was found.

Discussion

The prevalence of dementia in patients with coronary or peripheral artery disease was only 3% and comparable with the prevalence of dementia of 4.4% in subjects without manifest peripheral vascular disease, suggesting that the prevalence of dementia is independent of the presence of manifest peripheral vascular disease. On the other hand, in other studies demented subjects were shown to have more vascular risk factors than subjects without dementia.[17,18] These data suggest that vascular risk factors may have their influence on specific parts of the vasculature. The reverse association between blood pressure and dementia found in patients with stroke and in patients without vascular disease indicates that stroke patients may have a different vascular risk profile for the development of dementia. The relationship between blood pressure and dementia is complex and may change over time. Where in cross-sectional studies blood pressure levels in patients with Alzheimer's disease are usually lower, longitudinal studies have shown that patients with Alzheimer's disease are more likely to have arterial hypertension than controls 15 years prior to the onset of dementia, with a decrease in blood pressure a few years before the onset of dementia.[45] One of the explanations is that lesions in Alzheimer's disease may be present in areas involved in the regulation of blood pressure. It is important to recognize that dementia itself may influence risk factors for stroke. Therefore, we have planned a longitudinal part in our the study, to investigate the direction and nature of the relationship. Whether the 3 year follow-up is long enough remains to be awaited.

No difference in the prevalence of dementia was found between stroke subjects from the population based part of the study and subjects from the hospital cohort, which suggests that the hospital cohort very well reflects the one from the population. The high prevalence of 24.0% of dementia in patients with previous stroke that we report is in accordance with two other prospective studies in stroke patients.[7,8] Although there were some differences in inclusion criteria, these studies all indicate that approximately one fifth to a quarter of post stroke patients have dementia. Whether stroke is the result of extensive cerebral vascular disease and an epiphenomenon of vascular dementia, or stroke has actually caused dementia may depend on

the individual pattern of baseline characteristics and risk factors. Therefore, we think it is important to study vascular factors in relation to dementia, irrespective of its presumed cause, both in subjects without vascular disease and those with peripheral vascular disease and stroke.

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7

THROMBOXANE BIOSYNTHESIS AND POST-STROKE DEMENTIA

CHAPTER 7

INCREASED THROMBOXANE BIOSYNTHESIS IS ASSOCIATED WITH POST-STROKE DEMENTIA

Summary

Background and purpose. It has been suggested that daily intake of aspirin is associated with a reduction of cognitive decline, both in normal and in demented subjects, but the mechanism is unclear. We have studied the relationship between thromboxane (TX) A_2 biosynthesis, as reflected by the urinary excretion of 11-dehydro-TXB_2, and the presence of dementia in patients after acute stroke.

Methods. Patients from the Rotterdam Stroke Databank were screened for dementia between 3 and 9 months after stroke. Patients had a full neurological examination, neuropsychological screening, and on indication extensive neuropsychological examination. For the diagnosis of dementia the DSM-III-R criteria were used. Urine samples were taken at the time of screening. Urinary 11-dehydro-TXB_2 was measured by means of a previously validated radioimmunoassay.

Results. Dementia was diagnosed in 71 patients and urine samples were available in 62. Median value (range) of 11-dehydro-TXB_2 was 399 (89-2105) pmol/mmol creatinine for demented patients versus 273 (80-1957) for 69 controls with stroke but without dementia (P=0.013). No difference was found between 44 patients with vascular dementia, 404 (89-2105) pmol/mmol creatinine, and 18 patients with Alzheimer's disease plus cerebrovascular disease, 399 (96-1467) pmol/mmol creatinine (P=0.68). In a stepwise logistic regression analysis, where possible confounders such as use of antiplatelet medication, cardiovascular risk factors, and type of stroke were taken into account, increased urinary excretion of 11-dehydro-TXB_2 remained independently related with the presence of dementia (Odds Ratio 1.12, 95% confidence interval 1.03-1.22 per 100 pmol/mmol creatinine). The difference in metabolite excretion rates between demented and non-demented patients was most prominent within the subgroup of ischemic stroke patients who received aspirin (P<0.01).

Conclusions. Increased thromboxane biosynthesis in the chronic phase after stroke is associated with the presence, but not the type of post-stroke dementia, and is particularly apparent in patients on aspirin, thereby suggesting the involvement of extra-platelet sources of thromboxane A_2 production in this setting.

Introduction

Dementia is one of the most important causes of disability in the elderly. The increased aging of the population calls for strategies that reduce both the occurrence and severity of dementia, in order to lighten the burden on society in terms of health care, disability, hospital and institutional care. It has been estimated that in 10% to 40% of all demented patients vascular lesions or thromboembolic processes represent the identifiable cause of dementia.[1] Several studies[2,3,4,5,6,7] have addressed the hypothesis that aspirin might have a beneficial effect on the occurrence and progression of dementia and on cognitive function in general. In one randomized trial, patients with multi-infarct dementia benefited from aspirin therapy, with improvement of cognitive performance scores and cerebral perfusion values.[2] This study, however, was not placebo controlled. In another trial, subjects with a high risk for cardiovascular disease showed better cognitive performance after five years of treatment with antithrombotic therapy, i.e. aspirin, warfarin, or both, than those treated with placebo.[5] The effect, however, was small, and the patients studied represented a subgroup from a trial that was designed for another purpose. In two non-randomized population based studies, either no effect of aspirin on cognitive function was found at all,[6,7] or a small, not statistically significant, positive effect was reported.[4] Finally, a positive effect on cognitive performance of NSAIDs or aspirin was reported in patients diagnosed as possible or probable Alzheimer's disease.[3]

These studies suggest a positive effect of aspirin treatment on cognitive performance, both in demented patients, and in otherwise healthy elderly subjects. The evidence, however, is scarce, the effect, if any, is modest, and the underlying mechanisms largely unknown. Aspirin may be beneficial through its antiplatelet effect[8] by preventing recurrent cerebral infarcts, or through its anti-inflammatory properties. The dose-requirement for the apparent beneficial effect of aspirin on cognitive performance would favor the antiplatelet effect as the most likely explanation. In a previous study, we reported an association between platelet activation, as reflected by thromboxane metabolite excretion, in the acute phase of stroke and stroke severity.[9] There was also a non-significant trend for enhanced platelet activation in patients with the worst 3-month outcome, measured on the Rankin scale, a handicap scale that reflects the degree of independence of the patient, taking cognitive performance into account.

In this study, we have prospectively investigated the relationship between in vivo thromboxane biosynthesis during the chronic phase after stroke and the presence of post-stroke dementia.

Subjects and Methods

Study patients

Patients were recruited from the Rotterdam Stroke Databank, a prospective registry of patients with transient ischemic attack (TIA), ischemic stroke, or a primary intracerebral hemorrhage, admitted to the department of Neurology of the Dijkzigt University Hospital Rotterdam in the Netherlands. From March 1, 1993 until January 15, 1996, 825 consecutive patients were entered into this registry of whom 300 met the entry criteria for the Dutch Vascular Factors in Dementia Study.[10] Patients had to be 55 years or older, and they had to be admitted to our neurology ward with a TIA, cerebral infarction or intracerebral hemorrhage. Reasons for exclusion were in short as follows: 24% was too young, 15% died within 3 months after stroke, 6% did not give consent, 5% had had a TIA and no neurological signs on examination, 4% moved out of the region, 5% was untestable because of severe aphasia, 2% were not native Dutch speaking, and 3% were excluded for various other reasons. Of the remaining 300 patients, 71 were demented, and in 62 of these urinary samples were available for thromboxane metabolite measurements. Seventy-one control patients, including patients with TIA, ischemic stroke and intracerebral hemorrhage, frequency matched for age and sex were randomly taken from the 229 non-demented stroke and TIA patients. Urinary samples were available from 69 of the 71 control subjects. All patients were screened according to a strict protocol consisting of a full neurological examination, standardized blood tests, at least one and usually two CT scans of the brain, duplex scanning of the carotid arteries, and a cardiological analysis that included standard 12-lead ECG and, if indicated, 24-hour ECG monitoring and echocardiography. The nature and time course of the symptoms were recorded by means of a detailed checklist.[11] Patients with a cerebral infarction were further subdivided according to a clinical classification: total anterior circulation stroke (TACS), partial anterior circulation stroke (PACS), lacunar stroke (LACS), and posterior circulation stroke (POCS).[12] Apart from the neurological history, the following vascular risk factors were recorded: smoking habits, hypercholesterolemia (history of hypercholesterolemia and/or fasting total cholesterol level >6.5 mmol/L),[13] hypertension (history of hypertension and/or systolic blood pressure >160 mm Hg and/or diastolic blood pressure >90 mm Hg, treated or not), diabetes mellitus (history of diabetes mellitus type I or II and/or a random blood glucose of 8 mmol/L together with an HbA_{1c} level of 6.30% or more, treated or not),[14] atrial fibrillation (history of atrial fibrillation and/or atrial fibrillation on ECG), and a history of intermittent claudication, angina

pectoris, prior myocardial infarction, retinal infarction or stroke. We recorded the medication, especially antiplatelet and anticoagulant treatment, taken by the patients at the time of urinary sampling, which was between 3 and 9 months after the onset of stroke. As customary in the Netherlands, the vast majority of patients in our study were treated with a dose of 30 mg of aspirin daily. Only patients with a cardiac indication for aspirin (n=16) were treated with a higher dose varying between 80 and 100 mg daily. Nine patients used NSAIDs on a regular, but not daily basis. Eight of them were also using aspirin, and only 1 used NSAIDs and no aspirin. In the analysis, this patient was grouped in the non-aspirin group.

Five patients with cerebral ischemia as qualifying event, were not treated with antithrombotic medication at the time of assessment. One of them had a thrombocytopenia, which prevented aspirin treatment, the other 4 had recurrent systemic bleedings, of whom a 3 a gastric bleeding and 1 recurrent urinary tract bleeding. On the other hand, 5 patients with an intracerebral hemorrhage as qualifying event received antithrombotic treatment at the time of assessment, because they had already an indication for antithrombotic treatment prior to their hemorrhage. In one patient, oral anticoagulant treatment was restarted in the chronic phase after the bleeding because of a prosthetic aortic valve. In two patients with atrial fibrillation, one with a history of TIA, and one with a history of recurrent myocardial infarction, aspirin was started in the chronic phase after oral anticoagulant treatment was stopped in the acute phase of the hemorrhage. In 2 patients with a history of TIA, aspirin was restarted in the chronic phase.

Assessment of cognitive function and dementia

Premorbid cognitive function was assessed by means of an interview with a close informant and the score on the Blessed Dementia Scale.[15] Cognitive function was assessed through a neurological examination and by a series of neuropsychological screening instruments between 3 and 9 months after onset of stroke. We performed the Mini-Mental State Examination (MMSE),[16] Geriatric Mental Status-organic scale (GMS),[17] and the Dutch version of the cognitive and self contained part of the Cambridge Examination for Mental Disorders of the Elderly, the CAMCOG.[18] In patients in whom dementia was clinically suspected, extensive neuropsychological evaluation was performed. Based on information from a close relative, the results of extensive neuropsychological evaluation, and the clinical impression on examination, the diagnosis of dementia was assessed by a diagnostic panel that consisted of a neuropsychologist, two neurologists, and a physician of the Rotterdam Stroke Databank. For the diagnosis of dementia, the DSM-III-R criteria[19] were applied. The NINDS-

AIREN research criteria[20] were used to distinguish between patients with VAD and those with AD+CVD. The latter were patients with progressive cognitive deterioration existing before the onset of stroke, without a history or signs of cerebrovascular disease until the present stroke occurred, thus fulfilling the clinical criteria for possible Alzheimer's disease.[21] The degree of handicap was also assessed between 3 and 9 months after onset of stroke, by means of the modified Rankin scale.[22]

Measurements of thromboxane biosynthesis

Urine samples were collected 3 to 9 months after stroke, thus avoiding short-term fluctuations in thromboxane biosynthesis related to the acute phase of stroke.[9,23] The creatinine concentration was measured and samples of 50 mL were immediately frozen and stored at -20°C until extraction. Analytical measurements of 11-dehydro-TXB_2, a major enzymatic metabolite of TXB_2 in man, were performed blinded to the diagnosis of dementia. Immunoreactive 11-dehydro-TXB_2 was extracted from 10 mL aliquots of each coded urine sample (the pH was adjusted to 4.0 with formic acid) on SEP-PAK C18 cartridges (Waters Associates) and eluted with ethyl acetate. The eluates were subjected to silicic acid column chromatography and further eluted with benzene/ethyl acetate/methanol (60:40:30, vol/vol). Immunoreactive 11-dehydro-TXB_2 eluted from silicic acid columns was assayed at a final dilution of 1:30 to 1:1000, as described previously.[24] The urinary excretion rate of 11-dehydro-TXB_2 was expressed as picomoles per millimole of creatinine.

Statistical analysis

Data were analyzed by means of the Statistical Package for the Social Sciences (SPSS) and Egret statistical software. Values of 11-dehydro-TXB_2 between groups were compared with the Mann-Whitney *U* test. Differences in baseline characteristics between demented and non-demented patients were compared using Student's t-test, chi-square test, and Fisher's exact test where appropriate. Values of $P<0.05$ (two-sided testing) were considered statistically significant. A logistic regression analysis, in which potential confounders such as age, gender, the use of antiplatelet medication, the cardiovascular risk factors hypertension, hypercholesterolemia, atrial fibrillation, smoking habit, and type and site and of stroke, were taken into account, was performed to investigate whether 11-dehydro-TXB_2 was independently related to the presence of dementia.

Results

Seventy-one (23.7%) of the 300 stroke patients were diagnosed as demented. Three (6.5%), 54 (25%), and 14 (39%) of the patients with TIA, ischemic stroke, and intracerebral hemorrhage, respectively, were demented. Patients with an intracerebral hemorrhage had a higher risk of dementia (OR, 95%CI=2.31, 1.04-5.08), and patients with TIA a lower risk (OR, 95%CI=0.19, 0.06-0.67). The mean age of the demented patients was 73.3±7.7 years, compared to 62.8±8.0 years of the non demented patients (P<0.001). Of the demented patients 52% were female compared to 37% of the controls (P=0.03).

Table 7.1 Clinical characteristics of stroke patients in relation to the presence of dementia

Characteristic	N	Demented N (%)	Control N (%)	*P*
Age, mean±SD, year	131	73.8±7.9	73.6±8.2	0.90
Gender				
Male	69	29 (47)	40 (58)	0.20
Female	62	33 (53)	29 (42)	
Type of stroke				
TIA	12	3 (5)	9 (13)	0.10
Cerebral infarction	99	46 (74)	53 (77)	0.73
Intracerebral hemorrhage	20	13 (21)	7 (10)	0.09
Clinical subtype of cerebral infarction				
TACS	11	7 (15)	4 (8)	0.22
PACS	46	20 (44)	26 (49)	0.58
LACS	30	11 (24)	19 (35)	0.20
POCS	12	8 (17)	4 (8)	0.13
Severity				
Rankin score of ≤ 3 at follow-up	103	38 (61)	65 (94)	<0.001
Rankin score of > 3 at follow-up	28	24 (39)	4 (6)	
Antithrombotic medication				
Aspirin	77	36 (58)	41 (59)	0.90
Oral anticoagulant	34	12 (19)	22 (32)	0.10
Neither aspirin nor oral anticoagulant	20	14 (23)	6 (9)	0.03

N indicates number of patients; TACS, total anterior circulation stroke; PACS, partial anterior circulation stroke; LACS, lacunar stroke; and POCS, posterior circulation stroke.

Urine samples were available from 62 demented patients and 69 controls. Table 7.1 shows the baseline characteristics in relation to dementia

for both groups. No statistically significant difference in age, gender, type of stroke, and clinical subtype of cerebral infarction existed between the two groups, although patients with TIA were numerically more frequent in the control group, and more patients with intracerebral hemorrhage were present in the group with dementia. Patients who neither used oral anticoagulant nor antiplatelet medication were more frequent in the dementia group (P=0.03). However, no statistically significant difference existed in the number of patients using aspirin or anticoagulant medication between the two groups.

Patients with dementia had a significantly higher 11-dehydro-TXB_2 excretion (median, range, 399, 89-2105, pmol/mmol creatinine) than non-demented patients, (273, 80-1957, P=0.01), as detailed in table 7.2. No difference in 11-dehydro-TXB_2 excretion was found between the two major types of dementia, 404 (range:89-2105) pmol/mmol creatinine for vascular dementia, and 399 (range:96-1467) for Alzheimer's disease plus cerebrovascular disease. Impaired performance on cognitive screening tests was associated with increased 11-dehydro-TXB_2 excretion (P=0.003 for the MMSE, and P=0.03 for the CAMCOG). The correlation, however, between cognitive scores and level of 11-dehydro-TXB_2 was only modest, r^2=0.10 (P<0.001), and r^2=0.05 (P<0.001), for MMSE and CAMCOG, respectively. Patients with severe strokes, defined as a Rankin score >3 at follow-up, had significantly higher metabolite excretion levels than patients with minor stroke, P=0.0002. The group of patients treated with aspirin had significant lower excretion levels of 11-dehydro-TXB_2 than untreated patients (P<0.0001).

Because aspirin treatment has a major impact on urinary 11-dehydro-TXB_2, by largely suppressing platelet TXA_2 biosynthesis,[8] we investigated the relationship between metabolite excretion and dementia both in the presence and in the absence of aspirin therapy. In order to avoid an imbalance in the subgroups, patients with an intracerebral hemorrhage were excluded, as almost none of them used aspirin. Figure 7.1 depicts the individual values of 11-dehydro-TXB_2 for patients with cerebral ischemia, with and without aspirin. Only in the aspirin group 11-dehydro-TXB_2 excretion rates were significantly higher in demented patients than in controls, (P=0.01). When hemorrhagic stroke patients were added, the results remained the same (P=0.007). In patients not on aspirin, the median values were 494 (range 167-1957), and 431 (range 105-2105), for non-demented and demented patients, respectively, (P=0.73). For the aspirin group the corresponding values were 196 (range 80-631) and 290 (range 89-1935), respectively (P=0.01). Thus, in patients who did not have dementia at follow-up, aspirin treatment was associated with 60% lower rate of thromboxane biosynthesis than in the absence of antiplatelet therapy, and

only 2 out of 39 aspirin treated subjects had metabolite excretion in excess of the median value of untreated subjects. In contrast, in patients who were demented at follow-up, aspirin treatment was associated with 33% lower rate of TXA_2 biosynthesis and 13 out of 35 treated subjects had metabolite excretion in excess of the median value of untreated subjects. However, no firm conclusions can be drawn because of the relatively small numbers and the large variability.

Table 7.2 Median and range of 11-dehydro-TXB_2 excretion rates as a function of dementia, cognitive function, severity of stroke, and medication

Variable	N	11-dehydro-TXB_2	*P*
Dementia			
No	69	273 (80-1957)	0.01
Yes	62	399 (89-2105)	
Type of dementia			
Alzheimer's disease with CVD	18	399 (96-1467)	0.04†
Vascular	44	404 (89-2105)	0.04†
Possible	4	396 (220-796)	0.23†
Probable	40	404 (89-2105)	0.06†
Cognitive function			
MMSE ≤ 24	64	399 (89-2105)	0.003
MMSE > 24	67	269 (80-1313)	
CAMCOG ≤ 76	60	380 (89-2105)	0.03
CAMCOG > 76	71	272 (80-1957)	
Severity of stroke			
Rankin score of ≤ 3 at follow-up	103	286 (80-1313)	0.0002
Rankin score of > 3 at follow-up	28	635 (89-2105)	
Antithrombotic medication			
No oral anticoagulant or aspirin	20	610 (188-1467)	
Aspirin	77	214 (80-1935)	<0.0001‡
Oral anticoagulant	34	479 (105-2105)	0.44‡

† Vs non-demented patients.
‡ Vs no oral anticoagulant or aspirin.

In the logistic regression analysis, increased urinary excretion of 11-dehydro-TXB_2 remained independently related to the presence of dementia, with an Odds Ratio of 1.12 (95% confidence interval 1.03 - 1.22) per 100 pmol/mmol creatinine.

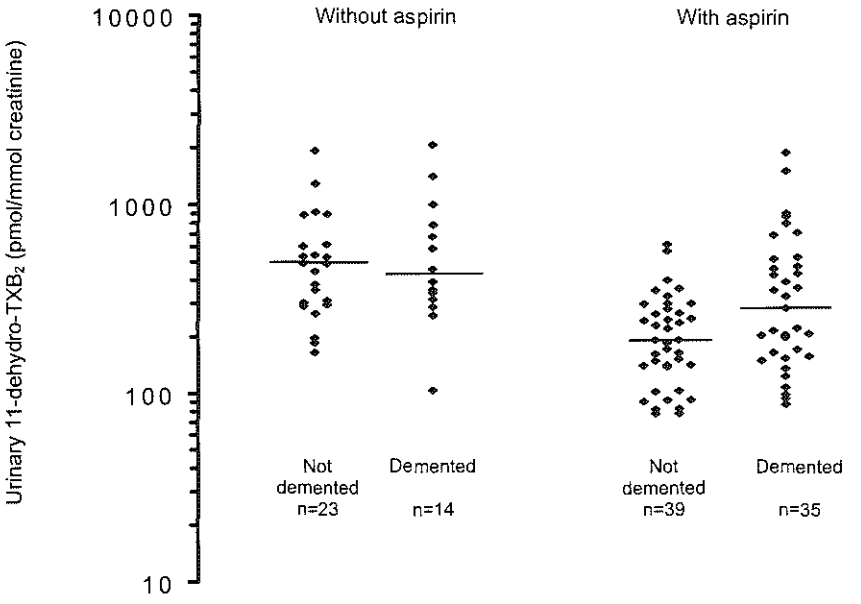

Figure 7.1 Individual urinary 11-dehydro-TXB_2 excretion rates depicted on a logarithmic scale for demented and non-demented patients with cerebral ischemia, as a function of aspirin therapy. The horizontal bars represent median values for each subgroup of patients.

Discussion

The main finding of the present study is that patients with post-stroke dementia more often show increased thromboxane biosynthesis than non-demented controls. Elevated levels of circulating platelet microparticles have been described earlier in a small study of patients with multi-infarct dementia.[25] However, the interpretation of blood indexes of platelet activation is hampered by sampling-related artifacts.[26]

Among other factors associated with increased 11-dehydro-TXB_2 in our univariate analysis were atrial fibrillation, unfavorable outcome and absence of antiplatelet treatment, which is consistent with the results of a previous study in patients with acute ischemic stroke.[9] Atrial fibrillation was also identified as risk factor for dementia, in a population based study,[27] as well as in a stroke population.[28] However, in a multiple logistic regression analysis, in which these confounders were taken into account, increased

urinary excretion of 11-dehydro-TXB_2 remained independently related to the presence of dementia.

Increased thromboxane biosynthesis may also reflect severe vascular disease, which may lead to dementia. However, this seems unlikely in the light of a recent study in patients with peripheral arterial disease,[29] which has clearly demonstrated that vascular disease per se is not associated with enhanced thromboxane biosynthesis.

The role of aspirin in our setting remains puzzling, since the association between dementia and increased thromboxane biosynthesis was most prominent in patients with aspirin treatment. This may reflect the play of chance, since the subgroup analysis included a small number of patients. On the other hand, this finding may suggest that stroke patients who show increased thromboxane biosynthesis that cannot be completely suppressed by aspirin have an increased risk of dementia. This might imply that patients with post-stroke dementia have an important aspirin-insensitive source of thromboxane biosynthesis. The involvement of PGH-synthase-2 (COX-2) in producing the substrate for thromboxane-synthase within the context of an ongoing inflammatory process in the brain would be compatible with this working hypothesis. Among the cell types endowed with thromboxane synthase and capable of expressing COX-2 in response to inflammatory cytokines and growth factors are monocytes and macrophages.[30] Moreover, transcellular biosynthesis of thromboxane A_2 may occur through the biochemical cooperation of cells expressing COX-2 (e.g. vascular endothelial cells) with aspirinated platelets.[31] Aspirin is considerably less potent in inhibiting human monocyte COX-2 than platelet COX-1 activity.[32] Thus, plasma aspirin concentrations achieved at conventional antiplatelet dosage are inadequate to suppress COX-2-dependent eicosanoid biosynthesis. Cipollone et al.[32] have recently reported that in unstable angina, episodes of aspirin-insensitive TXA_2 biosynthesis may reflect extra-platelet sources possibly expressing COX-2 in response to a local inflammatory milieu. If the same mechanism is operative in the setting of cerebral ischemia and inflammation, this might explain the rather conflicting results obtained with aspirin in observational studies,[2,4,5] as well as the apparent protection against Alzheimer's disease associated with non-aspirin NSAIDs.[3] These drugs (e.g. ibuprofen) are equally potent in inhibiting human platelet COX-1 and monocyte COX-2.[30]

We conclude that: 1) Patients with post-stroke dementia more often show increased thromboxane biosynthesis than non-demented stroke patients; 2) Increased thromboxane biosynthesis is not associated with the type of post-stroke dementia; 3) The association between thromboxane biosynthesis and the presence of post-stroke dementia is particularly

apparent in patients on aspirin treatment, suggesting that patients with post-stroke dementia have an aspirin-insensitive source of thromboxane biosynthesis, possibly related to COX-2 expression in the brain. The availability of specific COX-2 inhibitors offers the opportunity of testing this hypothesis with a properly designed randomized trial.

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8

GENERAL DISCUSSION

CHAPTER 8

GENERAL DISCUSSION

This thesis describes urinary 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) excretion, both in the acute and chronic phase after cerebral ischemia. Urinary 11-dehydro-TXB_2 excretion is under specific conditions a measure of in vivo platelet activation. To study the dynamics of platelet activation in the acute phase after cerebral ischemia, repeated measurements were performed every 6 hours up to 72 hours after onset of symptoms. In addition, we measured urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) excretion to elucidate the origin of potential vasoactive isoprostanes in this setting. A single measurement was performed in patients in the chronic phase after cerebral ischemia or intracerebral hemorrhage, between 3 and 9 months after onset of symptoms. Both in the acute and in the chronic phase after cerebral ischemia, the relationship of increased thromboxane biosynthesis with other risk factors for stroke, stroke characteristics and medication was investigated. In particular, we studied the relationship between thromboxane excretion, the presence of atrial fibrillation and aspirin treatment. Finally, urinary 11-dehydro-TXB_2 in the chronic phase after stroke was measured to explore the role of thromboxane biosynthesis in patients with post-stroke dementia.

Main findings

Thromboxane biosynthesis in the acute phase after cerebral ischemia

Approximately 75% of the patients who did not receive aspirin had increased urinary 11-dehydro-TXB_2 excretion during the first 3 days after onset of symptoms. In more than 60% of the patients this occurred repeatedly. In the patients with repeatedly enhanced thromboxane biosynthesis, no uniform pattern in metabolite excretion was found. Enhanced thromboxane biosynthesis was present in only 53% of the patients who were treated with aspirin, repeatedly in 40%. Mean values of urinary 11-dehydro-TXB_2 excretion decreased within 24 hours to normal range in patients treated with aspirin, whereas untreated patients had repeatedly

increased values throughout the study period. Repeatedly increased thromboxane biosynthesis was related to stroke severity, the presence of atrial fibrillation and antiplatelet medication. Peak levels of thromboxane excretion were higher in patients with more severe strokes or atrial fibrillation. In patients without atrial fibrillation and aspirin treatment mean metabolite excretion values were approximately within the normal range, whereas in patients with atrial fibrillation, thromboxane biosynthesis remained elevated even during aspirin treatment.

Thromboxane biosynthesis in the chronic phase after stroke

Increased levels of urinary 11-dehydro-TXB_2 were found in approximately 60% after cerebral ischemia and in 70% after intracerebral hemorrhage. Persistent enhanced thromboxane biosynthesis was related with the presence of atrial fibrillation and severity of symptoms at follow-up. It could be substantially suppressed by aspirin treatment.

Lipid peroxidation in the acute phase after cerebral ischemia

No evident peaks in urinary 8-epi-$PGF_{2\alpha}$ excretion were found. The correlation of 8-epi-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 excretion was only modest. Whereas urinary excretion of 11-dehydro-TXB_2 could be suppressed by aspirin, excretion levels of 8-epi-$PGF_{2\alpha}$ did not change after aspirin treatment.

Thromboxane biosynthesis and post-stroke dementia

Increased thromboxane biosynthesis was found to be associated with the presence of post-stroke dementia. No differences were found in thromboxane excretion levels between patients with Alzheimer's disease with cerebrovascular disease and those with probable or possible vascular dementia. The association between thromboxane biosynthesis and the presence of post-stroke dementia was particularly apparent in patients on aspirin treatment.

Methodological aspects

Does urinary 11-dehydro-TXB_2 excretion reflect platelet activation?

The method we used to measure urinary metabolites of thromboxane A_2 was introduced in 1987 and validated by Ciabattoni et al.[1] Urinary 11-

dehydro-TXB_2 excretion reflects in vivo thromboxane biosynthesis. We considered enhanced thromboxane biosynthesis to reflect platelet activation if it could be suppressed by low dose aspirin since platelets contain no DNA and are therefore not able to replace the cyclooxygenase enzymes blocked by aspirin. Other sources of thromboxane biosynthesis, such as monocytes and macrophages which are able to express cyclooxygenase-2 in response to inflammatory cytokines and growth factors,[2] are less sensitive to suppression by aspirin,[3] and can probably not be blocked by low doses of aspirin.

Measurement of urinary 11-dehydro-TXB_2 excretion

The method we used to measure 11-dehydro-TXB_2, described by Ciabattoni et al.[1] has been validated. However, it is a laborious and expensive procedure, and not suitable for routine clinical practice, at least at present. It is valuable in research projects in which large numbers of samples can be measured in one setting. In this setting, the role of thromboxane biosynthesis in vascular disease can be studied, but also many other research questions, for instance its relationship with dementia and migraine can be addressed.

Only when less expensive tests become available, which can easily determine in vivo thromboxane biosynthesis in the individual patient, thromboxane measurements can be applied in daily clinical practice. In the near future, it may be of use in the adjustment of antiplatelet medication, particularly in the individual patient with persistent platelet activation, when different modes of antiplatelet therapy probably become general available in the prevention of vascular events,[4] or in differentiating 'atypical TIA's' from cerebral ischemia.[5]

The diagnosis of dementia

As described in the introduction of this thesis, the diagnosis of dementia in stroke patients may be difficult.[6] For clinical practice and the fast growing number of clinical trials on cognitive functioning, large number of patients have to be screened for cognitive decline and dementia. This should be done with tests with the highest possible sensitivity and specificity.

At the time we started our studies, dementia screening instruments used in clinical practice were usually developed to detect cognitive deficits compatible with Alzheimer's disease. With these screening instruments, certain aspects of cognitive functioning, such as slowing of intellectual functioning, abstraction, retrieval, recognition, and visuospatial abilities are

less well measured. This means that these short tests are a priori less suitable for detecting subcortical dementia or dementia that may have both cortical and subcortical deficits, like dementia after stroke. Most existing dementia screening instruments predominantly contain verbally mediated items that tend to exaggerate the extent of cognitive deficits in patients with a left-hemispheric stroke. Likewise, tests that emphasize verbal capacities probably underestimate the consequences of right-hemispheric lesions.[7,8,9] In stroke patients, in particular those with a left-hemispheric stroke, a paresis may hamper a good performance at items that depend on constructional abilities. Moreover, subtests that refer to praxis, frequently accompanying aphasia, can raise problems in stroke patients. In patients with a right-sided hemispheric stroke neglect can be a confounding factor in test scores. The MMSE[10] is a frequently used mental status test in patients with a recent stroke, but it has several disadvantages due to its emphasis on language and focus on cortical functions. Tatemichi et al. used the MMSE as a screening instrument in stroke patients and found that the MMSE can be of use when adjustments are made for the false-positive rates.[11] Nevertheless, the absence of tasks sensitive to subcortical dysfunction may make the MMSE a useful, but not ideal screening instrument for patients with a recent stroke. The 3MS[12] seems more suitable for the detection of subcortical pathology than the MMSE. Grace et al. compared the original MMSE and the modified version in a stroke population and found that the 3MS and MMSE had a similar overall classification accuracy, which was adequate for patients with left-hemispheric strokes and poor for patients with right-sided strokes.[13] The authors, however, believe that the 3MS is a clinically more useful screening instrument in stroke patients because its false-negative rate is lower and it demonstrated a higher sensitivity in a stroke population.

Considering the drawbacks of the available dementia screening instruments for stroke patients, we thoroughly evaluated premorbid cognitive functioning as well as cognitive functioning after stroke.[14] Premorbid cognitive functioning was assessed by means of an interview with a close informant and the score on the Blessed Dementia Scale.[15] Between 3 and 9 months after stroke onset, cognitive functioning was assessed by a neurologist, based on clinical observation, the information of a close informant, and the score on the Blessed Dementia Scale. In case of any suspicion of cognitive decline, patients were invited for an extensive neuropsychological examination. The 'gold standard' for the diagnosis of dementia was based on the results of this extensive neuropsychological examination, clinical presentation, and information from a close relative. Although it is an elaborate work, this procedure is probably more sensitive and specific for the detection of dementia in stroke patients than screening

with one of the instruments followed by further evaluation in the screen positive patients. The latter may lead to under-diagnosis of dementia by missing the false negative patients. However, the method we used is not suitable for general clinical practice, because it is too time consuming. In order to investigate the value of screening instruments which can potentially be used in stroke patients in future studies, we performed a battery of screening instruments, independent of the diagnostic procedure described above. The MMSE, the Geriatric Mental Status,[16] and the Dutch version of the cognitive and self-contained part of the CAMDEX,[17,18] the CAMCOG, were administered in all patients. The value of this test in screening for dementia after stroke is described elsewhere.[14]

Thromboxane biosynthesis in stroke

Episodic platelet activation in the acute phase after cerebral ischemia

In the majority of patients, we found enhanced thromboxane biosynthesis during the first 3 days after cerebral ischemia which showed striking intraindividual fluctuation and could be suppressed by low dose of aspirin. Given the 45-minute half-life of 11-dehydro-TXB_2,[19] elevated levels would not be expected beyond 24 hours if thromboxane dependent platelet activation occurred as a single episode at the time of the event. We therefore concluded that platelet activation occurs repeatedly in the first few days after cerebral ischemia.

This finding raised more questions than answers. What is the meaning of increased platelet activation after stroke? Is it a result of the stroke? Does it have a causal relationship with stroke? Is it related with prognosis after stroke or increased stroke recurrence? Is it a reflection of the general vascular status of the patient?

We investigated the relationship with other vascular risk factors, and found that increased platelet activation was independently related with the presence of atrial fibrillation, stroke severity, and use of aspirin. No relationship with other vascular risk factors, or stroke characteristics was found, suggesting that increased platelet activation did not reflect generalized vascular disease.

The next question was whether a causal relationship existed between the extent and duration of platelet activation and stroke severity and outcome. In our study, repeatedly increased thromboxane production was not a statistically significant independent prognostic factor for outcome when added to stroke syndrome or stroke severity in a multiple logistic regression model, probably because of the small sample size. However, the finding that

aspirin could profoundly suppress increased thromboxane biosynthesis, together with our finding that episodic platelet activation was present in the acute phase after ischemic stroke, provided a rationale for testing the efficacy and safety of aspirin in this setting. The results of the International Stroke Trial (IST)[20] and the Chinese Acute Stroke Trial (CAST)[21] combined showed a highly significant, but modest reduction of 9 per 1000 patients treated in the overall risk of stroke recurrence or death in hospital.[22]

Thromboxane biosynthesis and atrial fibrillation in the acute phase after stroke

Atrial fibrillation, stroke severity and the absence of aspirin therapy were associated with increased platelet activation. These factors may reflect the fact that atrial fibrillation is more likely to cause large, often severe cortical infarctions. Moreover, patients with atrial fibrillation usually received no aspirin but anticoagulants, or no antithrombotic medication at all in the acute phase after stroke. However, in all patients studied in the acute phase which were analyzed together, atrial fibrillation remained an important determinant of 11-dehyro-TXB_2 excretion, even after adjustment for stroke severity. Atrial fibrillation increased metabolite excretion with 350 pmol/mmol creatinine. No interaction could be demonstrated between the effect of aspirin and atrial fibrillation. The absolute reduction in thromboxane excretion was approximately 300 pmol/mmol creatinine in both the patients with atrial fibrillation and those in sinus rhythm. We concluded that platelet activation in the acute phase of ischemic stroke is strongly associated with atrial fibrillation and that in fibrillating patients platelet activation is not sufficiently suppressed by aspirin treatment alone. Although in patients with atrial fibrillation low stroke recurrence rates were reported in the IST,[23] the risk of stroke recurrence in aspirin treated patients with atrial fibrillation is at least as high, but probably higher than in patients without atrial fibrillation.[24,25,26,27] Together with our finding that platelet activation is strongly associated with the presence of atrial fibrillation in the acute phase of stroke, and that aspirin alone does not adequately suppress platelet activation, this provides a rational for testing new antiplatelet regimens in this setting.

Increased platelet activation in the chronic phase after stroke

The fact that increased urinary thromboxane excretion was found in the majority of patients in the chronic phase after stroke, and in particular also in patients after intracerebral hemorrhage, suggests that increased platelet activation is a reflection of vascular risk factors, diffuse

atherosclerotic lesions, or the extent of vascular damage due to the stroke. We could not demonstrate a relationship between hypertension, diabetes mellitus, and hypercholesterolemia on the one hand, and elevated levels of 11-dehydro-TXB_2 excretion on the other. Yet, the majority of patients used aspirin. Together with the fact that a relatively small number of patients had one or more vascular risk factors probably explains why adjustment for aspirin intake did not eliminate its confounding effect in a multivariate analysis. However, enhanced thromboxane biosynthesis which could be suppressed by aspirin has been described in patients with hypercholesterolemia,[28] and in patients with diabetes mellitus.[29] It is unlikely that platelet activation merely reflects a generalized vascular disease because in patients with peripheral artery disease, diabetes mellitus and hypercholesterolemia, but not peripheral vascular disease per sé, were associated with enhanced thromboxane biosynthesis.[30]

Is persistent platelet activation a risk factor for stroke recurrence? The number of ischemic stroke recurrence in our study was too small to answer this question. However, in the 3 patients with recurrent ischemic stroke, thromboxane biosynthesis was significantly higher than in patients with no recurrences. This finding is in line with those of Davì et al. who reported that patients who experienced vascular events (myocardial infarction, cardiac death, ischemic stroke) during 2 years of follow-up had significantly higher levels of 11-dehydro-TXB_2 at baseline than patients who remained event free.[30] Whether persistent platelet activation is a risk factor for recurrent ischemic events in patients with ischemic and hemorrhagic stroke remains to be investigated in larger studies with long follow-up.

Suggestions for further research

To further elucidate the relationship between thromboxane biosynthesis, stroke, and vascular riskfactors, a nested case-control study is warranted. In addition it should be investigated whether patients with recurrent ischemic stroke show persistent platelet activation and whether this is insensitive for aspirin treatment or not. Risk reduction in vascular events by aspirin is far from optimal. The relative risk reduction of ischemic stroke and other major vascular events of antiplatelet therapy in patients with atrial fibrillation was 22%,[31] whereas in patients with TIA or minor stroke of presumed vascular origin, the relative risk reduction by aspirin in any dosage above 30 mg was only 13%.[32] If stroke recurrence is associated with persistent platelet activation which is insensitive for treatment with low dose of aspirin, other antiplatelet regimens should be tested. This could include higher dosages of aspirin, but also inhibition of platelet aggregation by GP

IIb/IIIa receptor blockers,[33] clopidogrel, which is an antagonist of ADP induced platelet aggregation,[34] or a combination of aspirin and dipyridamole,[35] or aspirin and clopidogrel.

If persistent platelet activation is proven to be a risk factor for stroke recurrence, stroke patients should be screened for persistent platelet activation in order to adjust treatment. Then tests should be developed that can easily measure in vivo thromboxane biosynthesis.

Thromboxane biosynthesis and post-stroke dementia

We found an association between enhanced thromboxane biosynthesis and the presence of cognitive decline and dementia in the chronic phase after stroke. No differences were found in thromboxane excretion levels between patients with vascular dementia and patients with Alzheimer's disease and cerebrovascular disease. In addition, the association between thromboxane biosynthesis and dementia that we found was strongest in patients who were treated with aspirin. This may reflect a play of chance, since the subgroup analysis included a small number of patients. On the other hand, the finding suggests that increased thromboxane biosynthesis in post-stroke dementia is insensitive for low dose of aspirin, and therefore probably not a reflection of increased platelet activation. Aspirin insensitive thromboxane biosynthesis may reflect extra-platelet sources possibly expressing COX-2 in response to a local inflammatory milieu in patients with unstable angina.[3] The same mechanism may be operative in the setting of cerebral ischemia and inflammation. Among the cell types endowed with thromboxane synthase and capable of expressing COX-2 in response to inflammatory cytokines and growth factors are monocytes and macrophages.[2] This hypothesis is in accordance with the increased levels of 11-dehydro-TXB_2 that were reported in patients with Alzheimer's disease without cerebrovascular disease,[36] and the elevated levels of TXB_2 in postmortem the brain of patients with Alzheimer's disease.[37] In addition, it might explain the apparent protection of non-aspirin NSAIDs against Alzheimer's disease,[38,39] because these drugs are equally potent in inhibiting human platelet COX-1 and monocyte COX-2.[2]

The precise role of thromboxane biosynthesis in the pathogenesis of post-stroke dementia remains unclear, but the possible positive effect of NSAIDs suggests that enhanced thromboxane biosynthesis in this setting is more than a epiphenomenon. The availability of specific COX-2 inhibitors offers the opportunity for testing the hypothesis with a properly designed randomized trial.

Conclusions

Episodic platelet activation occurs in the acute phase of cerebral ischemia and is related to the presence of atrial fibrillation and severity of symptoms. Increased thromboxane biosynthesis can be suppressed by low dose of aspirin. Suppression of metabolite excretion in this setting is clinically relevant because aspirin gives a reduction in stroke recurrence and death of 9 per 1000 patients treated.

In patients with atrial fibrillation, low dose of aspirin alone seems insufficient to adequately suppress platelet activation in the acute phase of cerebral ischemia.

Formation of vasoactive prostanoids by lipid peroxidation does not appear to play a major role in the acute phase of cerebral ischemia.

Persistent platelet activation is present in the majority of patients in the chronic phase after cerebral ischemia, but also after hemorrhagic stroke. It is related with severity of symptoms and the presence of atrial fibrillation.

Increased thromboxane biosynthesis is present in post-stroke dementia, both in patients with vascular dementia and those with Alzheimer's disease with cerebrovascular disease. In this setting, it is not suppressed by aspirin treatment and therefore does not reflect platelet activation.

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SUMMARY
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SAMENVATTING

SUMMARY

Ischemic stroke is a major health problem in the western countries. It is the leading cause of disability and strongly burdens on the community health care system. As a result of the high incidence of stroke, a relatively small reduction of stroke or stroke recurrence will result in a large reduction in number of patients appealing to the health care system. As blood platelets appear to play an important role in ischemic stroke, we describe the role of platelet activation in the acute phase and in the chronic phase of stroke in relation with other vascular risk factors, stroke characteristics, and post-stroke dementia. We also studied the effect of antiplatelet medication on thromboxane biosynthesis in this setting.

In *chapter 1* the methods of thromboxane metabolite measurements are introduced and explained. A historical view of measures of platelet activation is given, leading to the introduction of measurement of urinary 11-dehydro-TXB_2, a metabolite of thromboxane A_2. It is discussed that thromboxane synthesis that can be suppressed by low dose aspirin is of platelet origin, and cyclooxygenase dependent, and thus reflects platelet activation. The diagnosis of dementia in stroke patients and the problems encountered when dementia criteria are implemented in these patients are also described.

In *chapter 2* the time course of thromboxane biosynthesis in 13 patients with acute ischemic stroke is described. Metabolite excretion rates were compared with those of 20 control patients with nonvascular neurological diseases. Elevation in 11-dehydro-TXB_2 excretion levels was found repeatedly in the vast majority of patients with ischemic stroke. In half of the patients elevated levels were found beyond 24 hours after onset of symptoms. In view of the 45 minutes half-life of the metabolite, this would not be expected if thromboxane-dependent platelet activation occurred as a single episode at the time of the event. We concluded that platelet activation occurred repeatedly after acute ischemic stroke. The biochemical evidence of episodic platelet activation provided the rationale for testing the efficacy and safety of aspirin in this setting.

The role of potentially vasoactive F_2-isoprostanes, which can be formed by free radical-catalyzed peroxidation of arachidonic acid but also in a cyclooxygenase dependent fashion, is described in *chapter 3* in relation to formation of the vasoactive eicosanoid thromboxane A_2, produced via the cyclooxygenase pathway. Consecutive urine samples were obtained during the first 72 hours after cerebral ischemia and urinary 11-dehydro-TXB_2 and 8-epi-$PGF_{2\alpha}$ were measured with radioimmunoassays. We found no elevated levels of urinary 8-epi-$PGF_{2\alpha}$, one of the most abundantly formed and potent

vasoconstrictive F_2-isoprostane. Urinary excretion levels of 8-epi-$PGF_{2\alpha}$ were only modestly correlated with those of 11-dehydro-TXB_2. Excretion levels of 8-epi-$PGF_{2\alpha}$ were not related with intake of antiplatelet medication. We concluded that platelet activation is not associated with concurrent changes in F_2-isoprostane biosynthesis and that F_2-isoprostane biosynthesis is largely independent of platelet cyclooxygenase activity.

Repeated periods of enhanced thromboxane biosynthesis were found in more than half of the patients. Increased thromboxane biosynthesis was independently associated with severity of symptoms, atrial fibrillation, and treatment with cyclooxygenase inhibiting drugs. We concluded that in the first few days of acute ischemic stroke, platelet activation occurs repeatedly in a cyclooxygenase dependent fashion and that this platelet activation is independently associated with stroke severity and atrial fibrillation.

The relationship between thromboxane biosynthesis, atrial fibrillation, stroke severity and antiplatelet medication in the acute phase of cerebral ischemia is further investigated in *chapter 4*. Data of 131 patients from three of our previous studies concerning thromboxane biosynthesis in the acute phase of cerebral ischemia were combined. Atrial fibrillation was present in 13% of the patients. Stroke severity and atrial fibrillation were associated with increased urinary 11-dehydro-TXB_2 excretion, whereas use of aspirin was associated with decreased metabolite excretion. Use of aspirin was associated with nearly normal values in patients without atrial fibrillation, but in patients with atrial fibrillation metabolite excretion remained elevated. We concluded that platelet activation in the acute phase of cerebral ischemia is strongly associated with atrial fibrillation and that in these patients platelet activation is insufficiently suppressed by aspirin treatment alone.

Chapter 5 describes thromboxane biosynthesis in the chronic phase after stroke, including patients with intracerebral hemorrhage. Enhanced platelet activation was found in more than half of the patients, and also in more than 70% of the patients after intracerebral hemorrhage. Similar to the findings in the acute phase after cerebral ischemia, atrial fibrillation, stroke severity and use of aspirin contributed independently to the level of metabolite excretion. The suggestion that platelet activation is a reflection of vascular risk factors, diffuse atherosclerotic lesions, or the extent of vascular damage due to stroke is discussed. We concluded that platelet activation is often present in patients in the chronic phase after stroke, including those with intracerebral hemorrhage. Persistent platelet activation, which is associated with atrial fibrillation and poor stroke outcome, can be substantially suppressed by aspirin treatment.

Chapter 6 provides the rationale and design of the Dutch Vascular Factors in Dementia study, in which the study described in *chapter 7* was embedded. From the Rotterdam Stroke Databank, 825 consecutive stroke patients were screened, of whom 300 subjects met the inclusion criteria of the study. Dementia was diagnosed in 71 (24%) of them between 3 and 9 months after stroke. Urinary 11-dehydro-TXB_2 excretion was measured in the chronic phase after stroke. Thromboxane biosynthesis was independently related with the presence of dementia, but not the type of dementia. The differences in metabolite excretion between demented and non-demented patients was most prominent within the ischemic stroke patients who were treated with aspirin. This finding suggests that patients with post-stroke dementia have an aspirin insensitive source of thromboxane biosynthesis. The possible sources are discussed. We concluded that increased thromboxane biosynthesis is associated with the presence, but not the type of post-stroke dementia. The association between thromboxane biosynthesis and the presence of post-stroke dementia is particularly apparent in patients on aspirin treatment, suggesting that patients with post-stroke dementia have an aspirin insensitive source of thromboxane biosynthesis.

In *chapter 8* the results of the studies are discussed and suggestions for further studies are made.

SAMENVATTING

Het herseninfarct en de gevolgen daarvan vormen een belangrijk probleem in de Westerse wereld. Het is een belangrijke doodsoorzaak en de gevolgen van een herseninfarct drukken sterk op het gezondheidszorg systeem. Doordat de incidentie van de beroerte zo hoog is, leidt een relatief kleine reductie in het optreden van beroertes of recidief beroertes tot een grote absolute reductie in de mate waarmee de gezondheidszorg wordt belast. Omdat bloedplaatjes waarschijnlijk een belangrijke rol spelen bij het ontstaan van een beroerte, hebben we de bloedplaatjes functie onderzocht in zowel de acute als de chronische fase van het herseninfarct en deze functie gerelateerd aan vasculaire risicofactoren, eigenschappen van de beroerte zelf, en het optreden van dementie na een beroerte. Daarbij werd ook het effect van aspirine op de bloedplaatjes functie onderzocht.

In *hoofdstuk 1* worden de methoden uiteengezet, waarmee metabolieten kunnen worden gemeten die vrijkomen bij activatie van bloedplaatjes. Er volgt een kort historisch overzicht van methoden om bloedplaatjesactiviteit te meten. Vervolgens beschrijf ik de methode waarbij het urinegehalte van 11-dehydro-tromboxaan B_2, een metaboliet van tromboxaan A_2, als maat voor bloedplaatjes activiteit kan wordt genomen. Besproken wordt dat tromboxaan, waarvan de vorming onderdrukt kan worden met lage dosering aspirine, afkomstig is van bloedplaatjes, en derhalve bloedplaatjes activiteit weerspiegelt. Verder ga ik in op de diagnostiek van dementie bij patiënten met een beroerte en de problemen die optreden als dementie criteria bij patiënten met een beroerte worden toegepast.

In *hoofdstuk 2* wordt het verloop in tijd van tromboxaan biosynthese beschreven bij 13 patiënten in de acute fase na een herseninfarct. Tromboxaan metabolieten in de urine werden vergeleken met die van 20 controle patiënten met niet-vasculaire neurologische ziekten. In de meerderheid van patiënten werd bij herhaling een verhoging van de uitscheiding van 11-dehydro-TXB_2 in de urine gevonden. Bij ongeveer de helft van de patiënten werd een verhoogde excretie van metabolieten zelfs 24 uur na het ontstaan van het herseninfarct gevonden. Gezien de halfwaardetijd van 45 minuten van het metaboliet, kan dit niet verklaard worden door een eenmalig verhoogde bloedplaatjes activiteit ten tijde van het herseninfarct. Wij concludeerden dat verhoogde plaatjes activiteit herhaaldelijk optreedt in de acute fase van het herseninfarct. Deze biochemische aanwijzingen voor episodische bloedplaatjes activiteit in de acute fase van het herseninfarct,

vormde een goed uitgangspunt voor klinisch onderzoek naar het effect en de veiligheid van aspirine in deze situatie.

In *hoofdstuk 3* wordt de rol van mogelijk vasoactieve F_2-isoprostanen onderzocht bij patiënten in de acute fase na cerebrale ischemie in relatie tot de vorming van tromboxaan A_2 dat uit arachidonzuur wordt gevormd onder invloed van het enzym cyclooxygenase. F_2-isoprostanen kunnen, met vrije radicalen als katalysator, door peroxidatie van arachidonzuur worden gevormd, maar ook met behulp van het enzym cyclooxygenase. Gedurende de eerste 72 uur na het optreden van cerebrale ischemie, werden bij 62 patiënten de opvolgende urineproducties opgevangen en met behulp van een radioimmunoassay onderzocht op het gehalte van 11-dehydro-TXB_2, een metaboliet van tromboxaan A_2, en 8-epi-$PGF_{2\alpha}$, één van de meest gevormde F_2-isoprostanen met vasoconstrictieve eigenschappen. We vonden geen verhoogde uitscheiding van 8-epi-$PGF_{2\alpha}$. De waarden van 8-epi-$PGF_{2\alpha}$ waren maar matig gecorreleerd met die van 11-dehydro-TXB_2. Er was geen relatie tussen de uitscheiding van 8-epi-$PGF_{2\alpha}$ en het gebruik van aspirine. De conclusie was dat verhoogde bloedplaatjes activiteit niet geassocieerd is met gelijktijdige veranderingen in de biosynthese van F_2-isoprostanen en dat F_2-isoprostaan biosynthese voor het grootste deel onafhankelijk is van cyclooxygenase activiteit van de bloedplaatjes.

In meer dan de helft van de patiënten werd bij herhaling verhoogde tromboxaan biosynthese gevonden. Deze was onafhankelijk geassocieerd met de ernst van het herseninfarct, boezemfibrilleren, en behandeling met cyclooxygenase remmende medicijnen. Wij concludeerden dat er in de eerste paar dagen na cerebrale ischemie herhaaldelijk bloedplaatjesactivatie optreedt, die cyclooxygenase afhankelijk is. Deze bloedplaatjesactivatie is onafhankelijk geassocieerd met de ernst van de beroerte en boezemfibrilleren.

De relatie tussen tromboxaan biosynthese, boezemfibrilleren, ernst van de beroerte, en het gebruik van bloedplaatjes remmende medicijnen wordt verder beschreven in *hoofdstuk 4*. De gegevens van drie van onze eerdere studies die betrekking hadden op tromboxaan biosynthese in de acute fase na het ontstaan van cerebrale ischemie werden samengevoegd en geanalyseerd. Het betrof een populatie van 131 patiënten. Boezemfibrilleren was aanwezig bij 13% van de patiënten. Ernst van de beroerte en boezemfibrilleren waren geassocieerd met verhoogde uitscheiding van 11-dehydro-TXB_2 in de urine. Gebruik van aspirine was geassocieerd met een verlaging in de uitscheiding van deze metaboliet. Bij patiënten zonder boezemfibrilleren was gebruik van aspirine geassocieerd met vrijwel normale uitscheiding van 11-dehydro-TXB_2 in de urine. Bij patiënten met

boezemfibrilleren en gebruik van aspirine bleef de metaboliet uitscheiding verhoogd. Geconcludeerd werd dat bloedplaatjes activatie in de acute fase na het ontstaan van cerebrale ischemie, sterk is geassocieerd met boezemfibrilleren en dat bij patiënten met boezemfibrilleren de bloedplaatjesactiviteit onvoldoende wordt onderdrukt met aspirine alleen.

Hoofdstuk 5 beschrijft tromboxaan biosynthese in de chronische fase na een beroerte, waarbij ook patiënten met een intracerebrale bloeding werden geïncludeerd. Verhoogde bloedplaatjes activiteit werd gevonden in meer dan de helft van alle patiënten, en, zeer verrassend, ook in meer dan 70% van de patiënten met een intracerebrale bloeding. Zoals we in de acute fase na cerebrale ischemie hadden gevonden, bleken boezemfibrilleren, ernst van de beroerte, en het gebruik van aspirine, onafhankelijk bij te dragen aan het niveau van tromboxaan excretie in de urine. De vraag of bloedplaatjes activiteit een weerspiegeling is van vasculaire risicofactoren, diffuse atherosclerose, of de uitgebreidheid van de schade aan de bloedvaten ten gevolge van de beroerte, wordt in dit hoofdstuk besproken. Geconcludeerd wordt dat verhoogde bloedplaatjes activiteit vaak voorkomt in de chronische fase na een beroerte, en ook bij patiënten met een bloeding. Deze aanhoudende bloedplaatjesactivatie, die geassocieerd is met boezemfibrilleren en een slechter neurologische functioneren, kan goed worden onderdrukt met aspirine.

In *hoofdstuk 6* wordt de achtergrond en de opzet van de 'Dutch Vascular Factors in Dementia Study' beschreven. Het cohort beschreven in *hoofdstuk 7* is uit deze studie afkomstig. Gedurende de studieperiode werden 825 opeenvolgende patiënten uit de Rotterdam Stroke Databank gescreend van wie er 300 aan de criteria voor de dementie studie voldeden. Tussen 3 en 9 maanden na de beroerte kon de diagnose dementie bij 71 (24%) van hen worden gesteld. Het gehalte van 11-dehydro-TXB_2 in de urine als maat voor tromboxaan biosynthese werd bepaald in de chronische fase na de beroerte. Tromboxaan biosynthese was onafhankelijk gerelateerd aan het optreden van dementie, maar niet aan het type dementie. De verschillen in metaboliet uitscheiding tussen patiënten met en zonder dementie waren vooral aanwezig in the groep patiënten met cerebrale ischemie die behandeld werden met aspirine. Deze bevinding suggereert dat de wijze van tromboxaan productie bij patiënten met dementie na een beroerte ongevoelig is voor aspirine. De mogelijkheden van deze aspirine ongevoelige tromboxaan biosynthese worden verder in het hoofdstuk besproken. De conclusie is dat verhoogde tromboxaan biosynthese geassocieerd is met het optreden van dementie, maar niet met het type dementie na een beroerte. De associatie tussen verhoogde tromboxaan productie en dementie na een beroerte is met name aanwezig bij patiënten die aspirine gebruiken en suggereert dat deze

patiënten een aspirine ongevoelige bron van tromboxaan biosynthese hebben.

In *hoofdstuk 8* worden de resultaten van de studies besproken en worden aanbevelingen gedaan voor verder onderzoek.

DANKWOORD
CURRICULUM VITAE &
LIST OF PUBLICATIONS

DANKWOORD

Mijn dank gaat uit naar velen met wie ik in de jaren voor, tijdens en nu ook na mijn opleiding heb samengewerkt.
Allereerst mijn promotor, Prof. Peter Koudstaal, die mij in het derde jaar van mijn studie wist te strikken en mij enthousiast wist te maken en te houden voor het vak. Beste Peter, ik heb me in al die jaren vaak afgevraagd hoe het nou kwam dat ik als ik jouw kamer verliet met een nieuwe stapel werk waar ik vooraf niet veel zin in had, het verlichte gevoel had met vakantie te gaan. Eigenlijk was na zo'n gesprek het meeste werk gedaan. Daarnaast dank ik je voor je geduld, je vertrouwen en de opleiding door jou in bredere zin.
Ook heel belangrijk is voor mij geweest de helaas veel te vroeg overleden Dr. Anet van Latum, die mij het pre-Windows tijdperk heeft doorgeloodst en mij de eerste statistiek en epidemiologie in praktijk heeft bijgebracht.
The collaboration with our Italian colleague's, Prof. Carlo Patrono from the department of pharmacology, University of Rome 'La Sapienza', and Prof. Giovanni Ciabattoni from the department of pharmacology, University of Chieti 'G. D'Annunzio' is gratefully acknowledged. Dear Prof. Patrono, it has been, and I hope it will be in the future, a great pleasure to work with you. Your input has been of great value, not only for the papers, but also for my understanding of the pharmacological aspects of the subject. I have very much appreciated your kind hospitality in Dublin and in Fiuggi Terme.
Dear Prof. Ciabattoni, I thank you for the fine collaboration, the swiftness and the accuracy in handling the valuable samples. I am looking forward to new projects in which we can work together.
De coauteurs, betrokken bij het NESTOR project dank ik voor de leerzame periode die ik met hen heb gewerkt; Prof. Grobbee, Prof. Kluft, Dr. Bots, Dr. Breteler, Dr. Haverkate, Drs. van Harskamp en Dr. van Swieten. Beste Frans, beste John, met name de middagen dat we met Inge de dementie diagnose bespraken zal ik niet snel vergeten.
Paul Naarding, die de samenwerking met de psychiatrie belichaamd in het dementie project. Paul, ik hoop dat er nog enkele mooie stukken van onze hand verschijnen.
Mijn speciale dank gaat uit naar Lydia Loman, die ogenschijnlijk onvermoeibaar doorging met haar werkzaamheden voor anderen. Lydia, ik kijk met gemengde gevoelens terug op onze samenwerking. Enerzijds met veel plezier want je had altijd tijd en alles werd geregeld, anderzijds met enig schuldgevoel omdat we je misschien overvraagd hebben. Ik ben je in ieder geval dankbaar voor alle inzet die je hebt getoond en hoop dat het je beter gaat.

De collega's van de neurovasculaire onderzoeksgroep van wie ik er enkele met naam wil noemen. Diederik Dippel, Stef Bakker en Hanneke Hilkemeijer met wie de samenwerking vlot en vertrouwd verloopt en niet te vergeten, Anne-Marie Wijnhoud, die sneller figuren maakt dan haar schaduw.
De verpleegkundigen van 6 Noord en 6 Midden, die onvermoeibaar, dag en nacht, met de urinemonsters over de gang hebben gelopen. Zonder hun hulp was het onderzoek niet mogelijk geweest.
De ondersteuning op de 22e door Monica, Lourens en Kris en later op de afdeling neurologie door Lourens en Ton was altijd plezierig, laagdrempelig en heb ik zeer gewaardeerd. Aan de 'buitenschoolse activiteiten' bewaar ik goede herinneringen.
Mijn vrienden, die voor mijn gemoedsrust uiteindelijk nog liever hun tong afbeten dan dat ze het P-woord in de mond namen. Eindelijk krijg ik weer meer tijd!
Mijn ouders en schoonouders, die al jaren geduldig en met vertrouwen op het feestje hebben zitten wachten en die waar mogelijk de helpende hand hebben geboden.
Tenslotte, mijn paranimfen, waarvan ik de een wat langer ken, en de ander wat beter. Om met de eerste te beginnen. Beste Henk, onze start was niet groots, maar desondanks ben je op meerdere fronten een zeer gewaardeerde collega gebleken met wie ik veel lief en leed heb kunnen delen. Bedankt.
Lieve Inge, uiteindelijk is onze samenwerking zeer productief gebleken. Een mooier resultaat had ik mij niet kunnen voorstellen. Onze zoektocht door de Rotterdamse verpleeg- en verzorgingshuizen heeft gelukkig meer dan een rijkelijk gevulde database opgeleverd. De publicaties zullen nu vanzelf volgen.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 26 juni 1964 te Rotterdam. Hij volgde zijn middelbare schoolopleiding aan de Scholen Gemeenschap 'de Krimpenerwaard', Krimpen aan den IJssel, alwaar hij in 1982 zijn HAVO diploma en in 1984 het VWO (Atheneum B) diploma haalde. In dat jaar werd begonnen met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. In april 1991 haalde hij zijn artsexamen. Tijdens de laatste jaren van zijn studie was hij student-assistent op de afdeling Neurologie van het Academisch Ziekenhuis Rotterdam en onder leiding van Prof.dr. P.J. Koudstaal en betrokken bij het opzetten van de Rotterdam Stroke Databank. Daarnaast verrichtte hij studies naar glucose metabolisme en lipoproteïnen bij patiënten met acute cerebrale ischemie. Van 1991 tot 1992 was hij Assistent Geneeskunde Niet In Opleiding (AGNIO) op de afdeling Neurologie van het Academisch Ziekenhuis Rotterdam onder leiding van Prof.dr. F.G.A. van der Meché. Van 1992 tot 1993 is hij gedurende 18 maanden werkzaam geweest in het kader van de wet gewetensbezwaren militaire dienst, bij de Stichting Afasie Rotterdam, onder leiding van drs. F. van Harskamp. Tijdens die periode verrichte hij ook onderzoek naar bloedplaatjes activiteit bij patiënten met acute cerebrale ischemie onder leiding van Prof.dr. P.J. Koudstaal. Van 1993 tot 1996 was hij arts-onderzoeker op de afdeling Neurologie van het Academisch Ziekenhuis Rotterdam en deed onderzoek naar vasculaire factoren bij dementie en bloedplaatjes activiteit bij patiënten met een beroerte, onder leiding van Prof.dr. P.J. Koudstaal. Van augustus 1996 tot september 2001 volgde hij zijn opleiding tot neuroloog. Zijn opleiders waren Prof.dr. F.G.A. van der Meché en Prof.dr. P.J. Koudstaal. Vanaf 1 september 2001 is hij werkzaam als neuroloog in het Academisch Ziekenhuis Rotterdam.

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